Striatal amplifiers of incentive salience

by

Alexandra Gold DiFeliceantonio

A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
(Psychology)
in The University of Michigan
2013

Doctoral Committee:

Professor Kent C. Berridge, Chair
Associate Professor Joshua D. Berke
Professor Robert T. Kennedy
Professor Terry E. Robinson

To Daniel McEntire Gold

And

In Memory of Mary DiFeliceantonio

Acknowledgements

No one accomplishes anything alone. First I want to thank the people who steered me towards my current academic path (in chronological order). I want to thank Daniel Gottlieb for being the first person to show me that science was everything -- creativity, structure, expression, and discovery -- and that it was something I could do. I want to thank Nicole Schramm-Sapyta for her incredible enthusiasm and for being my first role model as a female scientist. Thirdly, and most importantly, I want to thank my graduate advisor, Kent Berridge, for his excellent guidance, his continual encouragement, and for teaching me to tell stories with data. I cannot say enough good things about Kent as a mentor. Succinctly, I feel incredibly lucky to have had the opportunity to learn from him.

I would also like to thank my committee members – Joshua Berke, Robert Kennedy, and Terry Robinson – their input has been invaluable to the progress of this dissertation. I want to thank Robert Kennedy and his postdoctoral researcher Omar Mabrouk for dedicating time and resources to teaching me microdialysis, analyzing samples, and generally taking a risk with me on this project. I want to thank Josh and Terry for always finding time to talk with me about my data.

Fellow Berridge lab members –Jocelyn Richard, Daniel Castro, Eric Jackson, and Steve Mahler – have taught me too many things to list and have always been willing participants in discussions of the meaning of findings, the universe, octopi, etc. In lab, numerous undergraduate assistants helped with rat handling, prepping and cleaning of equipment, and video watching. I want thank David Springstead especially for the artwork used in many of the figures here. I would also like to thank Aaron Garcia, Ryan Selleck, and Stephen Burwell for assistance with immunohistochemistry.

I have been a part of a great group of friends and graduate students: Lindsay Yager, Kirsten Porter-Stransky, Anne Berry, Eila Roberts, Jeremiah Bertz, Ben Saunders, Liz Cogan, Shanna Harkey, Alaina Neary Case, Morgan Gustison, Jeff Pettibone, Howard Gritton, and Matt Howe. Thanks to Matt especially for finding me and for everything, just everything.

Finally, I want to say thanks to my family (biological and chosen): my parents, Martha and Louis (you too Stefanie), who seem to sincerely believe I can do anything I want to in life, my sister Jordan for all of your encouragement, and my grandfather Mac for ensuring I had every opportunity I could ask for. It's good to know there are always people rooting for you no matter what.

Table of Contents

Dedication	ii
Acknowledgements	iii
List of Figures	vii
Abstract	viii
Chapter : Introduction	**1**
Figures	13
Chapter 2: Opioid Stimulation of Central Nucleus of the Amygdala Enhances Incentive Salience of a Preferred Cue	**14**
Introduction	14
Materials and Methods	18
Results	23
Discussion	34
Figures	43
Chapter 3: Effects of Mu Opioid and Dopamine Receptor Stimulation in Dorsolateral Neostriatum on Incentive Salience of a Preferred Cue	**48**
Introduction	48
Materials and Methods	50
Results	58
Discussion	71
Figures	80
Chapter 4: Enkephalin in Dorsomedial Neostriatum Says "Eat More Now!"	**88**
Introduction	88
Materials and Methods	89
Results	98
Figures	109

Chapter 5: Disruption of Sign-tracking and Enhancement of Goal-tracking after Mu Opioid Receptor Activation in Ventrolateral Neostriatum **115**

Introduction 115

Materials and Methods 116

Results 122

Discussion 126

Chapter 6: General Discussion 134

Figures 153

References 154

List of Figures

Figure 1.1.	Model of incentive salience generation	13
Figure 2.1.	CeA DAMGO enhances focus	43
Figure 2.2	Individual Variation	44
Figure 2.3.	Cue Locked Enhancement	45
Figure 2.4.	Topography of Behavior	46
Figure 2.5.	Localization of DAMGO effects	47
Figure 3.1.	Approach and consummatory behaviors are enhanced by DAMGO	80
Figure 3.2.	Dorsolateral DAMGO enhances focus	81
Figure 3.3.	Individual variation for DAMGO microinjection	82
Figure 3.4.	Individual variation for amphetamine microinjection	83
Figure 3.5.	Dorsolateral not dorsomedial neostriatum enhances motivational magnets	84
Figure 3.6.	Environmental shift	85
Figure 3.7.	Reinforcer value shift	86
Figure 3.8.	Conditioned reinforcement	87
Figure 4.1.	Endogenous extracellular opioid peptides in response to palatable food consumption	108
Figure 4.2.	Details of endogenous enkephalin surge	110
Figure 4.3.	Map of microinjection causation of intense eating	112
Figure 4.4.	Mu but not delta	113
Figure 5.1.	DAMGO potently enhances M&M consumption in ventrolateral neostriatum	129
Figure 5.2.	DAMGO enhances appetitive behaviors directed at the food cup in a time-locked manner	130
Figure 5.3.	Individual Variation	131
Figure 5.4.	Microinjection sites within ventrolateral neostriatum	132
Figure 6.1.	Anatomical connectivity of the extended amygdala, midbrain dopamine nuclei, striatum, and cortex	151

Abstract

Research into the neural circuits that underlie the amplification of motivation has been focused on the traditional "reward pathway." Recent work, however, has implicated another striatal level structure, the central nucleus of the amygdala, in the amplification of motivation. Here, I extend these findings and demonstrate mu opioid receptor activation of the central nucleus of the amygdala enhances the motivational power of cues associated with reward. Recent findings in from human imaging studies have hinted that another striatal level structure, the neostriatum, may also participate in the amplification of motivation. Here, I demonstrate that mu opioid receptor activation in neostriatum enhances motivation for learned cues and primary rewards. Mu opioid receptor activation in dorsolateral neostriatum potently enhanced the attractiveness of cues in a manner similar to amygdala activation and did so in a manner not consistent with a habit hypothesis. However, consumption of primary rewards was not enhanced. Here, I demonstrated for the first time that enkephalin in dorsomedial neostriatum surges when rats consume a sweet, fatty food. Further, this consumption can be stimulated by microinjection of a mu opioid receptor activating drug. Although, dorsomedial neostriatal activation participated in motivation for primary rewards, activation did not have an effect on motivation for learned cues. Finally, in ventrolateral neostriatum, mu opioid receptor activation enhanced the attractiveness of a contiguous cue and motivation to consume primary rewards. These findings extend the neural substrates of motivation beyond traditional reward structures and have implications for the description and treatment of disorders of intense motivation such as drug addiction and binge eating.

Chapter 1

Introduction

For an animal to survive in a complex environment, prediction is essential. A system for learning what cues predict the presence of nutrient dense food, receptive sexual partners, and injurious stimuli is necessary for survival. An understanding of these systems was initially described by Pavlov. He demonstrated that animals could use an auditory stimulus to anticipate the receipt of food (Pavlov and Anrep 1927). Since this description, many models of Pavlovian learning have been advanced, each focusing on how a cue gains *predictive* value. A popular form of such models are temporal difference models such as the Rescorla-Wagner model and it's many updates and modifications (Rescorla and Wagner 1972; Wagner and Rescorla 1972).

A hallmark of these models is that the learned association remains stable once established and requires stepwise updating to change associative strength between two stimuli. However, actual behavior of an animal is more dynamic. For example, a previously learned food cue will elicit different responses in a hungry or sated individual (Dickinson and Balleine 1990; Corbit and Balleine 2003; Kessler 2009) and a previously disgusting salt stimulus can become palatable and desirable when a salt-deficiency is induced (Berridge and Schulkin 1989; Tindell, Smith et al. 2006; Robinson and Berridge 2013). This means that although the *predictive* value of a cue can remain stable the *incentive* value of the cue can fluctuate dramatically on a moment to moment basis. Cues

that have attained more than predictive value are said to have gained incentive salience (Bindra 1978; Robinson and Berridge 1993). Incentive salience is dynamically computed at each cue encounter, incorporating associative strength between a reward and it's predictive cue and neurobiological state of mesocorticolimbic circuits at the moment of cue re-encounter, influenced by drug states, appetite/satiety states, stress states, etc. (Zhang, Berridge et al. 2009). An individual's mesocorticolimbic state combines synergistically with learned conditioned stimulus (CS)- unconditioned stimulus (UCS) associations to generate incentive salience ('wanting[1]') on the fly, and the result of the combination can raise, lower, or even completely reverse the previously learned motivational value (Berridge, Flynn et al. 1984; Pecina and Berridge 2008; Tindell, Smith et al. 2009; Zhang, Berridge et al. 2009; Robinson and Berridge 2013).

Zhang and colleagues' (Zhang, Berridge et al. 2009) formal conceptualization of this idea is the depicted mathematical model (Figure 1.1). In this model, incentive motivation is conceptualized with the equation $V= (r_t*K)$; where V is incentive salience, r_t is the learned associations between a cue (Pavlovian CS) and its reward (UCS), and K is physiological state such as appetite or brain activation (Zhang, Berridge et al. 2009). This model relates to a prediction error model of learning (Rescorla and Wagner 1972; Wagner and Rescorla 1972), in that it uses this model's V (associative strength) as part of the r_t (learned associations of the cue). What an incentive motivation model allows is a dynamic motivational transformation of that learned association: multiplying r_t by K, the motivational state of the individual at the time of the cue encounter. A stimulus-response

[1] Here I will use 'wanting' in quotation marks to signify attribution of incentive salience to rewards and reward paired cue (Robinson and Berridge 1993). This refers to a motivational state of enhanced attraction, consumption and invigoration of/by rewards and reward cues. This 'wanting' is implicit and does require conscious processing, differentiating it from wanting without quotation marks.

(S-R) or habitual model of responding only allows the cue to elicit the same response no matter the motivational state (K) of the individual. This model describes the transformation of learned CSs into motivational, 'wanted' CSs. It does not, however, posit a process or neurobiological mechanism for this transformation. The aim of this dissertation is to probe the neurobiological mechanisms underlying the dynamic amplification of incentive salience.

Pavlovian conditioned approach as a method to test the generation and targeting of incentive salience

To test the underlying neurobiological mechanisms of incentive salience an appropriate behavioral measure of incentive salience attribution, intensity, and target is necessary. How can we measure the strength of incentive salience of different cues in animal models? Individual differences in the target of incentive salience have been found in autoshaping or "sign-tracking" experiments in rats (Flagel, Akil et al. 2009; Saunders and Robinson 2010; Yager and Robinson 2013), which model the 'motivational magnet' feature of 'wanted' Pavlovian cues, in that they elicit rapid approach and draw behavior to them like a magnet. In one version of autoshaping, phasic presentation of a lever CS (CS+ lever; sometimes called the sign) always predicts a reward UCS: a sucrose pellet delivered to a dish (CS_{dish}; sometimes called the goal). After learning the Pavlovian CS-UCS association, many individual rodents, fish, pigeons, dogs, and people come to approach and bite (in the case of animals) the discrete CS+ sign and are known as "sign-trackers" (Zener 1937; Breland and Breland 1961; Jenkins and Moore 1973; Boakes, Poli et al. 1978; Flagel, Watson et al. 2007; Nilsson, Kristiansen et al. 2008; Kessler 2009;

Tomie, Lincks et al. 2012). By contrast, other individuals come to approach the goal location where reward is delivered (CS_{dish}) during the CS+ sign presentation and are known as "goal-trackers" (Jenkins and Moore 1973). Goal-tracking vs. sign-tracking differences emerge in the first few days of Pavlovian training in rats, and remains stable (Lolordo and Rescorla 1964; Mansour, Fox et al. 1994; Mahler and Berridge 2009). Using sign- and goal-tracking as a behavioral measure of incentive salience, I can then manipulate candidate brain areas to test the underlying substrates of incentive salience amplification.

Traditional amplifiers of incentive motivation

The mesolimbic dopamine system has been mentioned as one system involved in the amplification of incentive motivation. One part of this circuit, the nucleus accumbens, has long been identified as a limbic motor interface, a region capable of translating motivational value into behavior (Mogenson, Jones et al. 1980). For example, microinjections of amphetamine or mu opioid receptor activating DAMGO into nucleus accumbens shell or core produces robust increases in cue-triggered instrumental responding for sugary rewards as measured in Pavlovian to instrumental transfer (PIT) or in neuronal incentive salience signals (Wyvell and Berridge 2000; Pecina and Berridge 2008). Similarly, microinjections of DAMGO or amphetamine raise conditioned reinforcement value or breakpoint values in instrumental tests that might reflect incentive salience (Kelley and Delfs 1991; Cunningham and Kelley 1992; Zhang, Balmadrid et al. 2003).

Is the accumbens special in this ability to multiply the motivational value of a learned association or is this a function shared by all striatal structures? We have found that DAMGO microinjections in other striatal-type structures such as the central amygdala (CeA) also increase cue-triggered "wanting" in PIT and likewise increase the motivational magnet strength of an individual's favorite reward cue in a sign-tracking versus goal-tracking test (Mahler and Berridge 2009, 2011). This means that there is at least one other area capable of amplifying "wanting" for learned cues. This dissertation will further explore the CeA as an area capable of generating motivation and test the neostriatum as another structure that may generate motivation.

Moving outside the nucleus accumbens: central nucleus of the amygdala

CeA has distinct inputs and outputs that distinguish it from other nuclei in amygdala (Killcross, Robbins et al. 1997; Swanson and Petrovich 1998). Anatomically, the CeA can be viewed as a serial output nucleus for the basolateral amygdala (BLA) (de Borchgrave, Rawlins et al. 2002), and as a serial starting point for the extended amygdala complex (Heimer, Van Hosen et al. 2008; McGinty, Hayden et al. 2011). Importantly, the CeA can be viewed as a striatal-level structure within macrocircuits that organize cortico-striatal-pallidal networks to generate motivated behavior (Swanson 2005; Phillips and Hitchcott 2009). The aim of this dissertation is to probe the function of striatal level structures and this allows us to conceive of the CeA as a striatal structure that generate incentive motivation.

Functionally, CeA is a site where mu opioid stimulation via DAMGO microinjection can markedly increase motivation to seek and consume palatable food

rewards, and CeA interacts with nucleus accumbens in opioid enhancements of food intake (Gosnell 1988; Eblen and Graybiel 1995; Dickinson, Campos et al. 1996; Phillips, Vacca et al. 2008; Cox, Benkelfat et al. 2009). CeA also plays special roles in translating learned Pavlovian information into active motivation (Berridge, Flynn et al. 1984; Hollerman and Schultz 1998; Everitt, Parkinson et al. 1999; Hall, Parkinson et al. 2001; Holland and Gallagher 2003; Corbit and Balleine 2005). For example, stimulation of mu opioid receptors in the central nucleus of the amygdala (CeA) magnifies the ability of cues to trigger incentive motivation toward sucrose or sex incentives and to act as CS motivational magnets (Mahler and Berridge 2009, 2011). Here, I will more stringently test the role of the CeA in focusing and targeting incentive salience on a preferred cue.

Moving outside the nucleus accumbens: neostriatum

Dorsal neostriatum, especially dorsolateral neostriatum, traditionally has been viewed in terms of motor functions of movement and habits in responding to reward cues (Schultz and Dickinson 2000b; Packard and Knowlton 2002a; Yin, Knowlton et al. 2004; Balleine and Ostlund 2007; Wise 2009). However, dorsal striatum recently has become implicated in motivation-related functions (Volkow, Wang, Fowler, Logan, Jayne, Franceschi et al. 2002; Volkow, Wang et al. 2006; Palmiter 2008b; Stice, Spoor et al. 2008; DiFeliceantonio, Mabrouk et al. 2012; Nummenmaa, Hirvonen et al. 2012). Furthermore, dorsolateral striatal function is needed for motivational cues to excite reward seeking behavior (Corbit and Janak 2007). Given the building evidence that dorsolateral striatum may have some motivational function, and that many studies probing the dorsolateral striatum have not included specific tests for motivation, increasingly attention has been focused on the putative role of the dorsolateral

neostriatum in generating motivation to reward-cues (Parkinson, Cardinal et al. 2000; Ito, Dalley et al. 2002; Vanderschuren, Di Ciano et al. 2005; Vanderschuren and Everitt 2005).

As described above, reward-cues can trigger intense motivational states, increasing attraction to the cue and urge for the associated reward (Robinson and Berridge 1993). But the intensity of a cue's attractiveness still fluctuates, depending on the brain state of the individual at the moment of encounter. A recovering addict may successfully resist drug cues on many encounters, yet on another occasion find the same cues irresistible, triggering relapse, and spiraling back into the pattern of addiction. Therefore, cue-evoked activations of dorsolateral neostriatum might represent a novel mechanism for the generation of incentive motivation that makes the cue tempting and attractive. Alternatively, dorsolateral neostriatum activations might reflect non-motivational functions such as mediation of learned predictions of reward to follow, learned stimulus-response habits, or incipient movements evoked by reward cues, as is previously suggested. The experiments in this dissertation will explicitly test these two hypotheses and attempt to demonstrate that dorsolateral neostriatum participates in generation of motivation in addition to its role in habit expression and motor learning.

In contrast to the dorsolateral neostriatum, which has most commonly been tested in terms of habit expression and formation, the dorsomedial neostriatum has been demonstrated to be important for the formation of action-outcome associations. For example, disruptions of dorsomedial neostriatal activity decreases rats' sensitivity to reinforcer devaluation, meaning they work continue to work for a diminished reward (Yin, Knowlton et al. 2005). This finding led to the hypothesis that dorsomedial

neostriatum stores information about the value of instrumental outcomes, and begs the question: Can dorsomedial neostriatum also generate motivation for sensory reward?

There is some evidence dorsomedial striatum tracks the receipts of sensory reward. In dorsomedial neostriatum, preproenkephalin mRNA levels track short term satiety state and not long term deprivation state (Will, Vanderheyden et al. 2007). The decrease in preproenkephalin mRNA after feeding is likely due to a release of enkephalin during eating that may drive the motivation to eat (Kelley, Baldo, Pratt et al. 2005), although there is no direct evidence for this claim. Here I test this hypothesis using advances in microdialysis collection and analysis techniques that allow for the in vivo detection of endogenous peptides in freely moving and eating rats.

The hypothesis that opioid circuitry in dorsomedial neostriatum participates in generating motivation to consume, or even over-consume, a palatable food reward is concordant with its anatomical wiring from limbic prefrontal cortical inputs (Ragsdale and Graybiel 1988; Gerfen 1989; Ragsdale and Graybiel 1990; Eblen and Graybiel 1995; Kincaid and Wilson 1996; Levesque and Parent 1998). These inputs in dorsal neostriatum are confined to "patch" or striosome compartments (Pert, Kuhar et al. 1976; Herkenham and Pert 1980; Gerfen, Herkenham et al. 1987), which may also project directly to dopamine containing neurons in the substantia nigra (Fujiyama, Sohn et al. 2011). These patches are mu opioid receptor rich, the receptor for the ligand enkephalin in the dorsal striatum, therefore manipulation of these patches by endogenous enkephalin levels or exogenous drug, may increase incentive motivation (Pert, Kuhar et al. 1976; Akil, Watson et al. 1984). This dissertation will explicitly test this hypothesis.

Opioid manipulation of more ventral areas of neostriatum has been demonstrated to produce intense eating (Bakshi and Kelley 1993b, a). This portion of striatum receives projections from forelimb and oromotor areas of sensory motor cortex and dopaminergic manipulation of this area often results in oromotor stereotypy (Kelley, Lang et al. 1988; Kelley, Gauthier et al. 1989b; Delfs and Kelley 1990; Dickson, Lang et al. 1994). Often, manipulations that generate motivation for primary rewards also increase motivation for learned cues, but this is not always the case (Bakshi and Kelley 1993b, a; Pecina and Berridge 2000; Zhang and Kelley 2000; Jackson 2009). So it is unknown if mu opioid receptor activation will enhance motivation for learned cues as it does for primary food rewards.

Summary of current studies

The goal of this dissertation is to determine if areas outside the traditional "reward" pathway are capable of generating intense motivation. Specifically, I manipulated mu opioid receptor activation in the central nucleus of the amygdala, dorsolateral neostriatum, dorsomedial neostriatum, and ventrolateral neostriatum; and I manipulated dopamine in the dorsolateral neostriatum. I found that these areas do participate in the generation of intense motivational states, but differ in the target (UCS or CS, predictive CS or contiguous CS) of that intense state.

Chapter 2: Opioid Stimulation of Central Nucleus of the Amygdala Enhances Incentive Salience of a Preferred Cue

In these experiments I will further examine the effect of mu opioid receptor stimulation of the central nucleus of the amygdala on the motivational magnet properties of a preferred cue. These experiments build off of those previously performed in our lab,

but focus intensely on goal-tracking animals. Previous work suggested that both sign- and goal-trackers showed enhanced motivation for their preferred cue. The current experiment used new cameras angled directly into the food magazine to demonstrate that goal-trackers showed a similar enhancement of incentive motivation for the preferred cue after mu opioid receptor stimulation.

Chapter 3: Effects of Mu Opioid and Dopamine Receptor Stimulation in Dorsolateral Neostriatum on Incentive Salience of a Preferred Cue

Having established that mu opioid receptor stimulation of CeA enhances the motivational magnet properties of learned cues, I sought to test another area with the potential to generate incentive motivation, dorsolateral neostriatum. Mu opioid receptor activation enhanced sign- and goal-tracking in a pattern similar to that seen after mu opioid receptor activation in CeA. Because this portion of neostriatum is well established to be involved in habit expression, I experimentally tested a habitual or a motivational interpretation. Specifically, I tested the ability of mu opioid receptor activation to maintain a motor ritual in the face of environmental and reinforcer outcome change. We found results consistent with a motivational hypothesis, that behavior remained flexible and not habitual. Finally, I tested whether mu opioid receptor activation could enhance the value of a conditioned reinforcer for an entirely new instrumental task, it did. These results suggest that dorsolateral neostriatum is involved in the generation of intense motivation as well as it established roles in habit expression.

To explore the neurochemical specificity of this enhancement of incentive salience attributed to the preferred target cue, I microinjected a low dose of amphetamine

into the DLS during autoshaping. After amphetamine microinjection, purely sign- and goal-tracking rats did not demonstrate an increase in responding. Those rats that were mainly sign-trackers, but showed some goal-tracking greatly increased the amount of goal-tracking they performed following amphetamine microinjection.

Chapter 4: Enkephalin in Dorsomedial Neostriatum Says "Eat More Now!"

Dorsomedial neostriatum has been implicated in encoding the value of outcomes for instrumental outcomes. In this set of experiments I tested whether dorsomedial neostriatum can generate intense motivation for a tasty outcome (M&M candy). First, we used advancements in microdialysis to determine that enkephalin levels surge after animals consume M&M candies. Then I microinjected a mu opioid receptor agonist into the dorsomedial neostriatum and observed a huge increase in consumption only when the microinjections where in the dorsal anteromedial quadrant of neostriatum. Delta receptor activation did not produce the same effect. Finally, I determined the enhancement was solely in motivation for the food and not in its hedonic impact with the affective taste reactivity test.

Chapter 5: Disruption of sign-tracking and enhancement of goal-tracking after mu opioid receptor activation in ventrolateral neostriatum

Opioid manipulation of more ventral areas of neostriatum has been demonstrated to produce intense eating (Bakshi and Kelley 1993b, a). It is unknown however, if mu opioid receptor activation in this same area produces enhanced motivation for learned cues in an autoshaping paradigm. Often, manipulations that generate motivation for primary rewards also increase motivation for learned cues, but this is not always the case (Bakshi and Kelley 1993b, a; Pecina and Berridge 2000; Zhang and Kelley 2000; Jackson

2009). Here I report that activation of mu opioid receptors does not enhance motivation for the CS_{dish} in goal-tracking animals, but disrupts sign-tracking and enhances goal-tracking in sign-trackers. Microinjections in this same area robustly increase feeding.

Figures

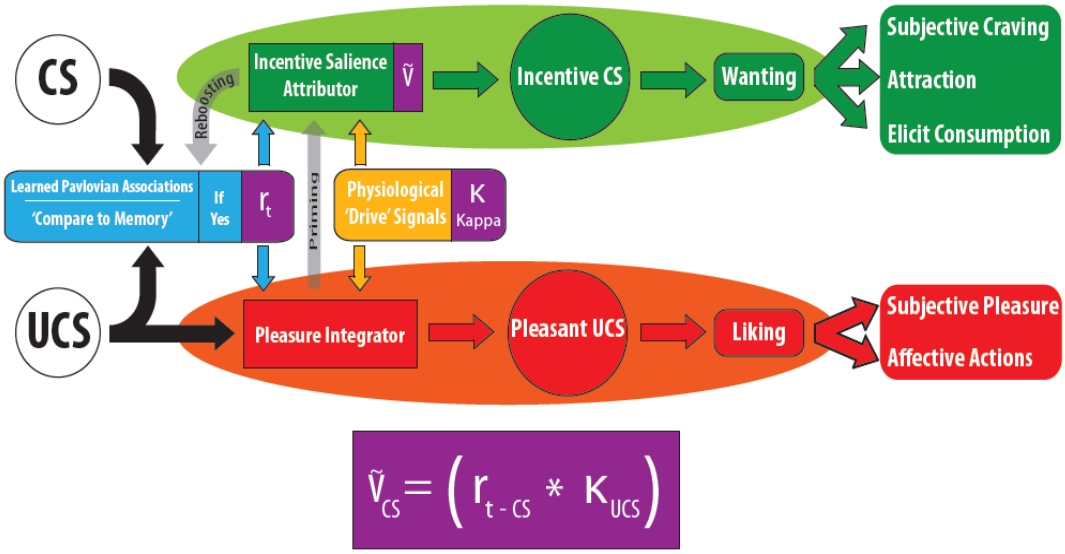


Figure 1.1. Model of incentive salience generation and a mathematical model of incentive salience generation. "Liking" and "wanting" are fractured into separable systems. Physiological signals (K) are capable of altering the incentive salience or pleasure generated at the moment of CS or UCS encounter.

Chapter 2

Opioid Stimulation of Central Nucleus of the Amygdala Enhances Incentive Salience of a Preferred Cue

Introduction

Reward cues (Pavlovian CSs) can carry incentive salience: eliciting craving for the reward, and making the cues themselves 'wanted', approached and even the target of consummatory acts such as ingestive licks, nibbles, and bites that normally belong to an associated food reward (UCS). Thus food cues can tempt a binge-eater to overindulge or drug cues can trigger relapse in a drug addict (Akil, Watson et al. 1984; Robinson and Berridge 1993; Berthoud and Morrison 2008; Kessler 2009), and such cues can attract appetitive-consummatory behaviors acting as 'motivational magnets'(Jenkins and Moore 1973; Rosse, Fay-McCarthy et al. 1993; Cetinkaya and Domjan 2006; Flagel, Watson et al. 2008).

However, reward cues are not always attractive, but rather vary across time in motivation potency. A cue's power to trigger temptation fluctuates especially when encountered in different physiological-brain states (e.g., drug intoxication, stress, hunger,

satiety) (Mansour, Fox et al. 1994; Zhang, Berridge et al. 2009; Smith, Berridge et al. 2011). Particular activations in mesocorticolimbic brain states, we will suggest, are why particular cue encounters may make addicts relapse into excessive consumption even after the same cue has been successfully resisted many times before(Wyvell and Berridge 2000; Tindell, Smith et al. 2009; Zhang, Berridge et al. 2009; Smith, Berridge et al. 2011). Some brain activations may also focus "wanting" more narrowly onto a single target, as well as elevating intensity [19].

Useful individual differences in the target of incentive salience have been found in autoshaping or "sign-tracking" experiments in rats (Flagel, Akil et al. 2009; Saunders and Robinson 2010), which model the 'motivational magnet' feature of incentive salience for Pavlovian cues. In one version of autoshaping, phasic presentation of a lever CS (CS+ Lever; sometimes called the sign) always predicts a reward UCS: a sucrose pellet delivered to a dish (CS_{dish}; sometimes called the goal). After learning the Pavlovian CS-UCS association, many individual rodents, fish, pigeons, dogs, and people come to approach and bite the discrete CS+ sign and are known as "sign-trackers"(Breland and Breland 1961; Boakes, Poli et al. 1978; Kessler 2009). By contrast, other individuals come to approach the goal location where reward is delivered (CS_{dish}) during the CS+ sign presentation and are known as "goal-trackers" (Jenkins and Moore 1973; Boakes, Poli et al. 1978). Goal-tracking vs. sign-tracking differences emerge in the first few days of Pavlovian training in rats, and remains stable (Mahler and Berridge 2009; Meyer, Lovic et al. 2012).

This difference in individual phenotype is related to underlying mesolimbic brain traits, but can also be experientially biased by environmental situations such as

encountering uncertainty in CS-UCS contingencies, receiving reward UCS directly without needing to approach a goal, receiving amphetamine or related drugs, or having been previously sensitized by drugs administered weeks earlier (Peterson, Frommer et al. 1972; Boakes, Poli et al. 1978; Timberlake, Wahl et al. 1982; Simon, Mendez et al. 2009; Anselme 2010; Holden and Peoples 2010; Robinson and Berridge 2010; Doremus-Fitzwater and Spear 2011). Some similarity in underlying mechanisms might be recruited in both sign-trackers and goal-trackers to attribute incentive salience to individualized targets, at least when mesocorticolimbic brain systems are in a stimulated state. Stimulation of mu opioid circuits in the central nucleus of the amygdala (CeA) was indicated to achieve that mesocorticolimbic state by an earlier study in our laboratory: producing elevation of incentive salience in both sign trackers and goal trackers, and simultaneously focusing that intense incentive salience onto a single Pavlovian target (Mahler and Berridge 2009).

Here, I explore further the idea that in autoshaping the two Pavlovian CSs (sign and goal) have potentially distinct roles: acting as 1) the *trigger* to elicit a phasic pulse of intense incentive salience, versus as 2) the *target* of focused incentive salience attribution (that becomes the most 'wanted' Pavlovian object of desire). That is, CeA opioid stimulation may make sign-trackers 'want' the CS+ Lever more, and similarly make goal-trackers 'want' the CS_{dish} more, each in a phasic pulse when triggered by CS+ encounter (Mahler and Berridge 2009). I hypothesize that the CS+ acts as the *trigger* in both sign-trackers and goal-trackers to evoke a temporary surge in the intensity of CeA-amplified incentive salience, which lasts seconds. However, the *target* CS that is attributed with focused incentive salience differs between sign-trackers and goal-trackers

during a state of CeA opioid stimulation. For sign-trackers, the target is the same trigger or CS+ Lever that predicts sucrose. By contrast, for goal-trackers the target is the CS_{dish} object/location where the UCS is delivered. Finally, I hypothesize that the breadth of focus for incentive salience attribution on the individualized target is also narrowed by CeA stimulation in a winner-take-all fashion. That is, individualized Pavlovian information-to-motivation links are amplified to make the most 'wanted' target even more intensely attractive after CeA opioid stimulation, while alternative targets may even decline in relative attractiveness.

However, a potential problem for our hypothesis is that goal-trackers may essentially lack incentive salience, as only sign-trackers appear to show high cue-triggered "wanting" (Lundy 2001; Flagel, Watson et al. 2007). Sign-trackers have been suggested to model addiction-like features of incentive salience much more than goal-trackers (Flagel, Watson et al. 2008; Flagel, Akil et al. 2009; Saunders and Robinson 2010, 2011), whereas goal-trackers might approach their dish using non-"wanting" mechanisms, such as cognitive expectancy mechanisms or via simpler S-R habit (Saunders and Robinson 2011). A potential reconciliation between such evidence and our hypothesis might be achieved if it could be shown that specific mesocorticolimbic states (e.g., CeA opioid stimulation) produce the higher intensities and sharper focus of incentive salience in goal-trackers. Specifically, our hypothesis is that CeA stimulated states cause goal-trackers to show pulses of high incentive salience that are equal in intensity to sign-trackers, though focused on a different target: the dish.

To test this hypothesis, it is necessary that goal-trackers in a state of mesocorticolimbic activation show the full cue-triggered sequence of motivated

appetitive-consummatory behaviors that characterizes a 'motivational magnet.' For a sucrose pellet UCS, these are sequences of approach, nibble, sniff, grasps and bite behaviors directed to the metal object (CS_{dish} or CS+ Lever). That sequence was not completely confirmed for goal-trackers in the earlier Mahler and Berridge study because the opaque metal wall of the goal dish precluded a clear camera view of actions inside, so that it was not possible to observe a goal-tracker's mouth performing nibble, sniff and bite behaviors in the dish (Mahler and Berridge 2009).

Here I aimed to more stringently test whether CeA stimulation enhances incentive salience using an additional close-up camera focused on the inside surface of the dish. This measured the full appetitive-consummatory sequences of approach, nibbles, sniffs, grasps and bites of the CS_{dish} in goal-trackers. We also aimed to more closely examine the winner-take-all aspect of narrower focusing on a single target induced by CeA DAMGO, in individuals that show nearly balanced mixtures of goal-tracking vs. sign-tracking, as well as in the more extreme phenotypes. Our results here confirm that CeA stimulation does make goal-trackers approach and 'consume' their metal CS_{dish} more, and in more focused fashion. The intensity and focus of the enhancement in goal-trackers' behavior is comparable to sign-trackers' enhanced behavior toward CS+ lever, consistent with the trigger vs. target hypothesis for Pavlovian incentive salience.

Materials and Methods

Subjects

Sprague Dawley rats (n=19; female) weighing 280-340 grams at the start of the experiment were pair housed on a reverse light/dark cycle. Water was provided *ad*

libitum; food was provided *ad libitum* except during weeks containing autoshaping training or test sessions, when rats were restricted to 90% free feeding weight and fed about 12gs of standard laboratory chow daily after each training session. All experiments were conducted in accordance with protocols approved by the University of Michigan Committee on Use and Care of Animals (UCUCA).

Surgery

Rats were anesthetized with ketamine (80mg/kg), xylazine (7mg/kg), and atropine (0.04mg/kg). The central nucleus of the amygdala was targeted by placing bilateral cannulae aimed at (CeA) AP ≈ -2, ML≈ 4, DV ≈ -5.8. Placement coordinates for cranial cannulae were calculated based on Paxinos and Watson (Paxinos and Watson 2007), and lowered into place with a stereotaxic apparatus (Kopf Instruments). Each rat was surgically implanted with chronic, bilateral, 14mm microinjection guide cannulae (23ga) positioned 2mm above the target CeA sites. Cannulae were anchored to the skull with bone screws and acrylic cement, and steel stylets were inserted to prevent their occlusion. All rats were given chloramphenicol sodium succinate (60mg/kg) to prevent infection as well as carprofen (5mg/kg) to provide pain relief. Carprofen was administered again 24 hours post-surgery.

Microinjections

Prior to tests, steel stylets were removed and cleaned, and 16mm microinjectors were inserted into the guide cannulae, pre-measured so that microinjector tips extended 2 mm below guides. Microinjections of DAMGO (Sigma-Aldrich) or vehicle were controlled by a syringe pump which delivered 0.2 µL over 90 seconds. DAMGO injections were 0.1µg of DAMGO dissolved in 0.2µL of aCSF vehicle; control vehicle

inject ions were of aCSF alone in the same rats, in counterbalanced order. Tips were left in the cannulae for 1 extra minute to allow for drug diffusion. Before any test rats were given one "sham" injection of 0.2μL vehicle to habituate them to the microinjection process.

Behavioral Autoshaping

Autoshaping training and testing was carried out in one of eight operant chambers (Med Associates) controlled by Med PC software, containing two retractable levers on opposite sides of a food receptacle. Each rat was always assigned to the same chamber for training and testing. Insertion of a lever on one side was designated as the CS+ that predicted sucrose pellet UCS delivery with 100% correlation. This CS+ Lever was a 4.5X2 cm retractable metal lever with a light emitting diode on its ventral surface. As CS+, the lever was inserted into the chamber through the wall for 8 seconds and accompanied by a 2.9 KHz tone. The CS+ was followed immediately by UCS presentation (delivery of sucrose pellet). Another lever was always present and designated as CS- because it bore no relation to UCS. Presses on the CS- lever were taken as measures of generalization or nonspecific motor activity. Sucrose UCS pellets (45mg) were presented in a metal dish $3cm^2$ at the bottom center of the same wall with the levers. The dish in which sucrose was delivered will be referred to from now on as CS_{dish} because the sight of the dish upon head insertion was the stimulus and action most contiguously paired in time and space with oral receipt of the UCS.

Training

Prior to Pavlovian CS-UCS pairings rats received one day of magazine training when 20 sucrose pellets were dropped into the CS_{dish}, approximately one pellet every 90

seconds. Autoshaping (CS+ paired with UCS) training sessions started the next day. Each Pavlovian session began with the illumination of the red house light and insertion of the control CS- lever at the beginning of the trial. Subsequent 8-sec CS+ Lever presentations were always paired associatively with a UCS reward presentation under a Pavlovian contingency. Then the CS+ Lever was retracted and a UCS sucrose pellet was immediately presented in the CS_{dish}. Each autoshaping session lasted about 40 minutes and consisted of 25 CS+ and UCS pairings with a variable intertrial interval of ~90 seconds.

Rats received 5 training days. By the 3rd day, every rat began to respond to the CS+ onset with an approach-consummatory CR predominantly focused toward either the CS+ Lever itself (in which case the rat was classified as a sign-tracker) or toward the CS_{dish} (in which case the rat was classified as a goal-tracker). The criterion for classification as sign-tracker was to approach, nibble, sniff, and bite the CS+ Lever at least three times more frequently than they did the sucrose dish during CS+ presentations on day 5. The criterion for classification as goal-tracker was to approach, nibble, sniff, and bite the dish at least three times more frequently than the lever during CS+ presentations, and additionally to approach the CS_{dish} three times more frequently when the CS+ Lever was present than in intervening baseline periods when the CS+ was absent (to ensure that CS+ Lever presentation was the trigger for a phasic elevation in goal-tracking behavior). All rats were successfully classified as either sign or goal-tracker by day 5.

Test for effects of opioid activation of CeA

Effects of DAMGO stimulation in CeA were tested by a within-subject comparison to the same rat's behavior on vehicle (control) on days 6 and 8 (in counterbalanced order across rats with 48hrs in between). That is, on day 6 a rat received either vehicle or DAMGO (0.1μg/0.2μL) microinjection before the autoshaping session. On day 8 the other microinjection was administered (drug or vehicle) and the test was repeated.

Behavioral Video Scoring

Rats were always videotaped from two angles. One camera was positioned under the transparent floor of the autoshaping chamber to provide a clear view of the rat's entire head and body wherever it was in the chamber. A second camera was directed from the side toward the inner surface of the CS_{dish} to provide a close up view of the rat's face and mouth movements when inside the dish. Both videos were analyzed off line in slow motion ($1/10^{th}$ to ½ actual speeds) by an observer blind to experimental conditions. For each trial, the 8 seconds before and 8 seconds during the 5^{th}, 10^{th}, 15^{th}, 20^{th}, and 25^{th} presentation of CS+ were selected for comparison (Mahler and Berridge 2009). Scored behaviors were *look* at the cue (orienting towards the cue by moving the head or forequarters toward it, without bodily approaching it), *approach* the cue, *nibble and sniff* the cue (contact of the nose or mouth on lever or dish, combined with rapid short (<0.5 sec) rhythmic 1-2 Hz bobbing movements of the head and nose (sniff), and of jaw, tongue, and/or teeth (nibble), similar to movements of normal eating of UCS) , and *bite* the cue (of jaw closing and contact by maxillary and mandibular incisors, often while

grasping the object with one or both paws, similar to movements that bite the actual UCS sucrose pellet).

Statistical Analysis

Within-subject ANOVAS comparing drug and vehicle days were performed for each anatomical area for the cue and pre-cue periods. Significant differences for individual dependent variables in autoshaping were determined by Bonferroni corrected t-tests. To avoid distortions in percentage change calculations arising from any zero baselines, a constant value of 1 was added to each rat's behavioral score for both CS_{dish} and CS+ Lever.

Histology

Rats were sacrificed immediately after the final day of testing by administration of a sodium pentobarbital overdose. Rats were decapitated and the brains were extracted and fixed in 10% paraformaldehyde solution for 1-2 days followed by a 25% sucrose solution in 0.1M NaPB for 2-3 days before slicing. 60 micron slices through the CeA were taken from each rat, mounted, dried, and stained with cresyl violet. Microinjection center was determined for each bilateral injection site and slides were compared with the stereotaxic atlas (Paxinos and Watson 2007) to determine placement in the CeA. Those rats with placements outside the CeA were excluded from analysis

Results

Overview: Mu opioid stimulation of central amygdala potently enhanced approach and appetitive-consummatory actions of goal-trackers toward dish but of sign-trackers toward lever.

In goal-trackers, DAMGO microinjections in CeA potently increased the number of appetitive-consummatory sequences directed toward their $CS_{dish.}$ The increase was selective to moments when the CS+ Lever was physically present. The increased sequences were always initiated by CS+-triggered approaches to the dish, followed by nibbles and sniffs of the dish rim and internal surface. In sign-trackers, these CeA DAMGO enhancements were matched by increased numbers of approaches and appetitive-consummatory sequences directed to the CS+ Lever. That is, CeA DAMGO similarly intensified the motivated cue-triggered behaviors that each phenotype directed at their own prepotent Pavlovian target.

In more detail, for goal-trackers, DAMGO in CeA selectively increased the number of CS+-triggered approaches, nibbles and sniffs to the goal CS_{dish} by 150%, compared to vehicle microinjection effects in the same rats. Simultaneously, CeA DAMGO conversely decreased goal-trackers' already low rate of approach to the CS+ Lever when it was present, indicating that the increase in the number of already-dominant goal tracking responses was at the expense of already-weaker sign-tracking responses ($F_{(4,8)}$=7.3, p<.01; $F_{(1,11)}$=8.639, p<.05). Similarly but conversely, sign-trackers increased their approaches, nibbles and sniffs of the prepotent CS+ Lever by 230% more under DAMGO compared to vehicle conditions, while oppositely decreasing their already low level of approaches to the goal or $CS_{dish.}$ (Overall $F_{(3,4)}$=11.1,p<.05; $F_{(1,6)}$=11.791, p<.05). For both sign-trackers and goal-trackers, CeA-induced effects on these appetitive-consummatory behaviors were manifest only when the CS+ Lever was physically present, and never in intervening baseline intervals when the lever was absent, regardless of target for approach. That is, even for goal-trackers whose dish target was always present, CeA

selectively enhanced phasic elevations of approach and consumption-related behaviors toward the dish only during the CS+ presentations, and did not enhance lower baseline levels of approaches or consummatory actions toward the dish during longer intervals in the absence of CS+ (goal-trackers: $F_{(4,8)}$=1.1, p>.1). Likewise, sign-trackers only enhanced approach/consummatory sequences during CS+ presentation, but that was perhaps less surprising since consummatory actions could not be directed to an absent lever; however, we note that DAMGO also failed to enhance sign-tracker's baseline levels of approach to the location in the chamber where the CS+ appeared, which in principle could still have been enhanced in lever absence ($F_{(3,4)}$= 1.27, p>.1)(Figure 2.1). Finally, a sequence was usually terminated by opening the mouth and dipping the head in the CS_{dish} (for goal trackers) or grasping, biting and depressing the CS+ lever (for sign trackers).

Approach to target

DAMGO enhanced approach to each rat's individualized target CS, as reflected in a) the alacrity or speed with the target CS was reached, and b) the ability of the CS target as a motivational magnet to pull all cue-triggered approaches exclusively to itself. In terms of approach latency, microinjection in CeA made both sign-trackers and goal-trackers approach their prepotent cue more quickly. Overall, rats reached their target within 1 to 3 seconds after trigger onset of CS+ Lever under vehicle conditions. While it is difficult to move much faster than a 2 second latency, it is possible to speed up longer >2 sec latencies toward the <2 sec floor. Here we found that all rats which took over 2 seconds to reach their targets after vehicle microinjection (mean ±SEM = 2.5 ±

0.15 sec latency) speeded up and reached their target in less than 1.5 sec after DAMGO in CeA (mean ± SEM = 1.4 ± 0.33 sec, $t_{(6)}$=3.7, p<.05).

Similarly, the strength of a motivational magnet can be assessed by the ability of a target CS to fully capture all appetitive approach behavior toward itself during whenever the CS+ lever or trigger is presented, and so to eliminate the defection of any approaches toward the alternative CS. Exclusivity of appetitive capture by a target CS can be dichotomized as either 100% complete capture (i.e., the rat exclusively approaches only its prepotent CS, and does not defect at all to the nonprepotent cue while triggers are present), or as incomplete capture (i.e., the rat approaches both CSs at least once during a trial). In principle, prepotent capture could also fail completely (so that the rat approached only the nonprepotent CS during a trigger), but that happened on less than 6% of vehicle cue presentations and was never observed after DAMGO in CeA. Instead the motivational magnet strength of the target CS essentially varied between 100% complete capture versus incomplete, and DAMGO selectively strengthened the target CS's completeness of capture for goal-trackers and sign-trackers. For rats overall, the incidence of 100% complete capture by the prepotent target CS, or sessions in which the rat never defected even once to the non-prepotent CS during any recorded trigger presentation, rose from 9/19 rats (47%) under vehicle to 15/19 rats (79%) under DAMGO (McNemar's test, p<.05). Even for goal-trackers considered separately, completeness of capture rose from 5/12 rats (41%) under vehicle to 11/12 rats (92%) under DAMGO (McNemar's test, p<.05). Accordingly, the probability of any visit by the rat to the nonprepotent cue during a trial fell from 29% under vehicle (this 29% includes approximately 5% contributed by 2 rats that exclusively approached their nonprepotent

CS on few stimulus presentation) to just 6% under DAMGO ($F_{(1,18)}$=6.0, p<.05). Similarly, the probability of visiting both CSs during a trigger fell from 24% under vehicle to 6% under DAMGO for all rats ($F_{(1,18)}$=6.4, p<.05).

This DAMGO pattern of endowing the prepotent CS with ability to completely capture 100% of all cue-triggered appetitive behavior suggests DAMGO made the prepotent target CS into a stronger "motivational magnet" for both goal-trackers and sign trackers. In summary, when a rat's CeA was in an opioid-stimulated state its prepotent target CS attracted approach more quickly to itself as soon as the trigger stimulus appeared, and more exclusively to itself (i.e., away from the alternative nonprepotent CS). These appetitive features applied to goal-trackers as well as sign-trackers.

Focus on target.

In terms of relative focus of incentive salience between the two CSs, for all rats, opioid stimulation by DAMGO in CeA increased the proportion of approaches, nibbles and sniffs directed by each rat to its already prepotent CS, while simultaneously decreasing the already low proportion of nibbles and sniffs toward its nonprepotent CS ($F_{(11,8)}$= 5.8, p=.01). In more detail, 71% of all nibbles and sniffs by goal-trackers were directed at their prepotent CS_{dish} under vehicle conditions, and that proportion rose under DAMGO stimulation to 77% at the CS_{dish} ($t_{(11)}$=-2.52, p<.05). Simultaneously, the proportion of responses directed by goal-trackers toward their non-prepotent CS+ Lever fell from 29% under vehicle to 23% under DAMGO (Figure 2.1). Conversely, for sign-trackers 68% of nibbles and sniffs were directed at their prepotent CS+ Lever under vehicle, and that proportion increased to 78% under DAMGO stimulation ($F_{(11,8)}$= 5.8, p=.01). Simultaneously, the proportion of responses directed by sign-trackers toward

their non-prepotent CS_{dish} fell from 32% under vehicle to 22% under DAMGO in CeA. This narrowing of focus on the individual's own prepotent target, occurring at the detriment of attraction to the alternative nonprepotent cue, is compatible with a "winner take all" property of incentive salience enhancement induced by CeA opioid stimulation. Under DAMGO, all rats more nearly ignored their nonprepotent cue, instead focusing the increase in approaches, nibbles and sniffs only toward their prepotent cue, whichever stimulus that was for an individual rat. In short, CeA DAMGO microinjections made the sign-trackers better sign-trackers and made goal-trackers better goal-trackers. This can be seen most clearly by plotting each rat individually for its number of nibbles and sniffs directed at the $CS_{dish,}$ and simultaneously the number directed at the CS+ Lever on each trial. DAMGO stimulation shifts each animal towards a more extreme preference. This demonstrates an enhancement of focus and intensity for both goal-trackers and sign-trackers (Figure 2.2).

In the middle: Individuals that balance targets still enhance only the prepotent one

The 'winner-take-all' feature applied even to relatively balanced individuals that showed substantial attraction to both CSs. One way to see this is to assess every individual separately, to trace the effect of CeA DAMGO on individualized response patterns. Every individual's signature can be plotted as a point in a space defined by two orthogonal axes of goal-tracking strength versus sign-tracking strength, and the effect of CeA stimulation is visible as moving the individual to a second point location (Figure 2.2). This individual-by-individual analysis revealed that DAMGO virtually always enhanced appetitive-consummatory response sequences only toward each individual's own prepotent target. Even if the initial bias approached balance as closely as 60:40, only

responses toward the prepotent CS (e.g., 60) were enhanced by DAMGO. Thus, the prepotent CS is a stronger 'motivational magnet' for every individual under CeA DAMGO stimulation, while the weaker CS typically gets weaker.

A more collective way to assess that 'winner-take-all' feature is to isolate for statistical analysis the intermediate one-third of the population, which typically shows mixed sign-tracking and goal-tracking responses in more nearly equal proportions (compared to the extreme one-third of goal trackers, or the opposite one-third of extreme sign-trackers) (Flagel, Akil et al. 2009; Saunders and Robinson 2010) (Figure 2.2). Split into three groups of one-third each, the middle group divided their CS+-triggered responses under vehicle in roughly 60:40 proportions between targets (some preferring the CS+ lever and others the CS_{dish}). DAMGO microinjection into CeA selectively enhanced only the prepotent target even for this middle group, which increased its preference ratio to 75:25 ($F_{(1,5)}$=7.8, P<.05). The absolute number of nibbles and sniffs for this middle group directed to the prepotent target also more than doubled from 1.2 under vehicle conditions to 3.1 after DAMGO stimulation ($F_{(1,5)}$=7.7, p<.05). Conversely the number of responses directed at the nonprepotent cue trended downward, if anything for this group (.3 to .2, n.s.). Taken together, these results show that the attribution of "wanting" becomes more narrowly focused on a single Pavlovian target for all individuals, as well as intensified in level, after DAMGO microinjection in CeA.

Temporal pattern phasic enhancements of approach & consummatory behaviors.

In terms of response timing, enhancements of nibbles and sniffs on the prepotent cue were always limited to phasic bouts lasting only 8 sec for both sign-trackers and goal-trackers, each bout triggered by the insertion of CS+ Lever, lasting for its duration,

and terminating almost immediately when the lever was retracted ($F_{(5,7)}$=31.9, p<.001). For goal-trackers, DAMGO microinjection in CeA amplified the number of cue-triggered approaches and consummatory actions toward the CS_{dish} by over 50% during each CS+ presentation, but did not alter the low baseline level in the absence of the CS+ Lever (Drug*Cue interaction $F_{(1,11)}$=5.4, p=.04; Figure 2.3). Likewise DAMGO microinjections in CeA doubled the number of sign-trackers' approach and consummatory CRs to CS+ Lever (5.6 per 8 sec presentation) while not altering the baseline number of approaches to the same location when CS+ was absent ($F_{(3,4)}$=1.27, p>.1). These patterns demonstrate that approaches and consummatory acts were always temporally locked to the insertion of the CS+ Lever, and that CeA stimulation enhancement was similarly time-locked and triggered by presentations of CS+ Lever, even for goal-trackers, for which the prepotent target CS_{dish} was always present.

CeA DAMGO intensifies microstructure of appetitive-consummatory behavior at prepotent target

A more fine-grained behavioral (frame-by-frame to 1/5 speed) video analysis of the detailed microstructure pattern of nibble-and-sniff movements directed toward the prepotent CS suggested that motivated behaviors also became more frenzied after CeA stimulation, and in the same way for goal-trackers and sign-trackers. To show the DAMGO change, the nibble-and-sniff behavior was choreographed in a randomly selected subset of animals under both vehicle and CeA DAMGO trials using a visual notation system (Mahler and Berridge 2009) (Figure 2.4). DAMGO in CeA increased the temporal rate of early-phase nibble and sniff movements to the prepotent CS that normally began an appetitive-consummatory sequence, as well as the number of those

actions. As a consequence, the bout of intensified nibbles and sniffs endured several seconds longer and so postponed the occurrence of slower bite movements that typically ended an 8 sec sequence (Figure 2.4; Duration of nibble-sniff bout before first bite vehicle vs. DAMGO $F_{(1,12)}=5.9$, $p<.05$). Increases in rate, number, and bout duration of these rapid early-phase sniff-nibble movements gave a more frenzied appearance to consummatory CRs under DAMGO. That pattern also suggested that incentive salience enhancement particularly promoted appetitive behavior and consummatory *initiation*, without necessarily potentiating late-phase consummatory termination acts involving the bite and swallow movements of actual UCS ingestion. In other words, the DAMGO stimulation of CeA appeared to make the metal cue take on food-like incentive properties, but did not make the rats mistake the lever or dish object for food.

Anatomical specificity of DAMGO enhancement in CeA

Microinjection cannulae tips that produced enhancements were located bilaterally well within the CeA in 19 rats (out of 25; Figure 2.5). In order to assess whether DAMGO was likely to be contained within the borders of CeA, we applied the earlier observation by Mahler and Berridge that DAMGO microinjections in CeA filled a tissue volume of approximately $0.43mm^3$ surrounding the microinjection tip (based on radius of Fos plumes produced at the same dose used here) (Mahler and Berridge 2009). If a similar radius applied here, we estimated that 90% of the entire DAMGO impact volume would have been contained inside CeA for 15 out of the 19 rats that had tips within CeA. Over 75% of the DAMGO impact volume would have been contained within CeA for another two rats, and over 50% contained within CeA for the final two rats. By contrast, we observed that other rats with placements outside the CeA (e.g., in IPAC) would not

have entered CeA, and did not express a DAMGO enhancement of their prepotent target, by contrast to the 19 placements contained within CeA that did ($F_{(2,4)}$=2.8, p<.1). Thus I am confident that DAMGO enhancement effects observed here were mediated essentially by receptors within CeA.

Further, a more precise localization of function for incentive salience enhancement in a subregion of CeA was potentially indicated by a spatial clustering of the most effective sites in a mid-anterior subregion of CeA, compared to other sites in CeA (Figure 2.5). Sites where DAMGO enhancements exceeded >200% in the number of CS+-triggered approaches, nibbles and sniffs to the individual's pre-potent target (e.g., more than twice the vehicle-control level for the same rat) all fell into this restricted mid-anterior subregion (Figure 2.5). To quantitatively probe this function localization, a hotspot was tentatively defined anatomically by outlining the outer border of the cluster of contiguous placements where DAMGO enhancements exceeded >200% over vehicle, and comparing the magnitude of increase for sites inside the hotspot versus outside the hotspot but still in CeA. Hotspot sites produced a DAMGO enhancement that averaged 236% in behavioral magnitude whereas other CeA sites that fell outside this hotspot averaged only 145% enhancement ($T_{(18)}$=-3.9, p<.05). Although the number of sites here is too small to draw a firm conclusion, we note that a similar anatomical mid-anterior clustering of the most potent sites in CeA was also found by Mahler and Berridge (Mahler and Berridge 2009), suggesting that this anterior CeA hotspot may well be real for CS motivational magnet enhancement.

Sign-tracker vs. goal-tracker differences in absence of CeA DAMGO stimulation

Do goal-trackers ordinarily attribute less incentive salience to their prepotent target than sign-trackers do to theirs, in absence of special mesocorticolimbic stimulation? Under vehicle conditions, our goal-trackers may have shown a slightly lower intensity cue-triggered increase in incentive salience than sign-trackers at least in one sense. That is, goal-trackers had a lower cue-triggered relative increase when incentive salience was calculated as a percentage increase in approaches, nibbles and sniffs to the prepotent target triggered by the CS+ presentation, over immediately prior pre CS+ levels (Goal-trackers = 163% , sign-trackers = 339%; $F_{(1,17)}$=6.873, p<.05). By contrast, administration of DAMGO raised both groups to higher and equal levels of relative CS+-triggered increase (Drug*phenotype $F_{(11,8)}$=2.15, P<.05). However, while the lower relative increase under vehicle may reflect lower CS+-triggered levels of incentive salience in goal-trackers than in sign-trackers under vehicle condition (Flagel, Clark et al. 2011), it also reflects higher pre CS+ baseline levels in goal-trackers (ST=.1 per 8 seconds, GT=.9 per 8 seconds; $F_{(1,17)}$=5.402, P<.05), and the absolute levels of cue-evoked nibbles and sniffs did not statistically differ between sign- and goal- trackers here under vehicle conditions (ST= 2.45 per 8 seconds, GT= 2.18 per 8 seconds; $F_{(1,17)}$=.106, P>.1) . Still, we agree with the proposition of Robinson and colleagues that goal-trackers may ordinarily attribute lower incentive salience than sign-trackers (Flagel, Watson et al. 2007; Flagel, Clark et al. 2011; Saunders and Robinson 2011) (when in an ordinary mesocorticolimbic state involving no physiological stimulation), and we note that even under vehicle condition our goal-trackers had a mild physiological state of hunger that could induce mesocorticolimbic reactivity because all of our rats were maintained at roughly 90% *ad libitum* body weight (fed 12-15 g chow daily to keep them at that

weight). By contrast, studies by Robinson and colleagues typically have tested rats in a completely sated state of permanent *ad libitum* access to chow (Flagel, Clark et al. 2011; Saunders and Robinson 2011). Recent pilot data from our lab suggests that such differences between mildly-hungry and fully-sated testing may matter for incentive salience, and that the phenotype difference between goal-trackers vs. sign-trackers may best be observed when rats are fully sated. Using similar autoshaping procedures, studies in our lab have found that fully sated goal-trackers show significantly slower latencies of CS-triggered approaches to CS_{dish} than fully-sated sign-trackers to CS+ Lever, and that hunger enhances approach for both phenotypes (ST= 1.5 ±.28, GT= 3±.67; $p<.05$; personal observations, Springstead and DiFeliceantonio).

Discussion

Central nucleus of the amygdala focuses incentive salience.

Goal-trackers and sign-trackers ordinarily differ in their targets of incentive salience, so that it has been suggested that sign-trackers may uniquely attribute incentive salience to discrete CSs for reward in ways relevant to addiction (Flagel, Watson et al. 2007; Flagel, Akil et al. 2009; Saunders and Robinson 2010; Flagel, Clark et al. 2011; Saunders and Robinson 2011). Our results add a degree of richness to this picture by confirming that goal-trackers can achieve similar high intensities of incentive salience pulses, though focused on a different type of Pavlovian CS target, especially when their brains are in a state of mesocorticolimbic activation induced by mu opioid stimulation in CeA.

Our results indicate that goal-trackers in a state of mesolimbic activation attribute intense incentive salience to their own prepotent CS_{dish} target. This occurs as a phasic pulse that makes the dish into a 'motivational magnet' as strong as the CS+ lever is to sign-trackers, during moments while the CS+ trigger is present (here about 8 sec each presentation). That is, mu opioid stimulation of CeA enhanced incentive salience levels that each phenotype attributed to its own prepotent Pavlovian target. Our results also suggest that both sign-trackers and goal-trackers share the same trigger stimulus, namely presentation of the CS+ Lever.

After DAMGO microinjection in CeA, goal-trackers were more intensely attracted to the delivery CS_{dish} where sucrose arrives (goal), specifically at moments when the CS+ Lever was present. DAMGO in CeA enhanced the number of goal-trackers' approaches and number of appetitive-consummatory nibble and sniff sequences toward the CS_{dish} target, each time the CS+ Lever appeared, without at all enhancing those motivated behaviors towards the same object during intervening baseline periods when the CS+ was absent. Sign-trackers, after DAMGO in CeA, were similarly attracted at those same CS+ Lever moments to their prepotent sucrose-predicting CS+ cue (sign), so that the CS+ lever acted as both their trigger and target. The enhancement of attraction to the prepotent cue was accompanied by a simultaneous reduction of attractiveness of the alternative cue. In short, this "winner takes all" pattern of incentive salience was always limited to one CS target, corresponding to the individual's own prepotent Pavlovian stimulus, at the expense of the other CS.

Synergy of incentive salience generation.

A synergy between mesocorticolimbic state and trigger presence in generating incentive salience is revealed by the need for two simultaneous conditions: CS+ Lever presence (phasic trigger; present for only 8 sec per occurrence) and CeA opioid stimulation (which presumably was relatively constant during the 40-min test). Simultaneous necessity of CS+ presence and stimulated brain state has been computationally modeled for incentive salience enhancement by Zhang et al. (Zhang, Berridge et al. 2009) as: $\tilde{V}(S_t)=r(r_t,K)+(\gamma V(S_{t+1})$. In that Zhang model $\tilde{V}(S_t)$ is the intensity of incentive salience triggered at the moment (t) when the trigger CS+ (S) appears. Here the target of the pulse of "wanting" was selectively always the individual's own prepotent target CS_{dish} or CS+ Lever, but the trigger was always CS+ Lever, reflecting the r_t carried by its Pavlovian correlation with sucrose UCS in the past. That r_t essentially corresponds to a memory cache formed by previous reward encounters and prediction errors, drawing on a temporal difference model of reward learning (Sutton and Barto 1981). Mesocorticolimbic reactivity, which is influenced by CeA state, is represented by K, a multiplicative gain factor that interacts with r_t at the moment of CS+ Lever encounter. Stimulation of CeA mu opioid receptors by DAMGO here can be understood as having elevated K>>1, thus dynamically elevating the multiplied product of $\tilde{V}(S_t)$ to produce excessive incentive salience at those particular moments. Applying this model to our results, the cached memory value was not changed intrinsically by opioid stimulation of CeA during the test, but the reactivity was heightened of mesocorticolimbic circuits that phasically generate "wanting" to the r_t association, and attributes the incentive salience directionally toward the Pavlovian prepotent target. The

rise in intensity of target "wanting" was triggered each time the CS+ was inserted and was revealed in more frenzied appetitive and consummatory behaviors directed at the prepotent target.

Sign-trackers' and goal-trackers' phenotypes: differences and similarities.

Our finding of similarities for CeA enhancement of incentive salience in sign-trackers and goal-trackers does not deny that sign-trackers ordinarily differ from goal-trackers in many important neurobiological and psychological ways. For example, Flagel, Robinson and colleagues have shown that sign-trackers have higher tonic levels of mRNA for dopamine D1 receptors in the nucleus accumbens, whereas goal-trackers have higher mRNA for D2 receptors, tyrosine hydroxylase, and the dopamine transporter in the ventral tegmental area (VTA) (Flagel, Watson et al. 2007). Psychologically, the same group has reported that only sign-trackers assign incentive salience to the CS+ Lever, and that sign-trackers ordinarily assign high intensities of incentive salience to their target CS but goal-trackers do not (Flagel, Watson et al. 2007; Flagel, Clark et al. 2011). For example, Flagel and colleagues reported that sign-trackers show higher dopamine elevations than goal-trackers in nucleus accumbens to CS+ Lever presentations (Flagel, Clark et al. 2011). Behaviorally, only sign-trackers learn to perform a new instrumental response to obtain CS+ presentation (i.e., instrumental conditioned reinforcement) (Flagel, Akil et al. 2009; Robinson and Flagel 2009; Flagel, Clark et al. 2011). Such observations have led to suggestions that sign-trackers attribute high levels of incentive salience, whereas goal-trackers rely upon non-"wanting" psychological processes of S-R habit or of cognitive expectations (Robinson and Flagel 2009; Flagel, Clark et al. 2011). In

conformance with that situation, I agree that non-hungry and pharmacologically non-stimulated goal-tracking rats may attribute less incentive salience than sign-trackers.

Our findings apply especially to heightened states of mesocorticolimbic reactivity, induced here by CeA opioid stimulation, which generate intense levels of incentive motivation. I conclude that, when in a heightened mesocorticolimbic state, goal-trackers and sign-trackers showed intense and comparably high elevations of incentive salience, narrowly attributed to their own particular target. Those pulses of intense incentive salience attribution to the target phasically came and went with the presence of the shared CS+ trigger, while simultaneously reducing the attractiveness of the competing alternative target in the same moments. This capacity for similarity in intense incentive salience states may also be related to why some psychological, pharmacological, or neurobiological manipulations are able to shift potential goal-trackers to become sign-trackers, or vice versa (Simon, Mendez et al. 2009; Holden and Peoples 2010; Doremus-Fitzwater and Spear 2011).

CeA modulation of corticolimbic circuitry with DLS.

What features of CeA allow its opioid stimulation to both magnify incentive salience intensity and narrow the target focus of attribution even more than usual to a single Pavlovian CS? Opioid circuits in central nucleus of amygdala may particularly aid the translation of previously learned information, in the form of a static Pavlovian CS-UCS reward association, into dynamic incentive salience that motivates behavior at the moment when CS is subsequently re-encountered (Dores, Akil et al. 1984; Hall, Parkinson et al. 2001; Phelps and LeDoux 2005; Swanson 2005; Mahler and Berridge 2009). Thus CeA is in an excellent position to modulate "wanting" of Pavlovian CSs.

The central nucleus of amygdala receives distinct inputs that might be important to reward processing, including gustatory inputs from the parabrachial nucleus in pons, and has important outputs, including indirect modulation of mesolimbic dopamine neurons in the ventral tegmentum (Gauthier, Parent et al. 1999; Lundy 2001). CeA also has been suggested to be embedded within the larger extended amygdala macrosystem (Swanson and Petrovich 1998; de Olmos and Heimer 1999; Alheid 2003; Heimer, Van Hosen et al. 2008), the lateral (or central) division of which begins in CeA and connects to the bed nucleus of stria terminalis (BNST), sublenticular extended amygdala (SLEA) and interstitial posterior limb of the anterior commissure (IPAC) (Zahm 2006). The extended amygdala system shares special features with caudal portions of the medial shell of the nucleus accumbens (Reynolds and Zahm 2005; Heimer, Van Hosen et al. 2008). The CeA also can be viewed in light of macrocircuit concepts described by Swanson (Swanson 2005; Heimer, Van Hosen et al. 2008), in which CeA is a striatal-level component (GABAergic), receiving inputs from the basolateral nucleus of amygdala (BLA) as a cortical-level component (glutamatergic), and sending outputs to BNST, SLEA and IPAC as pallidal-level components (GABAergic). A striatal-level status may be especially noteworthy for CeA's status as an incentive salience generator, in that other several other striatal-level structures also can generate intense enhancements of incentive salience when neurochemically stimulated (Wyvell and Berridge 2000; Pecina and Berridge 2008; Smith, Berridge et al. 2011)These include nucleus accumbens (ventral striatum) and even regions of neostriatum (dorsal striatum). Thus, CeA having striatal-level features may be important to its capacity for opioid stimulation to intensify CS "wanting".

In analyses of emotional learning, CeA has often been considered to be an output relay for BLA (Pare, Quirk et al. 2004). Comparing BLA to CeA, BLA inputs have been indicated to be especially important for pure Pavlovian learning functions such as formation of specific cue-reward associations or learning of new positive cognitive incentive values, whereas the CeA may be more involved in the active translation of learned information into motivation and generating incentive salience at moments of CS re-encounter (Gallagher, Graham et al. 1990; Corbit and Balleine 2005; Lee, Gallagher et al. 2010; Wassum, Cely et al. 2011).

Opioid neurotransmission in CeA appears to be especially important to dynamic amplification and focusing of incentive salience that makes a Pavlovian cue into a motivational magnet. Endogenously, CeA neurons receive mu opioid stimulation from local enkephalin neurons of amygdala and from B-endorphin axons projecting from the hypothalamic arcuate nucleus (Jackson and Berridge 2008; Poulin, Castonguay-Lebel et al. 2008; Le Merrer, Becker et al. 2009). DAMGO microinjection in CeA may mimic such endogenous opioid sources, increasing FOS gene transcription in CeA neurons (Mahler and Berridge 2009). Opioid stimulation may promote GABAergic disinhibition of output structures (Morris and Dolan 2001; Zhu and Pan 2004), to modulate and stimulate mesocorticolimbic dopamine circuits, via indirect projections such as to the lateral hypothalamus and peduncular pontine nucleus which in turn project to VTA (Gonzales and Chesselet 1990; Gauthier, Parent et al. 1999; Zahm 1999; Heimer, Van Hosen et al. 2008; Day, Jones et al.). Here, DAMGO microinjections into CeA may well have potentiated mesolimbic dopamine circuits to nucleus accumbens as a step in

amplifying the intense bouts of incentive salience observed in appetitive-consummatory behavior (Wyvell and Berridge 2000; Jackson 2009; Smith, Berridge et al. 2011).

Clinical implications.

Brain mechanisms that generate intense levels of incentive motivation may be especially relevant to addiction. Addiction and related compulsive pursuit disorders involve intense motivations that often have two important features: incentive specificity and temptation fluctuation. The first feature is that the 'wanted' target is usually specific. At moments of peak urge, a particular incentive may be 'wanted' much more than anything else. Drug addicts mostly 'want' drug rewards, and some addicts may even 'want' a particular drug, whereas binge eaters 'want' food, and perhaps a particular food. Other compulsive motivations have their own specific targets and triggers (sex, gambling, shopping, etc.). The focusing of incentive salience attributed to a prepotent target, at the expense of other competing targets, here made the one stimulus more 'wanted' above all else. Conceivably, related CeA circuits might similarly be involved in sharpening the focus of "wanting" on a single incentive target in intense compulsive disorders like drug addiction and binge eating.

The second feature in drug addiction and other compulsive motivations is temporal fluctuation in the cue's temptation power: a reward cue may be resisted many times successfully, only to elicit overpowering attraction on a subsequent encounter that triggers relapse. Why does the same Pavlovian CS+ trigger greater temptation on some occasions than on others? Our data suggest that one factor is the mesocorticolimbic reactivity state at the moment of cue re-encounter, which modulates the intensity of incentive salience that is triggered. Mesocorticolimbic reactivity can be enhanced by mu

opioid activation of CeA related circuitry, by mesolimbic dopamine or opioid stimulation of nucleus accumbens, and by drug-induced sensitization of those mesocorticolimbic circuits, all of which exploit the motivational plasticity of mesocorticolimbic circuits that evolved for natural appetite states (Wyvell and Berridge 2000, 2001; Tindell, Smith et al. 2006; Jackson 2009; Mahler and Berridge 2009; Smith, Berridge et al. 2011). All may similarly amplify the intensity of phasic pulses of incentive salience triggered by a predictive CS+. I suggest that temporal fluctuation of mesocorticolimbic circuit states involving CeA opioid activation could dynamically amplify incentive salience attributed to a previously-resisted CS at a particularly intense moment of temptation, creating a more powerful "wanting" for its reward that could drive relapse in maladaptive drug addiction, binge eating and related addiction-like disorders.

Figures

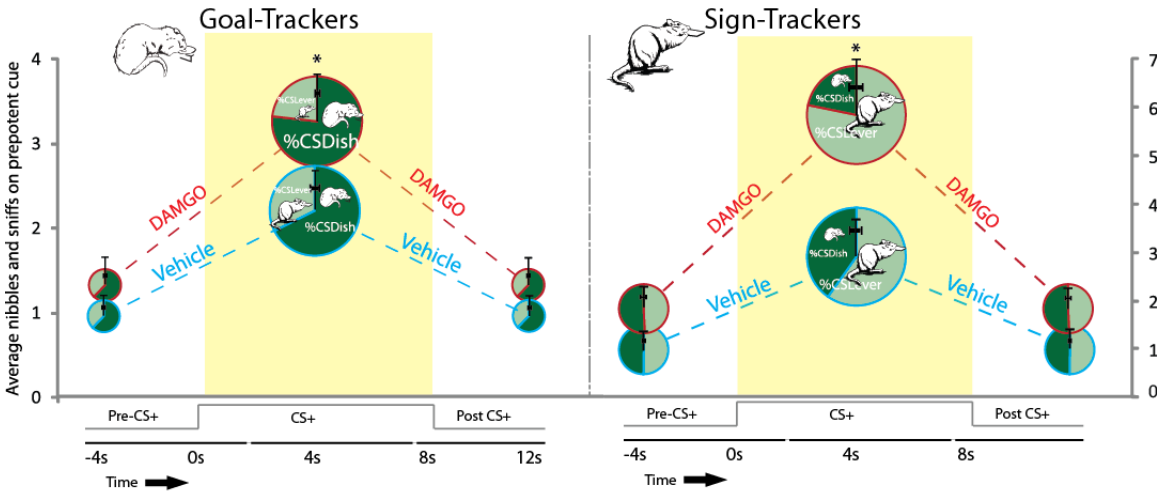


Figure 2.1. CeA DAMGO enhances focus. CeA DAMGO microinjection amplifies and focuses appetitive-consummatory behaviors directed toward the prepotent cue for both sign-trackers and goal-trackers. Both the amount of approaches, nibbles and sniffs directed at the prepotent cue and proportion of all approaches, nibbles and sniffs directed at the prepotent cue is increased, while the proportion directed toward the nonprepotent CS is decreased. Yellow background indicates periods when the CS+ is physically present; white backgrounds indicate before and after CS+ presentations * indicates $p<0.05$. Pie-graph circles show the proportion of appetitive-consummatory behaviors directed by that phenotype to CS+ Lever (sign) vs. CS_{dish} (goal).

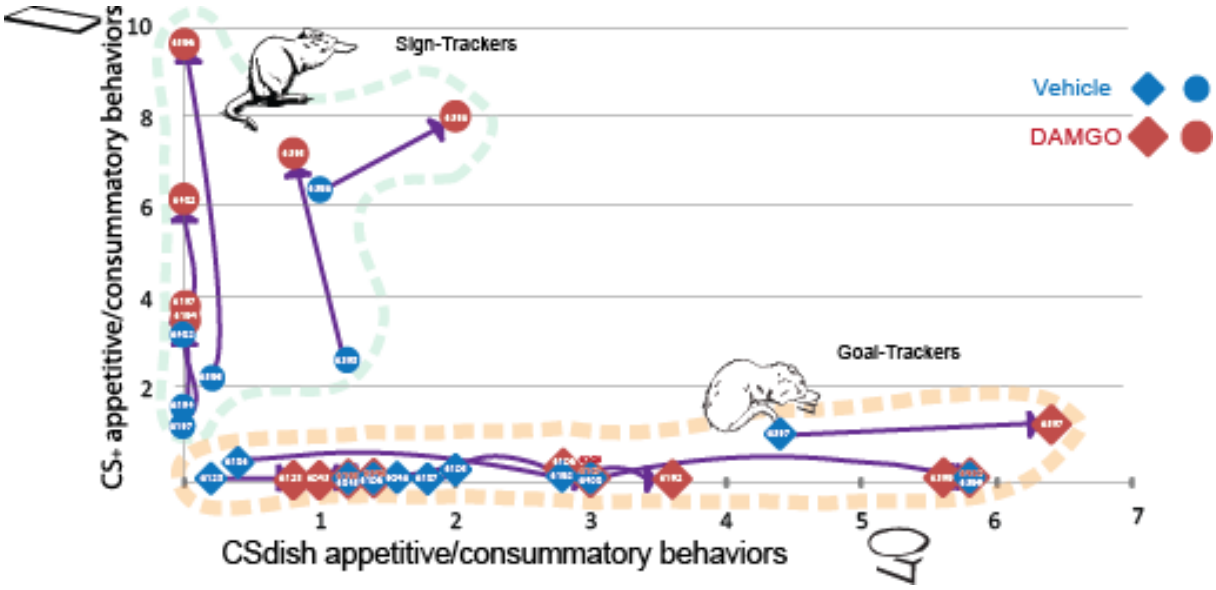


Figure 2.2 Individual Variation. DAMGO microinjection into the central nucleus of the amygdala makes sign-trackers into more intense sign-trackers and goal-trackers into more intense goal-trackers. In this scatter plot, each individual rat is represented by two dots: a blue dot in vehicle condition and a connected red dot in DAMGO condition. Sign-trackers are circles and goal-trackers are diamonds. Vertical axis plots the number of sign-tracking behaviors toward CS+ Lever (sign). Horizontal axis plots the intensity of goal-tracking behaviors toward CS_{dish} (goal). DAMGO always intensifies the pre-existing preference of an individual that was already prepotent.

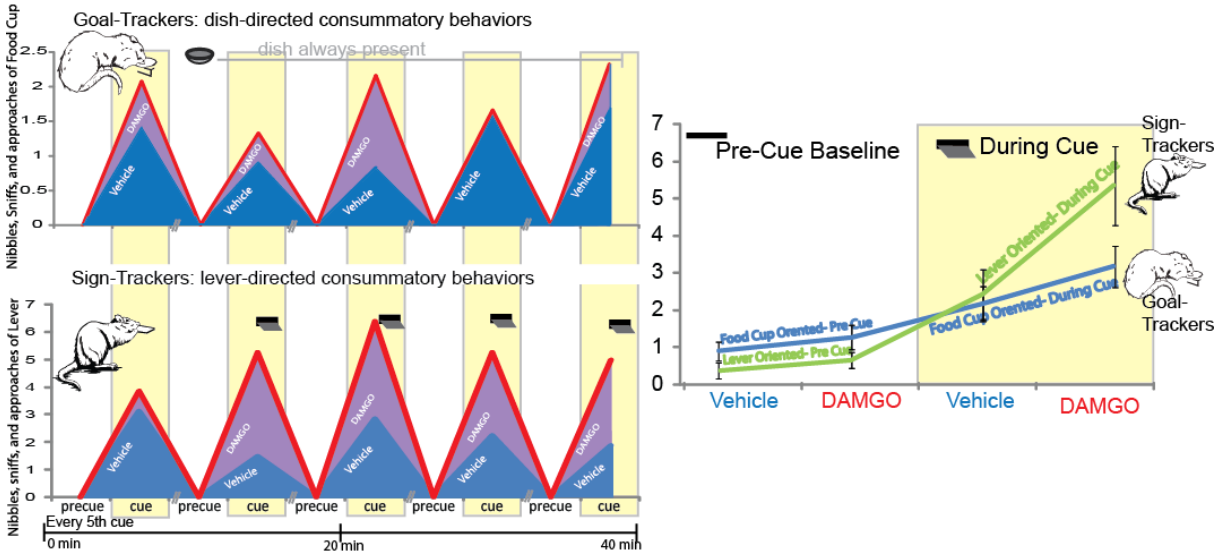


Figure 2.3. Cue Locked Enhancement. DAMGO microinjection increases nibbles and sniffs to the prepotent cue during cue presentations (CS+ Lever insertions into chamber; yellow backgrounds) only, but not during inter-cue intervals. This cue-locked increase is similar for both sign-trackers and goal-trackers, even though the CS_{dish} is always present for goal-trackers. On vehicle nibbles and sniffs increase during the cue periods ($p<.05$) and on DAMGO this cue-locked increase is greatly enhanced (DrugXCue $p<.05$). Left: temporal pattern of behaviors over successive CS+ Lever presentations and baseline intervals during the 40 min test session for goal-trackers (top) and sign-trackers (bottom). Pre-cue nibbles and sniffs were subtracted from all values depicted to normalize baseline levels. Right: total approaches, nibbles and sniffs to each CS during baseline intervals versus during CS+ presentations.

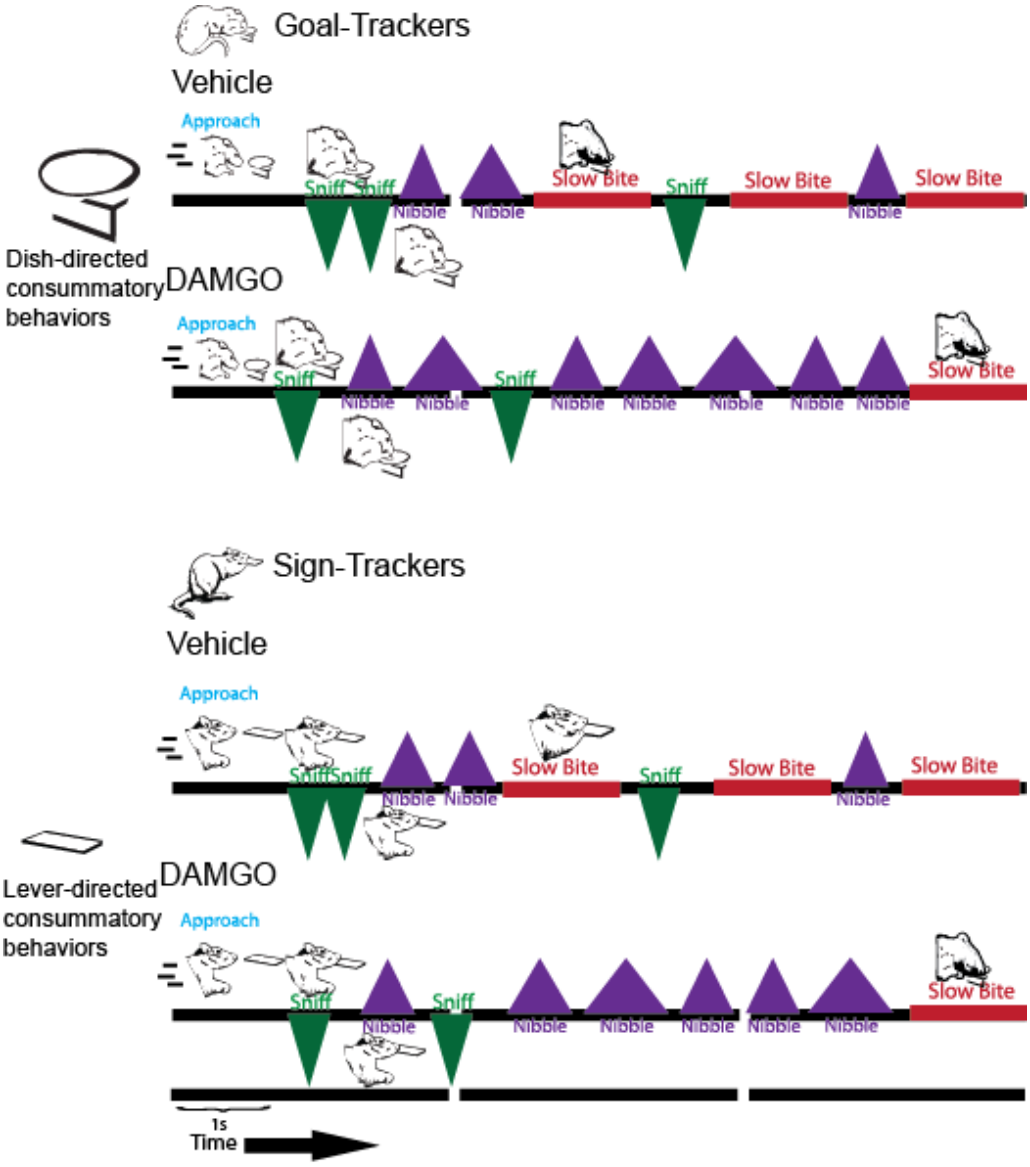


Figure 2.4. Topography of Behavior. DAMGO shifts the individual choreography of each rat's response to more anticipatory nibbles and sniffs and less terminal slow bites. Consequently, latency to the first slow bite is increased after DAMGO microinjection. Each choreograph shows a 'typical' instance compiled from several actual rats. Time proceeds from left to right during 7 second presentation of CS+. Green downward triangles denote individual CS sniff actions; purple upwards triangles denote nibble actions; red bars denote slower consummatory bites typically seen in later phases of actual ingestion.

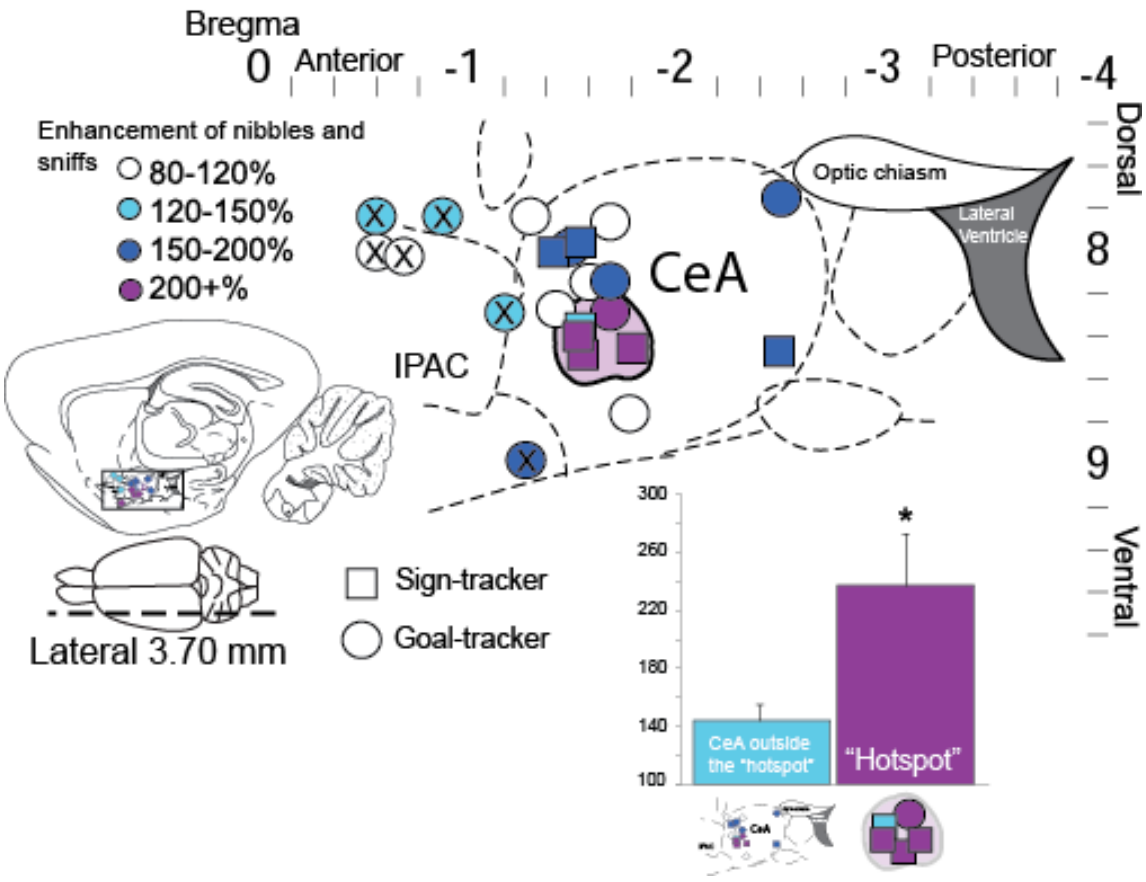


Figure 2.5. Localization of DAMGO effects. The center of each microinjection cannulae placement is represented as a circular point . The enhancement effect of DAMGO on that rat's CS+ lever-triggered 'motivational magnet' attraction toward its individualized target CS (lever or dish) is color coded and represented as % change from vehicle control level in the same rat. A DAMGO "hotspot" of maximal effect is highlighted in anterior CeA, defined as a contiguous cluster of anatomical placements that produced enhancements >200%. DAMGO placements outside of CeA did never increased nibbles and sniffs on the prepotent cue.

Chapter 3

Effects of Mu Opioid and Dopamine Receptor Stimulation in Dorsolateral Neostriatum on Incentive Salience of a Preferred Cue

Introduction

The dorsolateral subregion of neostriatum (i.e., the lateral 50% of the most dorsal 25% of neostriatum), and its dopamine and corticostriatal connections, has been viewed traditionally to mediate movement, action sequencing, stimulus-response (S-R) habits, simple learning processes (S-R), and the actor in actor-critic models of learning (Schultz and Dickinson 2000b; Packard and Knowlton 2002a; Balleine and Ostlund 2007; Wise 2009; Bornstein and Daw 2011). The dorsolateral neostriatum's involvement in addiction has been suggested to produce overly-strong S-R habit rituals of reward-seeking. On the other hand, reward motivation for learned rewards is viewed to be a function provided by more ventral or medial levels of striatum, especially the nucleus accumbens (Wise, Fotuhi et al. 1989; Robinson and Berridge 1993; Wyvell and Berridge 2000). Recently, however, dorsal levels of neostriatum have begun to be increasingly recognized to participate in reward and motivation functions too (Volkow, Wang, Fowler, Logan, Jayne, Franceschi et al. 2002; Volkow, Wang et al. 2006; Palmiter 2008a; Stice, Spoor et al.

2008; Wise 2009; DiFeliceantonio, Mabrouk et al. 2012; Nummenmaa, Hirvonen et al. 2012; Schneck and Vezina 2012) . For example, dorsal neostriatum activates to food or drug rewards and cues in neuroimaging and electrophysiological studies (Schultz and Dickinson 2000b; Volkow, Wang, Fowler, Logan, Jayne, Franceschi et al. 2002; Volkow, Wang et al. 2006; Stice, Spoor et al. 2008; Nummenmaa, Hirvonen et al. 2012). Opioid-stimulating microinjections in the anteromedial region of dorsal neostriatum generate intense motivation to eat (DiFeliceantonio, Mabrouk et al. 2012), and damage to dorsolateral neostriatum reduces cue-triggered "wanting" for reward (Corbit and Janak 2007).

Incentive salience is a motivation function that interacts specifically with Pavlovian learning to make reward-associated cues attractive and 'wanted' and potently trigger pulses of motivation to obtain and consume reward (Robinson and Berridge 1993; Mahler and Berridge 2009; DiFeliceantonio and Berridge 2012; Robinson and Berridge 2013). The intensity of a cue-triggered pulse of motivation is actively generated at the moment of cue re-encounter based on reactivity states of mesocorticolimbic circuits, not fixed by previous learning alone, and so can vary across encounters (Wyvell and Berridge 2000; Pecina, Schulkin et al. 2006; Mahler and Berridge 2009; Zhang, Berridge et al. 2009; DiFeliceantonio and Berridge 2012; Saunders and Robinson 2012; Robinson and Berridge 2013). Sudden increases in the attractiveness of Pavlovian cues can be detected using autoshaping or sign-tracking measures in animal neuroscience studies (Robbins and Everitt 2002; Robinson and Flagel 2009; Doremus-Fitzwater and Spear 2011; DiFeliceantonio and Berridge 2012). For example, neurochemical stimulation of amygdala circuitry that interfaces learning with motivation cam make a Pavlovian

conditioned stimulus (CS), such as a metal lever object that predicts sucrose reward unconditioned stimulus (UCS), to be attributed with more intense incentive salience, so that the Pavlovian metal CS is more 'wanted', approached and targeted with consummatory grasps, sniffs and ingestive licks, nibbles, and bites that normally belong to sucrose (Zener 1937 ; Jenkins and Moore 1973; Boakes, Poli et al. 1978; Meyer, Lovic et al. 2012).

Here we examined the role of the dorsolateral region of neostriatum (DLS) specifically in amplifying and directing the incentive salience of learned reward-cues upon re-encounters, to direct motivated behavior to specific cues as intensified 'motivational magnets.' Effects of mu opioid stimulation and dopamine stimulation in DLS were compared after DAMGO versus amphetamine microinjections.

Our results indicate that mu opioid stimulation of dorsolateral neostriatum focuses enhanced "wanting" on one single CS that is individually tailored or prepotent, at the motivational expense of an alternative CS, in a 'winner take all' style reminiscent of addiction (CS sign lever that predicts reward for individual sign-trackers; CS_{dish} goal that delivers reward for individual goal trackers). By contrast, dopamine stimulation only enhanced "wanting" for the most goal-proximal CS (CSdish), suggesting heightened attractiveness of cues that occur closest in time or space to a reward experience.

Materials and Methods

Subjects.

Sprague Dawley rats (n=26 for sign-tracking/goal-tracking; n=14 for moved cue sign-tracking; n=10 for satiety devaluation of moved sign tracking; n=29 for instrumental

conditioned reinforcement) weighing 280-350 grams at the start of the experiment were pair housed on a reverse light/dark cycle. Water was provided *ad libitum*; food was provided *ad libitum* except during weeks containing autoshaping training or test sessions, when rats were restricted to 90% free feeding weight and fed about 14gs of standard laboratory chow daily after each training session. Before surgery, all rats received 2-4 10 minute sessions of experimenter handling to acclimate them to being held. All experiments were conducted in accordance with protocols approved by the University of Michigan Committee on Use and Care of Animals (UCUCA).

Surgery.

All rats were anesthetized with ketamine (80 mg/kg), xylazine (7 mg/kg), and atropine (0.04 mg/kg). To prevent infection, chloramphenicol sodium succinate (60 mg/kg) was administered as well as carprofen (5 mg/kg) to provide pain relief. Carprofen and chloramphenicol were administered again 24 h post-surgery. All rats were allowed 5-7 days to recover from surgery before testing.

Chronic bilateral 14 mm (23 ga) guide cannulae aimed at dorsolateral neostriatum (AP 0-2.5, ML ±3-4, DV -3.5-4.5; coordinates marked from Bergman at flat skull; n=13) or medial control placements (AP 0-2.5, ML ±1.8, and DV -3.5-4.5; n= 13) based on Paxinos and Watson (Paxinos and Watson 2007). All guide cannulae tips were implanted 2 mm above intended target injection site. Cannulae were anchored to the skull with bone screws and acrylic cement. Steel stylets were inserted into guide cannulae to prevent occlusion. Localization of function was further determined by mapping the causal efficacy of neostriatal microinjection sites to enhance behaviors. Symbols in maps were sized to the maximum radius of "Fos plumes" surrounding DAMGO microinjections. Fos

plumes were measured in separate rats after a single microinjection in order to capture maximal spread and avoid serial plume shrinkage (Fos radius reflects the anatomical spread of drug impact, Fig 3).

Microinjections and drugs

Prior to all tests, steel stylets were removed and cleaned, and 16mm microinjectors were inserted into the guide cannulae, pre-measured so that microinjector tips extended 2 mm below guides. Microinjections of [D-Ala^2, N-MePhe^4, Gly-ol]-enkephalin (DAMGO; Sigma), amphetamine, or vehicle (aCSF; Harvard Apparatus) were controlled by a syringe pump which delivered 0.5 µL over 120s. DAMGO was dissolved in aCSF at 973 µM concentration (0.25µg/0.5µL), amphetamine was dissolved at 116.5 mM concentration (10µg/0.5µL). Microinjector tips were left in cannulae for 1 min following the injection to allow diffusion away from microinjector tips. Each rat received a "sham" injection 1 day prior to testing of vehicle to habituate them to the microinjection procedure.

Statistical Analysis.

Within subject repeated measures ANOVAs comparing drug and vehicle days were performed on data from autoshaping testing. Placement within neostriatum, cue period, and prepotent cue (sign- vs. goal-tracker) were between subjects variables. Between subjects multivariate and univariate ANOVAs were used to determine difference between drug and vehicle groups in moved cue and conditioned reinforcement tests. All t-test presented were corrected for multiple comparisons using the Bonferroni correction.

Histology.

Rats were sacrificed immediately after the final day of testing by administration of a sodium pentobarbital overdose. Rats were decapitated and the brains were extracted and fixed in 10% paraformaldehyde solution for 1-2 days followed by a 25% sucrose solution in 0.1M NaPB for 2-3 days before slicing. 60 micron slices through the neostriatum were taken from each rat, mounted, dried, and stained with cresyl violet. Microinjection center was determined for each bilateral injection site and slides were compared with the stereotaxic atlas (Paxinos and Watson 2007) to determine placement within the dorsal neostriatum.

Rats used for Fos analysis were anesthetized and transcardially perfused 75 min after bilateral microinjection of vehicle (dorsomedial n = 8; dorsolateral n = 6), DAMGO (dorsomedial n = 10; dorsolateral n = 9), or normal (dorsomedial n = 2; dorsolateral n = 2). Brains were extracted, frozen, and sliced at 40 μm. Slices were processed for c-Fos-like immunoreactivity using NDS, goat anti-cFos (Santa Cruz Biotechnology, Santa Cruz, CA) and donkey anti-goat Alexa Flour 488 (Invitrogen, Carlsbad, CA) (Pecina and Berridge 2005; Richard and Berridge 2011). Slices were mounted, air dried, and cover slipped with ProLong Gold antifade reagent (Invitrogen). The radius and intensity of plumes of c-Fos positive cells surrounding the microinjection site were mapped as described previously (Pecina and Berridge 2005; Richard and Berridge 2011).

Behavioral Autoshaping Training

All rats received the same autoshaping training procedures as previously described (Mahler and Berridge 2009; DiFeliceantonio and Berridge 2012). In brief, autoshaping training and testing for a particular rat was always carried out in one of eight

operant chambers (Med Associates) controlled by Med PC software, containing two retractable levers on opposite sides of a food receptacle. Rats first received one session of magazine training consisting of 20 sucrose pellets being delivered into the food dish. Pavlovian autoshaping training (CS+ paired with UCS) began started the second day. Training sessions began with illumination of the house lights, followed by insertion presentations of the CS+lever with a light emitting diode on its ventral surface and accompanied by an auditory 2.9 KHz tone. Each CS+lever/tone presentation lasted 8s before the lever was retracted back through the wall, which was followed immediately by delivery of one sucrose pellet into the food dish (UCS; Test Diet). Twenty-five CS+ UCS pairs were presented on a 90s variable inter-trial interval schedule during the 40 minute session. A control lever was always present in the chamber.

Training sessions were repeated over 5 consecutive days. By the 3rd training day, every rat began to respond to the CS+ onset with an approach-consummatory CR predominantly focused toward either the CS+lever itself (in which case the rat was classified as a sign-tracker) or toward the CS_{dish} (in which case the rat was classified as a goal-tracker). All rats' prepotent and non-prepotent cues were discernible by day 3.

Autoshaping Testing

Testing began on day 7 and continued day 9, when either DAMGO or vehicle was microinjected immediately prior to the autoshaping session (order counter-balanced across rats; one microinjection per day). One camera was positioned under the transparent floor of the autoshaping chamber to provide a clear view of the rat's entire head and body wherever it was in the chamber. This allowed scoring of both sign-tracking approaches and goal-tracking approaches, as well as scoring of consummatory

behaviors in sign-trackers. A second camera was directed from the side toward the inner surface of the CS_{dish} to provide a close up view of the rat's face and mouth movements when inside the dish. A test trial consisted of 25 CS-UCS autoshaping trials identical to trainings. Recordings were scored later offline by the experimenter blind to drug condition.

Behavioral Video Scoring: Autoshaping

Behavior of rats toward CS+ lever and dish were always video recorded from two angles simultaneously through two strategically positioned cameras. One camera was positioned under the transparent floor of the autoshaping chamber to provide a clear view of the rat's entire head and body movements wherever it was in the chamber. A second close-up camera was directed from the side toward the inner surface of the CS_{dish} to provide a detailed view of the rat's face and mouth movements when inside the metal dish. Both videos were analyzed off line in slow motion ($1/10^{th}$ to ½ actual speeds) by an observer blind to experimental conditions. For each trial, the 8 seconds before and 8 seconds during the 5^{th}, 10^{th}, 15^{th}, 20^{th}, and 25^{th} presentation of CS+ were selected for comparison (Mahler and Berridge 2009). Scored behaviors were *look* at the cue (orienting towards the cue by moving the head or forequarters toward it, without bodily approaching it), *approach* the cue, *sniff* the cue (contact of the nose and rhythmic nose flaring movements), and *nibble (contact of mouth or teeth* on lever or dish, combined with rapid short (<0.5 sec) rhythmic 1-2 Hz bobbing movements of the head), and rhythmic opening and closing movements of jaw, tongue, and/or teeth similar to movements of normal eating of UCS) , and *bite* the cue (of jaw closing and contact by

maxillary and mandibular incisors, often while grasping the object with one or both paws, similar to movements that bite the actual UCS sucrose pellet).

Moved cue training

Rats were trained in the autoshaping procedures described above. They received 5 sessions of 25 CS+ UCS pairings. For this experiment only identified sign-trackers were tested in the moved cue paradigm.

Moved cue testing

One classical feature of S-R habits is that they become stereotyped rituals of centrally-programmed movements (Carr and Watson 1908). Ritualized habits persist unchanged in the same movement sequence initially when objects are spatially rearranged, sometimes even resulting in collision with objects in new locations (Carr and Watson 1908). Therefore we pitted habit against motivated "wanting" of the CS+lever expressed by flexible pursuit.

After autoshaping training, rats were given microinjections of DAMGO or vehicle and placed in the operant chamber. For the first time, the CS+lever was inserted into the box from a new location, the opposite wall and opposite side from its previous location (Fig. 4b). Because each rat had to experience the sudden cue shift under either DAMGO or vehicle for the first time, a between subjects design was used.

Moved cue behavioral video scoring

In addition to all behaviors described above, approaches, looks, and nibbles and sniffs directed at the CS+lever entry slot were also scored. To determine typical and atypical responses to CS+lever onset, a choreograph of each response was created. When

a pattern of response emerged for each rat, this was designated as "typical," patterns that differed were "atypical." During moved cues testing, the first 3 cues were used for analysis, as these first cues best capture the sudden shift in the environment.

Free intake paradigm testing

On test days, rats received free access to palatable milk chocolate candies (M&Ms) in a 1-hr intake test (DiFeliceantonio and Berridge 2012). DAMGO or vehicle counterbalanced across days with 48 h between each testing session Rats were habituated for 4 days to clear plastic tub cages with ~3 cm of corn cob bedding, 20 g of pre-weighed M&Ms, 20 g of pre-weighed chow. Water was available through a drinking spout. M&M's remaining were counted and re-weighed, and videoed eating behavior was scored at a later date offline.

Free intake paradigm video scoring

Videos were scored by experimenters blind to the experimental condition of each rat. Seconds spent engaging in the following behaviors were recorded: eating M&Ms (actual chewing and consumption), eating chow, drinking, and chewing on non-food items. The following behaviors were recorded as a single event: sniffing M&Ms (anticipatory sniffs and approaches), sniffing chow, grooming, cage crossing, and rearing (Richard and Berridge 2011).

Devaluation testing

Rats received 3 days of autoshaping training. On the fourth day rats were exposed to one of two conditions: 1) exposure to sucrose pellets used in training, 2) no exposure. For the exposure group pellets were delivered into the magazine at a VI 30s interval and

were continuously delivered until rats refused to consume more. Rats typically ate about 60 pellets before refusing to eat more. After this point rats were removed from the operant chambers and placed briefly in their home cages. Rats in the no exposure group remained in the operant chambers for the average amount of time spent in the chamber by the exposed groups (30min) and then returned briefly to their home cages. Rats were then removed from their home cages and tested using the moved cue procedure described above.

Conditioned reinforcement testing

After 5 days of autoshaping training, rats were microinjected with either DAMGO or vehicle and immediately placed in the operant chamber. In this test the operant chamber had been altered. The food magazine was removed and the front panel now contained two nose ports on either side of a retractable lever (Fig. 5). Each nose port was randomly assigned as "active" (produced the CS+lever) or "inactive" (no consequence). Pokes into the active nose port resulted in a 2s presentation of the CS+lever and tone. The session lasted 30 minutes. Number of active and inactive nose pokes was recorded at the end of the session.

Results

Overview

In sign-trackers, DAMGO microinjection in the dorsolateral neostriatum (DLS) made rats approach their CS+lever more quickly, and upon reaching it more avidly sniff, grasp and nibble their prepotent metal lever cue. In goal-trackers, DAMGO microinjection in DLS instead made the rats more rapidly and intensely approach, sniff,

grasp and nibble their prepotent CS+ metal dish. Both enhancements occurred only at moments when the CS+lever trigger was physically present, and both occurred at the expense of approaches and nibbles of the alternative CS. By contrast, dopamine stimulation of DLS by amphetamine microinjections enhanced only goal-tracking responses in any rats.

Further, DAMGO in DLS did not simply activate an S-R habit or motor ritual, but instead enhanced several motivated features of behavior: 1) rats more flexibly followed their CS to a new location with greater alacrity after DLS opioid stimulation, abandoning their previously habitual ritual of approach to old location; 2) rats were more willing to learn a new response and work to earn their CS+lever after DAMGO in DLS, in an instrumental conditioned reinforcement test, and 3) the original learned conditioned response sequence of approach-sniff-grasp-nibble appeared never to have been a habit in the sense of persisting independent of outcome, because we found that satiety devaluation of sucrose UCS produced an immediate transfer of reduced approach to the CS+lever in a subsequent extinction test (no UCS).

Mu opioid receptor activation in dorsolateral neostriatum enhanced cue-locked "motivational magnet" properties of an incentive stimulus.

Classification of sign- and goal- trackers. Robinson and colleagues have created an index to classify animals into sign- and goal- trackers. This Pavlovian conditioned approach (PCA) index ranges from -1 (goal-tracker) to 1 (sign-tracker). There are three inputs to this score: approach bias which incorporates number of responses made on each cue, probability bias, which is the probability a cue will be contacted, and latency score, which is the difference in speed of approach for each cue. To create this score, either

computer scored data can be entered (as used by Robinson and colleagues) or hand scored video data (used here). Using computer scored data we identified 8 goal-trackers and 5 intermediate animals with no sign-trackers, likely due to the sensitivity of our levers in our operant chambers. Using the exact same equations, but using hand scored data as the input, we identified 8 goal-trackers, 4 sign-trackers, and 1 intermediate rat.

DAMGO microinjections into the dorsal neostriatum selectively enhanced the 'motivational magnet' power of each rat's prepotent target CS to elicit approach and consummatory actions regardless of their classification as sign- or goal- tracker by either method. All animals tested, including the intermediate, were used in analysis. Here sign- and goal-trackers are presented as those calculated using video scored data and video scored data is used unless explicitly noted. Each animal's behavior can be broken down into measures of approach (probability and speed) and consummatory actions, these are discussed separately below.

Approach. Here, the probability to make a single contact with the preferred stimulus for each cue was calculated according to Meyer et al. (2011). Using detailed video scoring data all rats approached their preferred cue with 100% probability and after DAMGO microinjection continued to approach their preferred cue with 100% probability (Figure 3.1). Probability to approach the non-preferred cue was 12% under vehicle conditions (GT=12.9%, ST= 8%). DAMGO microinjection enhanced the selectivity of responding by focusing behavior on the preferred cue by decreasing the probability to approach the nonpreferred cue to 6% (GT=8%, ST= 7.5%). Although an enhancement in probability to approach the preferred cue was not observed due to a ceiling effect (vehicle

= 100%), the decrease in the probability to approach the nonpreferred cue demonstrates an enhancement of focus of behavior after DAMGO microinjection.

Another measure of approach, speed of approach, was also enhanced by DAMGO microinjection. After DAMGO microinjections in dorsolateral neostriatum rats reached their target lever or dish nearly twice as fast upon CS+lever appearance ($t_{(11)}$=5.9, p<.05; Figure 3.1). Under vehicle conditions, rats approach their cue rapidly with a latency of 1.5s (ST=0.9 s, GT=1.7 s). This rapid approach was further increased after DAMGO microinjection, shortening latency to 0.8s (ST=0.6 s, GT=0.9 s).

Consummatory grasps, sniff, and nibbles of metal CS. Once rats reached their prepotent CS, opioid stimulation of DLS additionally made rats emit more frenzied appetitive/consummatory actions of grasping, sniffing, licking, and nibbling the metal lever or dish (DAMGO: $F_{(4,7)}$=7.371, p<.05; CUE: $F_{(4,7)}$= 59.130, p<.001; DAMGO*CUE: $F_{(4,7)}$= 10.520, p<.01, Figure 3.2). Overall, DLS DAMGO microinjection increased the number of consummatory grasps, nibbles and sniffs to over 150% of vehicle levels on the prepotent target CS (ST=168%, GT=142%)($t_{(11)}$=20.09, p<.01; Fig. 3.2), while never enhancing consummatory action on alternative CS (ST vehicle= 1.68, ST DAMGO= 0.69; GT Vehicle=1.4, GT DAMGO=1.3; t=.99, p>.3, Fig.3.2b). For sign-trackers DLS DAMGO microinjections specifically increased consummatory acts toward CS+ Lever, rising over 60% from 5.48 to 9.24 in grasps, nibbles & sniffs directed to metal lever (t(4)=3.595, p=.023). For goal-trackers, DLS DAMGO microinjections specifically directed the intense consummatory acts toward the metal $CS_{dish,}$ or dish associated with sucrose delivery, rising 40% from 4.2 after vehicle to 6.0 after DAMGO per 8-sec presentation of the CS+lever (before the UCS sucrose pellet arrived; t(7)=4.006,

p=.005). By contrast to the individual's prepotent CS, the alternative CS was never enhanced for consummatory actions in either phenotype. (ST vehicle= 1.68, ST DAMGO= 0.69; GT Vehicle=1.4, GT DAMGO=1.3; t=.99, p>.3, Fig.3.2b).Thus the prepotent dish or lever CS became more 'edible' in the sense of being perceived as eligible for ingestive-style consummatory actions after DAMGO microinjection in dorsolateral neostriatum ($F_{(3,48)}$=35.17, p<.001; Cue Type $F_{(1)}$=96.12, p<.001; DAMGO*Cue Type $F_{(1)}$=5.49, p<.05.).

DLS DAMGO enhancements of prepotent dish or lever attraction were always time-linked to triggering appearances of the CS+lever. Even for goal-trackers, the enhancement of CSdish as target of grasps, sniffs and nibbles on was temporally bound to the presence of CS+ lever (8 sec duration), coming as bouts with the insertion of the CS+lever into the cage and fading within seconds after it disappeared. This temporal pulse pattern suggested that the reward-predictive lever was the *trigger* of the incentive salience pulse, whereas the reward-proximal CSdish was the *target* stimulus that became more 'wanted' for goal trackers (DiFeliceantonio and Berridge 2012). At all other moments, when the CS+lever was absent, no enhancement of nibbles and sniffs or of approaches to lever or dish was detectable after DAMGO microinjections in either sign-trackers or goal-trackers ($F_{(4,7)}$= 3.091, p>.05; beam breaks $t_{(11)}$= 0.0, p>.9). This need for simultaneous opioid brain state and CS+lever presence for motivation enhancement reveals a synergy between the two inputs at the moment of cue re-encounter for the generation of incentive salience.

In the home cage, DAMGO in DLS never changed any measure of general locomotion general locomotion (rearing, cage crosses, treading), grooming ($F_{(4,6)}$=1.63, p>.2), eating duration or amount of food eaten ($t_{(9)}$=0.1, p>.9).

Amphetamine in dorsolateral neostriatum shifts 'mixed' individuals toward goal-tracking

Amphetamine microinjections in dorsolateral neostriatum more directionally enhanced the attractiveness of the CS_{dish}, the CS most proximal to UCS sucrose delivery. This effect was most visible for 'mixed' individual rats that tended to equally approach both it and the CS+lever stimulus during lever presentations (i.e., enhancing goal-tracking in mixed CS individuals that approached both CSs on 66% of trials). Amphetamine microinjection in DLS also sped up approach by about 70% to the goal CS, slashing the latency to reach the CSdish from 6.02 s after vehicle to 2.1 s after amphetamine microinjection. Latency to contact the CS+lever increased from .6s to 2s (p>0.05). Rats that were already goal trackers also showed a small (34%) further increment in in the number of nibbles and sniffs directed at the CS_{dish} (Vehicle = 3.65, Amphetamine = 4.9, p>.1). Under both conditions they approach the CS_{dish} on 100% of trials. All goal-tracking rats showed this increase. Similarly, exclusive sign-trackers nibbled and sniffed their CS+lever 32% more (Vehicle= 3.5, Amphetamine = 4.85, p>.2) after amphetamine microinjection while maintaining 100% probability of approach to the CS+lever under both conditions and showing no increase in approach or consummatory behaviors to the CS_{dish}. In summary, any animal that demonstrated goal-tracking during training, that goal tracking was potentiated after amphetamine microinjections, but if a rat

did not goal-track at all, exclusive sign-tracking, that sign-tracking became more intense (Figure 3.3).

In more detail for 'mixed CR' rats that showed the strongest effect, dopamine stimulation in DLS boosted the probability of mixed CR rats would approach the goal CS_{dish} from 66% after vehicle to 93% of trials after amphetamine microinjection ($F(1,5)=10, p=.025$). Sign-tracking responses to the CS+lever remained at 100% always after either vehicle or amphetamine. So amphetamine microinjection the appeal of the CS_{dish} in a nonexclusive fashion, without pulling mixed CR rats completely away from the CS+lever.

After mixed CR rats reached the CS_{dish}, amphetamine microinjection in DLS also increased the number of consummatory grasps, sniffs, and nibbles on the metal dish during the CS+lever extinction presentation (though no sucrose UCS was present). Mixed CR rats more than doubled their number of nibbles and sniffs on the metal dish from 1.4 per 8 sec presentation of CS+lever after vehicle to 3.6 after amphetamine microinjection in DLS. The approach-and-nibble-dish enhancement still required the simultaneous presence of the triggering CS+lever stimulus, and no amphetamine enhancement was detected when CS+lever, was absent ($F(1,5)=1$, $p=0.363$; $F(1,5)=1.359$, $p=0.296$).

Localization of opioid and dopamine CS attraction enhancements in dorsolateral neostriatum

Localization of function was determined by mapping behavioral enhancement magnitudes on to the sites that caused them, aided by Fos plume measures of spread of

neurobiological impact, helped confirm that the lateral half of the dorsal neostriatum was the principal site for CS motivational magnet enhancements. Fos plume diameters provide at least some information on the radius of spread of local impact on neuronal function surrounding a microinjection of DAMGO or amphetamine, and the diameter of microinjection impact is useful in mapping location of function revealed by neurochemical stimulation (Pecina and Berridge 2000; Richard and Berridge 2011). In dorsolateral neostriatum, Fos plumes produced by an initial DAMGO microinjection extended about 0.2 mm in total radius, containing both a small center of >150% Fos elevation (above vehicle baseline; 0.13mm radius, volume=0.009mm^3), and a larger 0.2mm radius surrounding sphere of moderate 150-200% Fos elevation above normal levels (0.033mm^3 volume). These were, similar to DAMGO plumes previously observed in dorsomedial neostriatum (DiFeliceantonio, Mabrouk et al. 2012).

DAMGO microinjections enhanced prepotent cue attraction as described above only in the lateral half of the dorsal neostriatum (DLS). A microinjection site was classified as being contained within DLS if >75% of a total plume volume was inside the lateral half of dorsal neostriatum (Figure 3.4). DAMGO microinjections failed to enhance at sites in the medial half of neostriatum (dorsomedial striatum) if the plume did not penetrate into the lateral half of dorsal neostriatum (Drug*Placement: $F_{(2,23)}$=5.37, p=.012). Dorsomedial sites for DAMGO microinjections did not increase approach frequency or speed, nor grasps, nibbles and sniffs on any cue ($F_{(3,10)}$=1.250, p=.343; all individual comparisons p>0.1).

DLS DAMGO enhances flexible pursuit of moved motivational magnet, not S-R motor rituals.

In potential support of a habit ritual interpretation, we identified here a motor ritual typically displayed after 3 days of training by sign-trackers using video analysis (Figure 3.5a). The ritual began with 1) a rat typically sitting slightly nearer its goal dish (3cm) than the lever location (5cm) in anticipation before the CS+lever was inserted. When 2) the CS+lever actually appeared, rats typically turned 30 – 90 degrees so as to immediately face the CS+lever. Next 3) each rat typically took a small forelimb step towards CS+lever (< 2 cm step), 4) followed with a small hind step movement to move the rear quarters (<2 cm step), and then 5) repeated forelimb and hind limb steps as needed until the head was within 1 cm of the lever, and then 6) merely leaned forward without moving the hindquarters to make oral contact with the lever, often accompanied by a forelimb grasp, and 7) initiated consummatory sequences of sniffing and nibbling the CS+lever (Figure 3.5a). Does DAMGO microinjection in DLS simply strengthen this motor ritual?

Here we used a manipulation that could be described as 'who moved my cheese?' similar to Carr & Watson's (1908) to test whether opioid stimulation of DLS would strengthen sign-tracking as a motor ritual or make it perseverate and decouple from action consequences when lever location is moved. In practice, this can most readily be tested by suddenly moving the CS+lever of sign-trackers to a new location on the opposite wall of the chamber from its previous appearances.

To test these alternative hypotheses, separate rats were trained as above, and sign-trackers were identified. On the test day after their DAMGO or vehicle microinjection,

the location of the CS+ lever was moved to a new location on the opposite wall from where it had previously emerged.

DAMGO or vehicle microinjections in dorsolateral neostriatum were administered on a test on the 4th day. On that same day, the location of where the CS+lever would emerge was suddenly moved to the opposite wall, approximately 20 cm from the original location. No lever emerged from the original location on this test day.

At the first CS+lever emergence in new location, rats typically abandoned their habitual pattern described above. Even after DAMGO microinjection in DLS rats made few if any approaches to the old location. Instead rats that received DAMGO microinjection made 20% fewer habitual visits to the old location on the first switched appearance of the CS+lever (0.68±0.3) than rats that received vehicle microinjections (0.78±0.6; Figure 3.5b).

Instead, rats immediately switched to the new location. The approach to the new location required a different movement pattern from the previous ritual. In the new movement sequence, rats typically 1) turned 90 – 180 degrees in opposite direction away from their old location and head movement, towards the opposing wall where the cue now appeared, 2) took a long forelimb step toward new location (i.e., >5 cm), 3) followed with a long hind limb step (>5 cm), 4) repeated steps # 2 and #3 at least 2 times and up to 5 times until the new location was reached (Figure 3.5b).

DAMGO microinjection in DLS facilitated this abandonment of original motor ritual and flexible shift to follow the cue to its new location. DAMGO microinjections sped up by 30% the first arrival at the new CS+lever location (vehicle speed = 4.65s ±0.88, DAMGO speed=5.9±0.94). Finally, once reached, DAMGO in DLS made rats

emit 38%more intense nibbles and sniffs, grasps and bites of the newly placed CS+lever than rats that received vehicle microinjection, suggesting they were more motivated to 'consume' the metal lever even in its new location (Vehicle= 2.79±1.2, DAMGO= 3.85±0.96; Figure 3.5b). Thus, opioid stimulation of DLS makes rats abandon more quickly any ritualized S-R habit involved in sign-tracking. Instead, DLS stimulation makes the rats flexibly follow the cue to its new location, despite requiring a new sequence of movements. This flexible shift and new motor pattern supports the idea that rats more strongly 'want' the attractive cue more after DLS opioid stimulation in a motivated fashion.

Sign-tracking does not show S-R habit resistance to UCS devaluation

One test of whether a conditioned response has become habitual is to assess whether it is insensitive to its outcome, in the sense of continuing to perseverate near original levels after the associated UCS reward is suddenly devalued, such as by inducing sensory-specific satiety (Colwill and Rescorla 1990; Dickinson and Balleine 1990; Balleine and Dickinson 1992). An S-R habit may persist after goal devaluation, at least until the UCS is re-encountered again after performing the response in a devalued state (Dickinson and Balleine 1990; Wassum, Cely et al. 2009). By contrast, incentive salience controlling a motivated Pavlovian response can decrement or increment levels of behavioral responding to the CS when UCS value is shifted, without further CS-UCS retraining, without further S-R retraining, and without re-tasting the UCS again before performing the response (Dickinson, Smith et al. 2000; Berridge 2012; Robinson and Berridge 2013). Therefore we tested whether UCS devaluation would reduce sign-tracking responses elicited by the CS+lever in an extinction test.

Sign-trackers (n=9) were trained as above for 3 days. Then the UCS was devalued on the 4th day by inducing sensory satiety to sucrose. Satiety (n=5) was induced by allowing rats to consume as much sucrose as they could within 45 min in the test chamber (~60 sucrose pellets and water spout available ad lib). Control non-devalued rats (n=4) were placed in the chamber for the same duration, but no sucrose pellets were given (water was still available). Approximately 5 min after this consumption or control session, CS extinction session was begun with the moved lever as described above (containing 5 presentations of CS+lever) and sign-tracking was assessed.

Sign tracking approaches to the lever were cut in half to 53% of control levels after satiety was induced by sucrose consumption (Figure 3.6). The speed to reach the CS on presentations that were approached was also slowed to one-third of the speed of non-devalued control rats. ($t_{(4)}$=2.89, p=.047; Figure 6). The number of consummatory grasps, sniffs, and nibble actions performed on the metal CS+lever was similarly cut to less than one-third of control levels after satiety devaluation of UCS ($t_{(4)}$=3.107, p=0.036), and by 71% ($t_{(3)}$=6.07, p=.009; Figure 3.6) .

Thus UCS devaluation produced an immediate reduction of conditioned responding elicited by CS+lever to less than ½ control levels by merely inducing a degree of satiety to the UCS. This pattern suggests that sign-tracking never strongly became an S-R habit that was insensitive to goal value, and instead that CS "wanting" was adjusted by physiological state in accordance with the incentive salience hypothesis (Zhang et al. 2009; Berridge, 2012; Robinson & Berridge, 2013).

DLS opioid stimulation enhances instrumental working to obtain CS+lever.

A final measure of increased "wanting" for a CS+ is whether individuals will learn a new response and work harder in order to obtain the CS. This can be measured as instrumental conditioned reinforcement, which requires rats that have previously learned the Pavlovian CS+-UCS association to learn an entirely novel instrumental response to earn presentations of the CS+lever alone (Pryor, Haag et al. 1969; Taylor and Robbins 1984; Meyer, Lovic et al. 2012). Does DAMGO microinjection in DLS enhance the acquisition and performance of instrumental conditioned reinforcement?

Rats (n=21)were trained in Pavlovian autoshaping for 5 days, and sign-trackers were identified as above if they directed approaches, nibbles and sniffs more than 3 times more frequently to the CS+lever as target than to the CS_{dish}. On the 6th day, sign-trackers were tested for instrumental conditioned reinforcement after receiving bilateral microinjections of either DAMGO or vehicle (between subjects) in the DLS. In this instrumental task, rats could perform a new nose poke response, by breaking a photo beam inside a designated hole in the wall that had not been present on previous days, in order to earn presentations of the CS+lever on an FR1 schedule. A second nose poke hole 10cm away earned nothing and served as a control for general motor activity.

DAMGO microinjections in DLS enhanced the number of the new instrumental nose poke response performed to earn the Pavlovian CS+lever presentation. Rats that received DAMGO worked harder to obtain the CS+lever, making 150% more nose pokes into the porthole, and earning 150% more 2-sec presentations of the Pavlovian CS+lever than control rats that received vehicle microinjections (38.00±7.5 to 57.10±5.54), DLS DAMGO microinjections selectively enhanced responding on the nose poke hole that earned the CS+lever, and did not enhance responding into the other control hole that

earned nothing (inactive nose vehicle= 17.27±2.1, DAMGO = 17.30±1.6; nose poke type*drug interaction $F_{(1,19)}$=5.17, p=.035, Figure 3.7).

By contrast to dorsolateral sites in neostriatum that produced enhancement of conditioned reinforcement, more medially placed sites in dorsomedial neostriatum produced no change in conditioned reinforcement after DAMGO microinjection ($F_{(2,6)}$=0.675, p=.544). Neither responding for the CS+lever (vehicle 52.8(±1.7); DAMGO 56.37(±9.39; $F_{(1,7)}$=0.071, p=0.789), or for the control hole was altered by opioid stimulation of dorsomedial sites (vehicle 33.12 (±6.45) ; DAMGO 25.37(±5.36; $F_{(1,7)}$=0.905, p=0.375). This fits the conclusion from our first experiment that dorsolateral sites in neostriatum, but not dorsomedial sites, support opioid enhancement of "wanting" for a prepotent CS.

Discussion.

Opioid stimulation of the dorsolateral of neostriatum, via microinjection of the mu agonist DAMGO, amplified the motivational magnet properties of CSs for sucrose reward in a directional and individualized fashion, focusing higher incentive salience on each individual's prepotent Pavlovian cue (pre-existing favorite) to make that CS even more attractive and 'wanted', at the expense of an alternative cue. That is, of DLS by DAMGO microinjections made sign trackers emit even more intense and focused sign tracking under the influence of mu opioid stimulation approaches and consummatory nibbles and sniffs of the predictive metal lever CS (while decreasing their residual goal-tracking responses). Likewise, DLS DAMGO microinjections made goal trackers emit more intense and focused approach, nibbles and sniffs toward the metal dish or goal that

delivered sucrose pellets. Although the dish was always available as a target, the enhancement occurred only when the CS+lever was physically presents, and not in the absence of a triggering stimulus. For all individuals, the motivation enhancement was triggered as temporal pulses by appearance of the CS+lever. This pattern of motivation enhancement for a previously learned reward cue was essentially identical to the pattern previously reported for opioid stimulation of the central nucleus of amygdala. The equivalence of the two sites suggest that both may use learned Pavlovian information as an input, and local opioid-stimulation state as a separate input, to determine the intensity and exclusivity of CS "wanting" at the moment of re-encounter.

Neostriatal opioid receptors are localized mainly in "patch" or "striosome" compartments (Pert, Kuhar et al. 1976; Herkenham and Pert 1980; Gerfen 1984; Crittenden and Graybiel 2011). In DLS, patches receive inputs from cortex limbic, prelimbic and anterior cingulate cortices (Ragsdale and Graybiel 1990; Eblen and Graybiel 1995; Kincaid and Wilson 1996; Levesque and Parent 1998; Haber, Kim et al. 2006; Crittenden and Graybiel 2011).

Mu opioid receptors on neurons in "patches/striosomes" in dorsolateral neostriatum may be in a unique position to control dopamine function, as such patch neurons possibly project directly to the substantia nigra pars compacta, to most directly influence striatal dopamine tone (Sato, Sumi-Ichinose et al. 2008; Fujiyama, Sohn et al. 2011). It is possible that opioid stimulation of CeA and the dorsolateral neostriatum produce similar enhancements of prepotent CS "wanting" because both interact through the substantia nigra to produce a focusing of intense motivation on learned cues

(Gonzales and Chesselet 1990; Rouillard and Freeman 1995; Fudge and Emiliano 2003; Lingawi and Balleine 2012).

Anatomical localization of CS "wanting" amplification

Neuroanatomically, the prepotent enhancement was generated only by sites in the lateral half of the dorsal half of neostriatum (dorsolateral quarter of neostriatum). Most effective sites were contained in the upper 25% level of dorsal neostriatum (e.g., within 1.5mm of the dorsal edge of neostriatum measured directly above each effective site). Both anterior and posterior sites in the lateral half of dorsal neostriatum appeared effective. At sites placed in the medial half of the dorsal neostriatum, DAMGO microinjections failed to alter autoshaped behavior toward CS+lever or CSdish in any individuals, even in the anterior region of dorsomedial neostriatum. This failure of dorsomedial neostriatum to control behavior directed toward learned Pavlovian CSs contrasts to our previous report that DAMGO in anterior portion of dorsomedial neostriatum produced robust enhancement of UCS consumption, and that consuming sweet foods triggered an endogenous enkephalin surge. That double dissociation suggests two conclusions. First, mu opioid stimulation in the lateral half of dorsal neostriatum promotes the incentive motivation attractiveness of learned Pavlovian CSs for food reward (but not necessarily for UCSs themselves). Second, by contrast, mu opioid stimulation in anteromedial portion of dorsal neostriatum promotes the attractiveness of the natural UCS of palatable food, but does not interact with previously learned Pavlovian associations to focus "wanting" on a particular CS+. This localization of motivation functions (UCS vs. CS targets) adds to other functions previously described for these regions (Vanderschuren and Everitt 2005).

DLS neurochemical differences between opioid and dopamine amplification

Neurochemically, a very different pattern of CS enhancement was produced by dopamine stimulation via amphetamine microinjection. The effect of dopamine stimulation was to directionally promote goal-tracking at the expense of sign-tracking. This dopamine effect was more tenuous or individually selective than the opioid effect, in that the goal-tracking enhancement was only observed in 'mixed' individuals that spontaneously showed both sign-tracking and goal-tracking CRs to comparable degrees when the CS+lever was present. Pure sign-trackers and pure goal-trackers failed to be modulated in their CS-directed behavior. Regarding potential mechanisms for differences between opioid and dopamine stimulation in DLS, mu opioid receptors are expressed especially on neurons located in patch or striosome compartments, and patch neurons may project most directly to pars compacta of substantia nigra, including to dopamine neurons (Crittenden and Graybiel 2011; Fujiyama, Sohn et al. 2011). Mu opioid receptor stimulation in DLS might thus be expected to preferentially alter patch-striosome neuronal function. By contrast, amphetamine promotes dopamine release that would stimulate dopamine D1-type and D2-type receptors on neurons in both patch and matrix compartments of neostriatum, as well as on presynaptic terminals of corticolimbic glutamate neurons (Gerfen 1984; Packard and Knowlton 2002b; Crittenden and Graybiel 2011).

Dopamine stimulation in DLS specifically enhanced the attractiveness of the UCS-contiguous CSdish, to enhance goal-tracking particularly in mixed individuals. For both sign-trackers and goal trackers the CS+ Lever, which has the highest predictive correlation with UCS delivery, was the trigger that controlled the timing of incentive

salience surges after DAMGO stimulation. For sign-trackers the Pavlovian target of focused "wanting" was the same physical CS+ Lever, whereas for goal-trackers the 'wanted' target was the CS_{dish}, which has the closest spatial and temporal contiguity with the UCS (because the rat's head was always inserted into that dish whenever the sucrose UCS was experienced). Contiguity has long been recognized as important to facilitate a Pavlovian association between a UCS and a CS, and contiguity may remain important even when contingency correlation dominates associative prediction (Zener 1937; Mackintosh 1974; Rescorla and Cunningham 1979; Delamater and Holland 2008). Contiguity also has been reported previously to be important for pharmacological dopamine-related enhancements of incentive salience (Tindell, Berridge et al. 2005; Simon, Mendez et al. 2009; Holden and Peoples 2010; Smith, Berridge et al. 2011), but see also (Doremus-Fitzwater and Spear 2011).

DLS and sensorimotor S-R habit functions

The dorsolateral or sensorimotor region of neostriatum is known to play roles in serial movement patterns and S-R habits. One line of evidence for DLS in habits has been that DLS apparently becomes increasingly recruited over time as learned actions become more over trained or habitual. For example, DLS lesions are reported not to disrupt cocaine self-administration early in training, but to produce disruptions of self-administration if made later after additional training (Murray, Belin et al. 2012). A related line of evidence is that learned reward-seeking actions become increasingly independent of outcome as overtraining proceeds, so that over trained learned behaviors perseverate even if the reward has been devalued by satiety or if the act-outcome contingency is

diluted by free rewards (Dickinson and Balleine 1990; Balleine and O'Doherty 2010). Lesions of the DLS reduce such perseveration, so that learned responses decline again after reward devaluation or contingency dilution (Yin, Knowlton et al. 2004, 2006). A third line of evidence for DLS involvement in habits is serial ritualization into stereotyped patterns that characterize habits (Graybiel 2008). Habitual actions can be computationally generated by a prediction error mechanism that once trained operates inflexibly (Daw, Niv et al. 2005). Rigid serial patterns of action are disrupted by lesions of the dorsolateral neostriatum, both for learned serial rituals (Yin 2010) and for instinctive rituals of serial actions (Cromwell and Berridge 1996). Further, neurons within the dorsolateral neostriatum track the chunking of serial actions into ritualized patterns, both for learned rituals (Barnes, Kubota et al. 2005) and instinctive serial rituals (Aldridge and Berridge 1998). In short, "habits are sequential, repetitive, motor, or cognitive behaviors elicited by external or internal triggers that, once released, can go to completion without constant conscious oversight" (Graybiel 2008), in which DLS plays an important role.

DLS opioid enhancement of CS attraction: "wanting" motivation or S-R habit?

With the above points in mind, it is crucial to ask whether DLS microinjections of DAMGO strengthened an S-R habit ritual to enhance approach and consummatory responses to a prepotent CS. We believe the evidence clearly shows the answer to be 'no'. First, there was no 'who moved my cheese?' perseveration of sign-trackers' well-established approach ritual to familiar-location when their CS+lever was suddenly moved to a new location. Instead, rats almost immediately abandoned their old ritual within a second or two, and switched to the new location using a new movement sequence of

opposite-direction turn and longer strides. Rats switched even faster after DAMGO and reached the new location with even greater alacrity, suggesting they more intensely 'wanted' the moved cue and were willing to flexibly follow it. Second, DLS microinjections of DAMGO also made rats 'want' the CS+lever cue more in the sense of being willing to learn an entirely new movement response to earn it. Rats learned a new nose-poke response, and performed at higher levels to earn CS+lever insertions as an instrumental conditioned reinforcer after DAMGO microinjection in DLS than after vehicle microinjections. Although conditioned reinforcement can be explained in several ways, the enhancement is certainly consistent with the notion that DLS opioid stimulation made rats 'want' the Pavlovian cue more intensely. Finally, sign-tracking itself as a behavioral response never quite became habitual in the accepted sense of persisting after UCS devaluation. Instead, sign-tracking immediately reduced after UCS devaluation by satiety induction. Devaluation sensitivity suggests that sign-tracking always remained a motivated response, integrating current biological state with learned Pavlovian information as incentive salience computation typically does (Zhang, Berridge et al. 2009; Berridge 2012; Robinson and Berridge 2013).

Dopamine stimulation in DLS by amphetamine microinjections also may have produced a motivational enhancement but of a different type. Amphetamine microinjection more selectively enhanced goal-tracking alone, and only in 'mixed' individuals that originally both sign-tracked and goal-tracked to comparable degrees. It is difficult to view this as S-R habit enhancement, since there was no enhancement in individuals that showed the strongest patterns to begin with, i.e., sign-trackers

In sort, these lines of evidence demonstrate the dorsolateral neostriatum participates

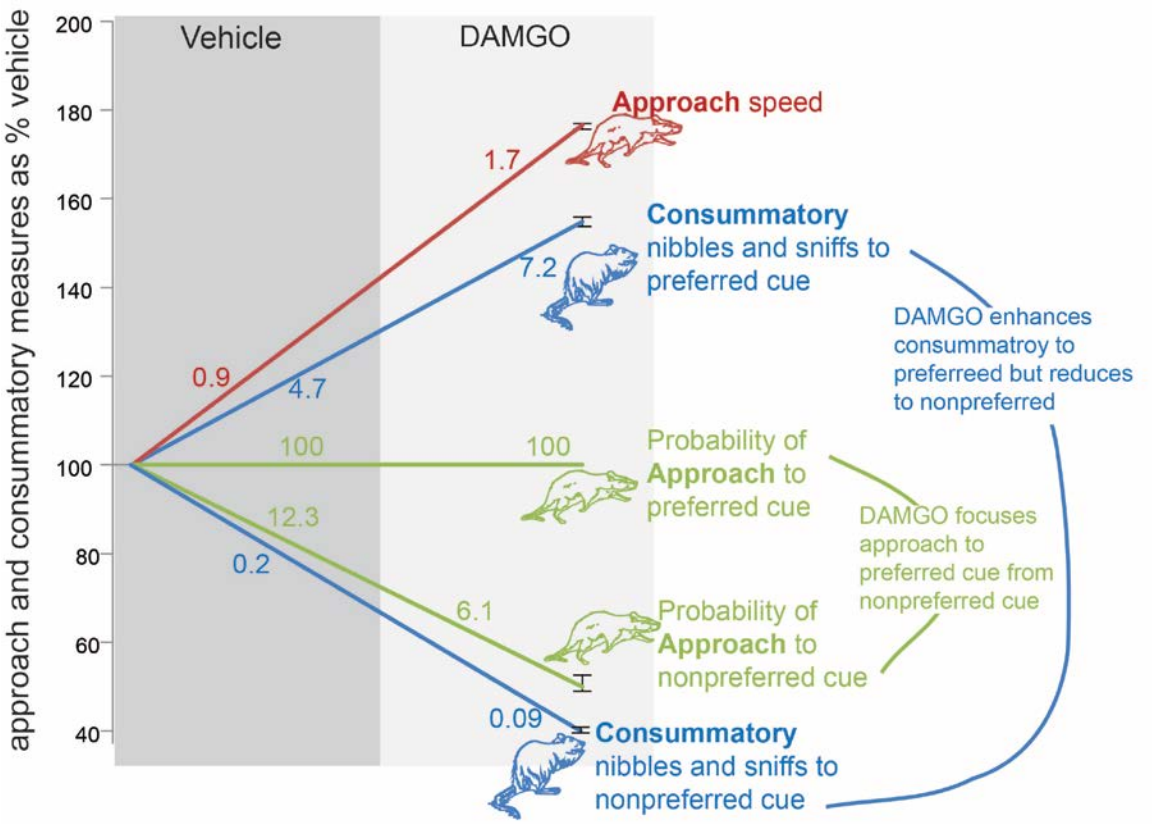
in generation of intense motivation as incentive salience assigned to a particular Pavlovian cue for reward. Although motivation generation by DLS may come as a surprise to traditions that view it more purely as sensorimotor or habit-based, there is increasing supporting evidence for a motivation role. In animals, DLS participates in restoring motivated food-related behaviors (Palmiter 2008a), and DLS neurons code flexible responses as well as stable responses when reward cues are suddenly changed (Graybiel 2008; Kubota, Liu et al. 2009). A neural signal in DLS for learned motivational value could possibly be reflected by the rise in extracellular dopamine observed when a cocaine cue is encountered as a conditioned reinforcer (Ito, Dalley et al. 2002). In line with an interpretation as a focuser of motivation, dopamine signaling within the dorsolateral neostriatum seems to correlate most closely with the focus or selectivity of a response, i.e. choosing the drug nose port over the inactive nose port, rather than compulsive drug taking (Willuhn, Burgeno et al. 2012). In human neuroimaging experiments, DLS activates to food stimuli correlated with motivation ratings of desire (Hollmann, Hellrung et al. 2012).

Conclusion

Enhancement of incentive salience attributed one particular reward CS, converting that CS into a more powerful motivational magnet that controls both the direction and intensity of behavior expands the role of the dorsolateral neostriatum in motivation. DLS can make a Pavlovian CS more 'wanted', in a winner take all fashion. This may have implications for disorders of intense compulsion and addiction. In particular, our findings suggest that DLS recruitment may magnify the amplitude and persistence of cue-triggered pulses of behavior focused on an incentive target, through a Pavlovian

motivation mechanism that can operate independently of simple S-R habits or sensorimotor automatisms.

Figures



Approach and consummatory behaviors are enhanced by DAMGO. Here both approach and consummatory behaviors are mapped as % vehicle. Raw values are shown next to end points. Speed of approach as well as consummatory behaviors to the preferred cue were enhanced by DAMGO. DAMGO decreased approaches and consummatory behaviors to the nonpreferred cue.

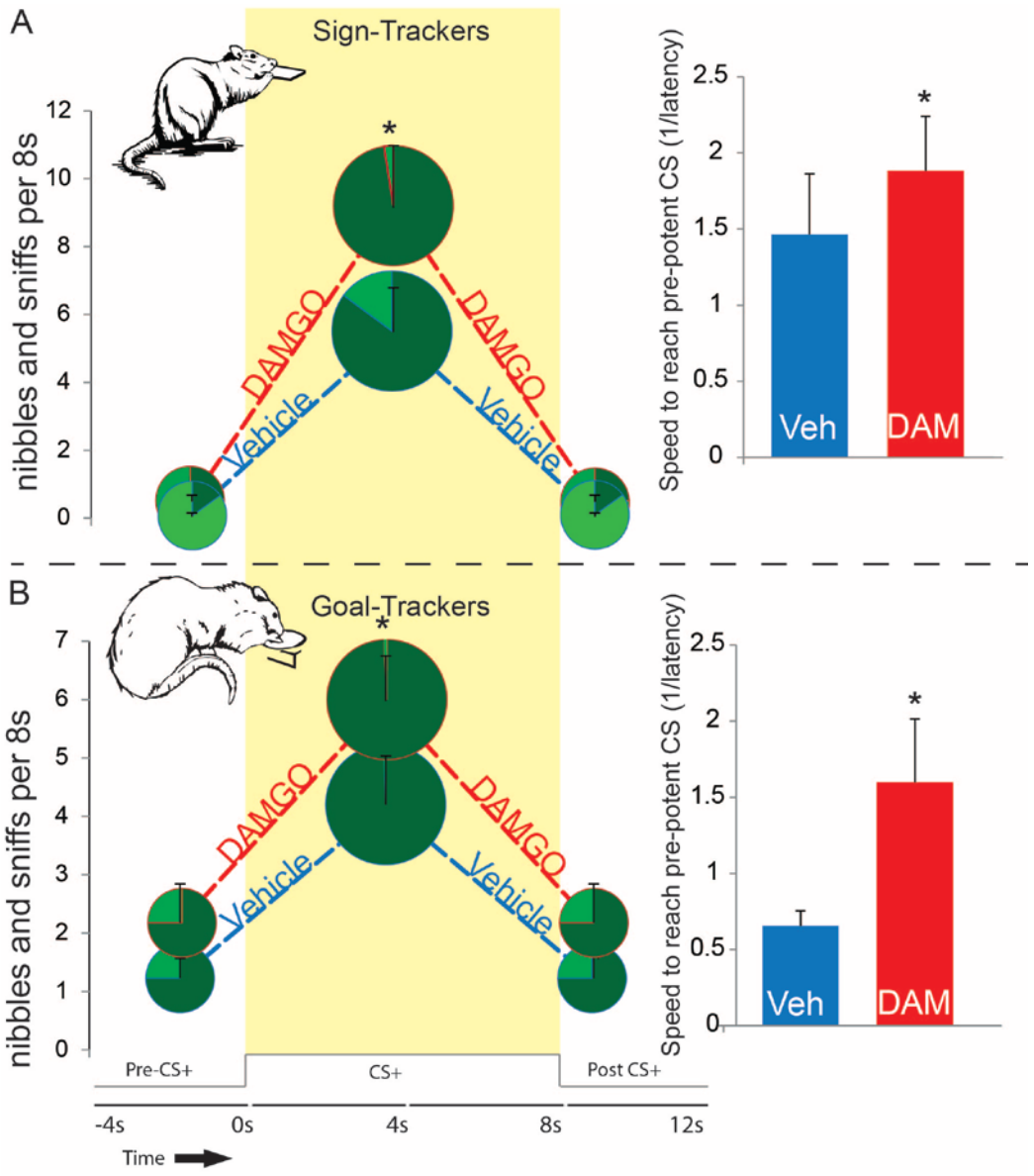


Figure 3.2. Dorsolateral DAMGO enhances focus. Dorsolateral DAMGO microinjection amplifies and focuses appetitive-consummatory behaviors directed toward the prepotent cue for both sign-trackers and goal-trackers. Both the amount of approaches, nibbles and sniffs directed at the prepotent cue and proportion of all approaches, nibbles and sniffs directed at the prepotent cue is increased, while the proportion directed toward the nonprepotent CS is decreased. Yellow background indicates periods when the CS+ is physically present; white backgrounds indicate before and after CS+ presentations * indicates $p<0.05$. Pie-graph circles show the proportion of appetitive-consummatory behaviors directed by that phenotype to CS+ Lever (sign) vs. CS_{dish} (goal).

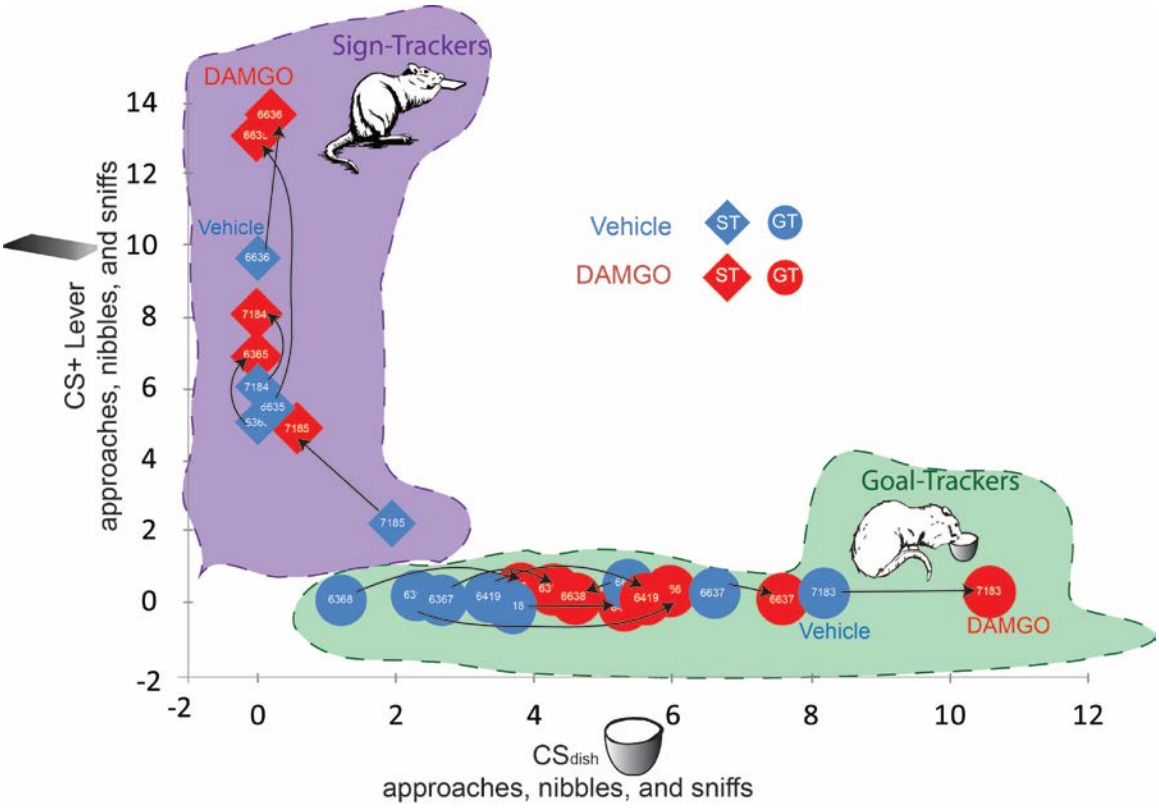


Figure 3.3 Individual variation for DAMGO microinjection. DAMGO microinjection into the dorsolateral neostriatum made sign-trackers into more intense sign-trackers and goal-trackers into more intense goal-trackers. In this scatter plot, each individual rat is represented by two dots: a blue dot in vehicle condition and a connected red dot in DAMGO condition. Sign-trackers are circles and goal-trackers are diamonds. Vertical axis plots the number of sign-tracking behaviors toward CS+ Lever (sign). Horizontal axis plots the intensity of goal-tracking behaviors toward CS_{dish} (goal). DAMGO always intensifies the pre-existing preference of an individual that was already prepotent. Inset: probability to approach the preferred cue for sign-trackers and goal-trackers.

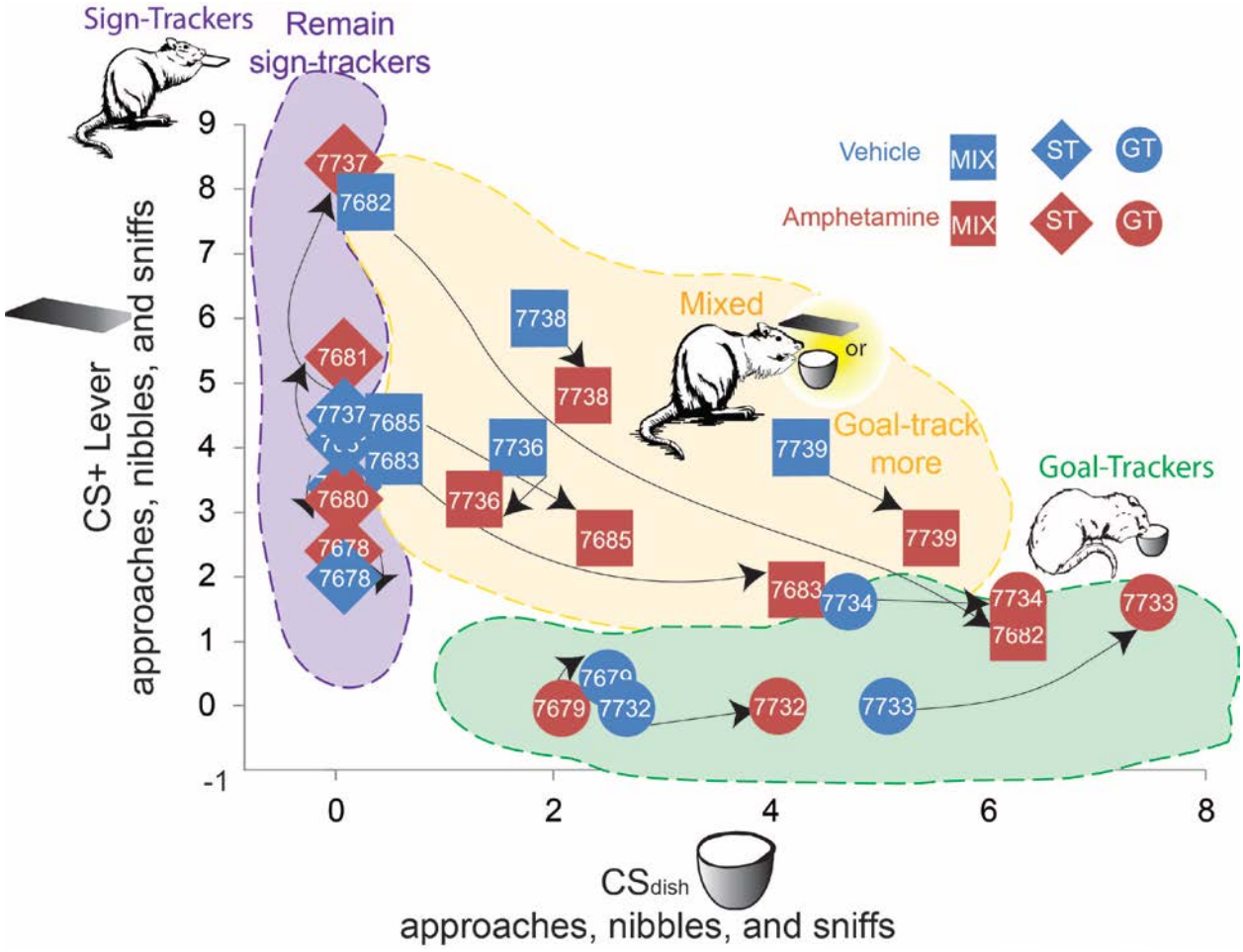


Figure 3.4 Individual variation for amphetamine microinjection. Amphetamine microinjection into the dorsolateral neostriatum had the most noticeable effect on animals that showed a mixed phenotype (yellow group), showing sign- and goal-tracking during training. These animals showed much stronger goal-tracking after amphetamine microinjection.

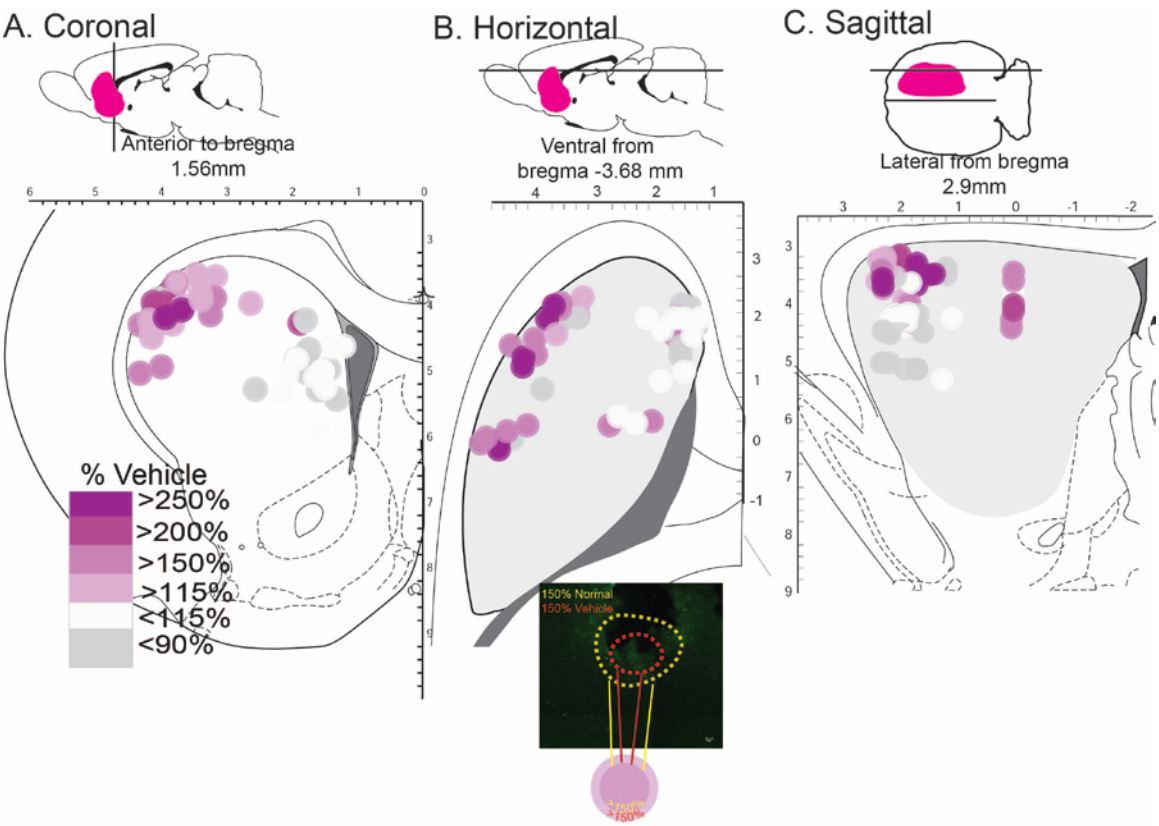


Figure 3.5. Dorsolateral not dorsomedial neostriatum enhances motivational magnets. Each microinjection in represented in 3 anatomical planes. The size of the symbol represents the area of maximal drug spread (inset). For clarity in mapping, the distance from the dorsal most portion of the striatum for each placement in its appropriate slice was measured and maintained here in the representative slice. There is a clear division of function between the dorsolateral and dorsomedial neostriatum.

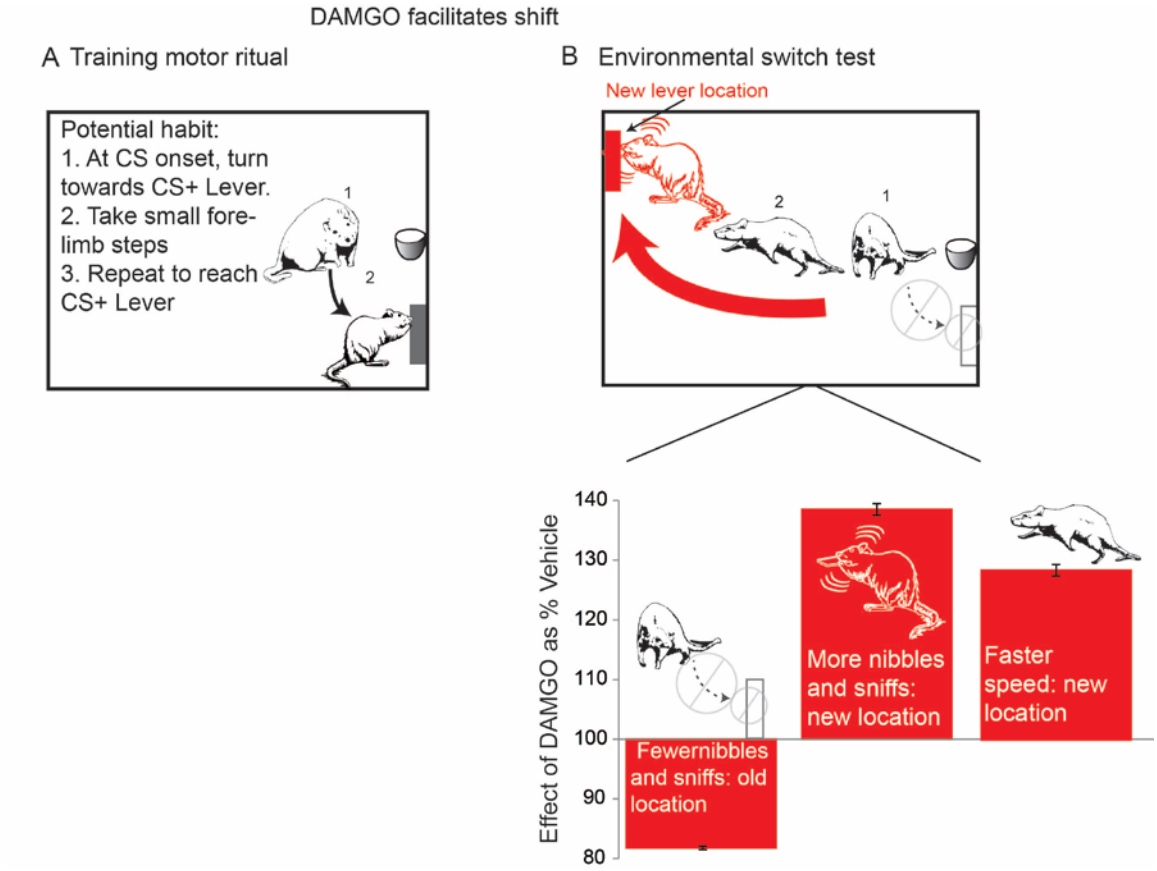


Figure 3.6. Environmental shift. The ritualized behavior exhibited by most rats during training is represented in Panel A. In Panel B the effect of the shift in the lever location is represented. The inset shows DAMGO facilitated the shift to the new location by reducing nibbles and sniffs and the old location and enhancing speed and nibbles and sniffs of the new lever location (inset).

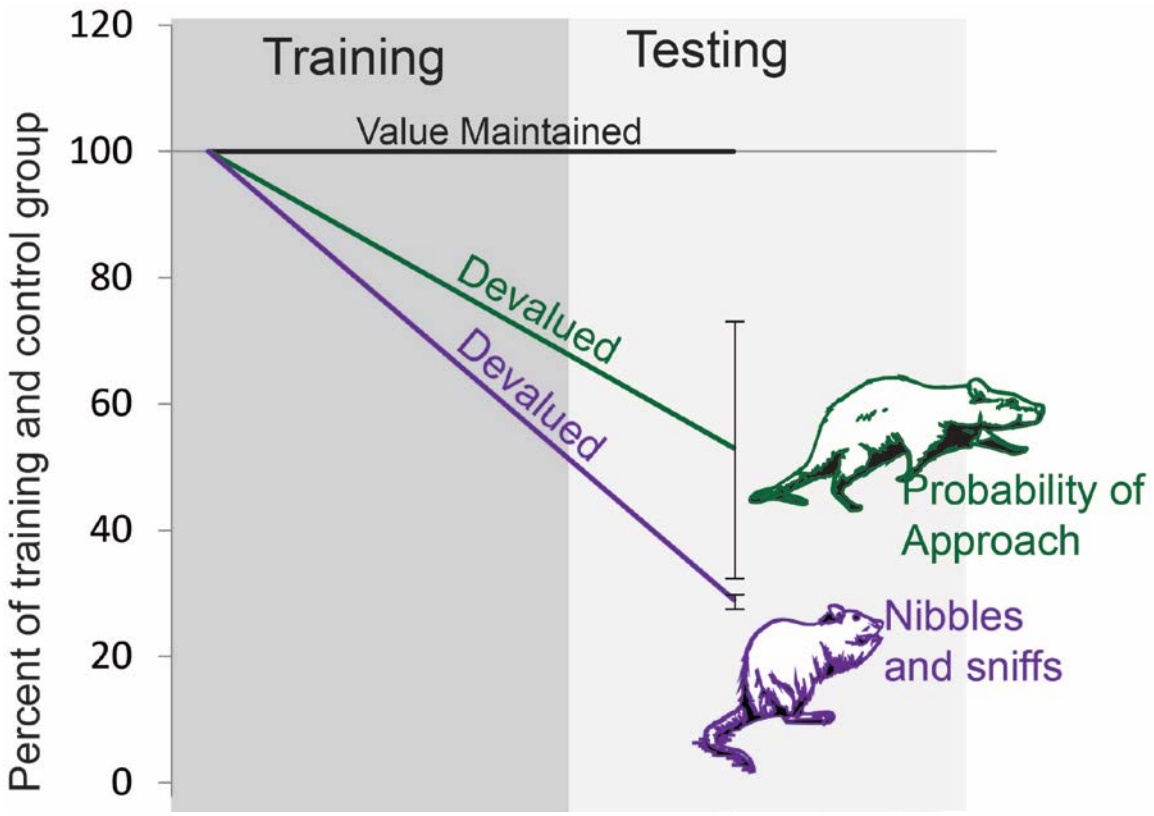


Figure 3.7. Reinforcer value shift. In this experiment the value of the sucrose pellet was devalued by sensory specific satiety in separate rats. Those rats that spent only time in the chamber are represented at 100% by the black line. The detriment in approaches and nibbles and sniffs of the lever seen in those rats that were pre-fed on sucrose pellets in resented by the green and purple lines respectively.

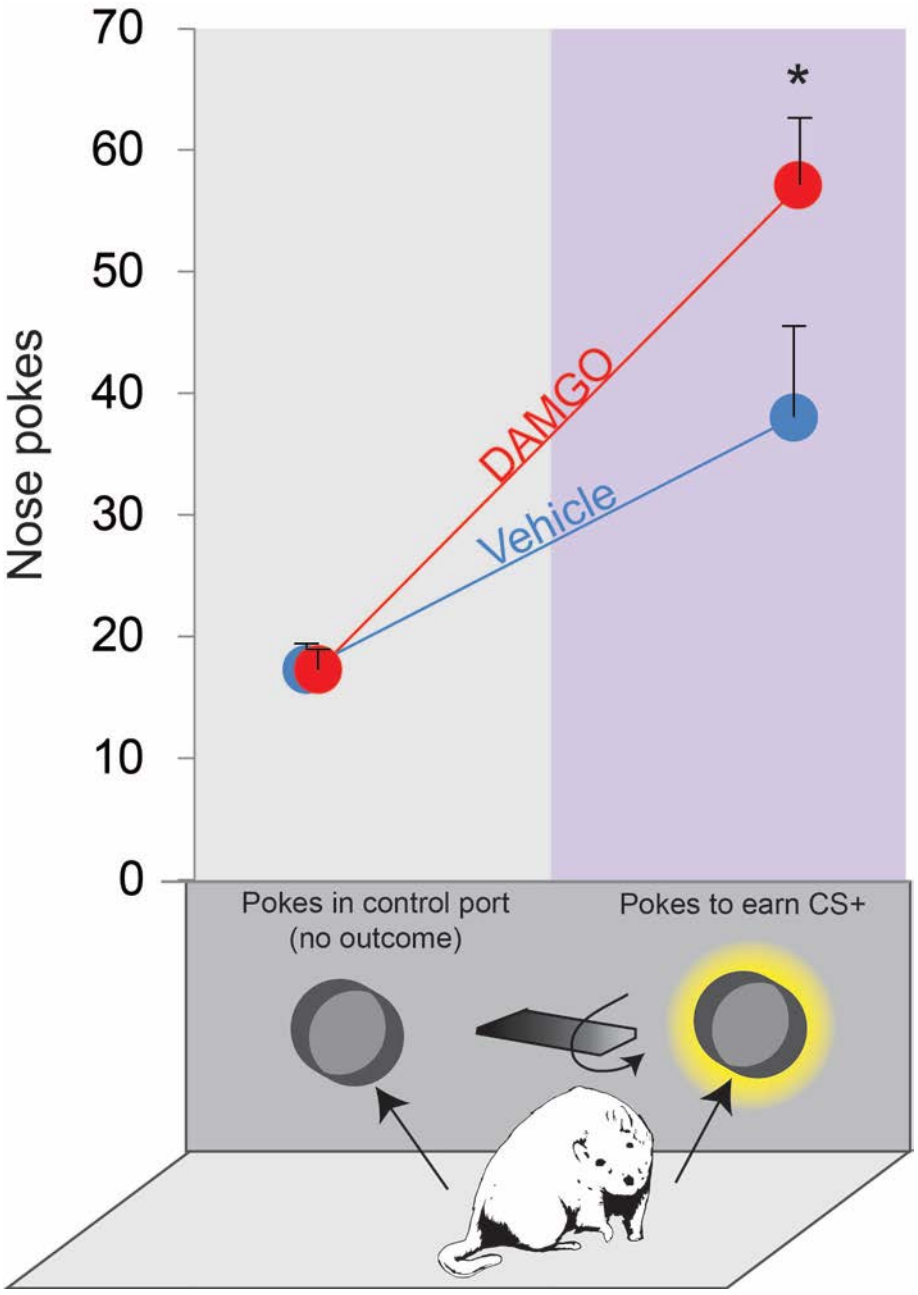


Figure 3.8. Conditioned reinforcement. After autoshaping training, rats were microinjected with either DAMGO or vehicle and were placed in the operant chambers. The chambers were now equipped with nose pokes around a center lever. Those rats microinjected with DAMGO made more pokes in the active port, earning more CS+ presentations that those rats that received vehicle. Pokes in the inactive port did not differ across condition.

Chapter 4

Enkephalin in Dorsomedial Neostriatum Says "Eat More Now!"

Introduction

Dorsal neostriatum has been traditionally viewed to mediate movement and habits (Parkinson, Cardinal et al. 2000; Balleine and Ostlund 2007; Graybiel 2008; Suto, Wise et al. 2011), and to respond to learned cues (Schultz and Dickinson 2000a), whereas ventral striatum is well known to generate reward and motivation to consume incentives (in large part mediated by opioid circuitry) (Pecina and Berridge 2005; Baldo and Kelley 2007). Dorsal striatum recently has also become implicated in reward-related functions (Volkow, Wang, Fowler, Logan, Jayne, Franceschi et al. 2002; Volkow, Wang et al. 2006; Palmiter 2008a, b; Stice, Spoor et al. 2008; Wise 2009; Nummenmaa, Hirvonen et al. 2012) and here we report that opioid signaling in an extremely dorsal region of neostriatum contributes to generating intense motivation to over-consume palatable food rewards. In dorsal neostriatum, mu opioid receptors are localized mainly in "patch" or "striosome" compartments (Pert, Kuhar et al. 1976; Gerfen 1984; Crittenden and Graybiel 2011). Patches or striosomes in neostriatum receive converging inputs from limbic regions of prefrontal cortex, including from orbitofrontal, prelimbic, and anterior cingulate regions (Ragsdale and Graybiel 1988, 1990; Eblen and Graybiel 1995; Kincaid and Wilson 1996; Levesque and Parent 1998; Graybiel 2008; Crittenden and Graybiel

2011). We focused here on the medial region of dorsal neostriatum, which has been implicated by previous studies in processing value of rewards (Yin, Knowlton et al. 2005).

Materials and Methods

Subjects

Sprague Dawley female rats (n = 42 for microinjection studies, n = 12 for sucrose taste reactivity, n=8 for M&M fragment taste reactivity, n = 9 for enkephalin microdialysis, and n = 5 for dynorphin microdialysis) weighing 280-370g at the start of the experiment were pair housed on a reverse light/dark cycle. Water and food (Purina Rat Chow) were provided ad libitum at all times for rats in microinjection experiments and food was restricted to 10g for one light/dark cycle prior to dialysate collection. All experiments were conducted in accordance with protocols approved by the University of Michigan Committee on Use and Care of Animals (UCUCA).

Surgery

All rats were anesthetized with ketamine (80 mg/kg), xylazine (7 mg/kg), and atropine (0.04 mg/kg). To prevent infection, chloramphenicol sodium succinate (60 mg/kg) was administered as well as carprofen (5 mg/kg) to provide pain relief. Carprofen was administered again 24 h post-surgery for all rats and chloramphenicol was administered 24 h post-surgery and as needed for taste-reactivity rats. All rats were allowed 5-7 days to recover from surgery before testing.

Intracranial microinjections. Chronic bilateral 14 mm (23 ga) cannulae aimed at anterior dorsomedial neostriatum (AP 1-2.5, ML ±1.8, and DV -3.5-4.5), posterior

dorsomedial neostriatum (AP 0-1, ML ±1.8, and DV-3.5-4.5), anterior dorsolateral neostriatum (AP 1-2.5, ML ±3-4, DV -3.5-4.5) and posterior dorsolateral neostriatum (AP 0-1, ML ±3-4, and DV-3.5-4.5) were implanted 2 mm above target injection site. Dorsoventral coordinate was marked from bregma at flat skull. Placement coordinates for cranial cannulae were calculated based on Paxinos and Watson (Paxinos and Watson 2007) and lowered into place with a stereotaxic apparatus. Cannulae were anchored to the skull with bone screws and acrylic cement. Steel stylets were inserted to prevent their occlusion. Rats used for c-Fos analysis received the same cannulae implantation except for those designated as "normal." These animals received all procedures except cannulae implantation.

Taste Reactivity. As above, chronic bilateral 14 mm (23 ga) cannulae aimed at anterior dorsomedial neostriatum (AP 1-2.5, ML ±1.8, and DV -4.5) were implanted. Additional oral cannulae were inserted to allow infusion of taste solutions directly into the mouth. Oral cannulae were constructed in house of ethyl vinyl microbore tubing (0.04 inch inner diameter; Cole-Parmer) and stainless steel (19 ga). Each was inserted just lateral to the first maxillary molar and run subcutaneously along the zygomatic arch to the top of the skull where the cannulae exited through the incision made for the cranial cannulae. There, they were secured using wire and acrylic cement. After 3 days of recovery, oral cannulae were cleaned and flushed with water daily to prevent buildup of food materials and blockage of the cannulae. Rats used for solid M&M taste reactivity received cranial microinjection cannulae only.

Microdialysis. Chronic unilateral cannula (CMA-12, Harvard Apparatus) aimed at anterior dorsomedial striatum (AP 1-2.5, ML ±1.8, and DV -4) was implanted. Lateralization of the unilateral cannula was varied across rats (Left=5, Right=4). Cannula was secured in place with bone screws and acrylic cement. A stainless steel wound clip was fixed to the acrylic cement to allow the rat to be attached to a tethering system that provided free movement around the apparatus. A dummy probe (CMA-12, Harvard Apparatus) was inserted into the cannula to prevent occlusion.

Drugs and intracranial microinjections

Prior to free food intake and taste reactivity tests, steel stylets were removed and cleaned. Microinjectors (16 mm) were inserted into the guide cannulae, pre-measured so that microinjector tips extended 2 mm below guides. Microinjections of [D-Ala^2, N-MePhe^4, Gly-ol]-enkephalin (DAMGO; Sigma), [D-Pen^{2,5}] Enkephalin, (DPDPE; Sigma) or vehicle (aCSF; Harvard Apparatus) were controlled by a syringe pump which delivered 0.5 µL over 120 s. DAMGO and DPDPE were dissolved in aCSF at 973 µM concentration (0.25µg/0.5 µL). Microinjector tips were left in cannulae for 1 min following the injection to allow diffusion away from microinjector tips. Each rat received a "sham" injection 1 day prior to testing of vehicle to habituate them to the microinjection procedure.

Behavioral Testing Procedures

Food Intake. Rats were habituated for 4 days to clear plastic tub cages with ~3 cm of corn cob bedding, 20 g of pre-weighed M&Ms, 20 g of pre-weighed chow, and a subset received chow shaped wood blocks. Water was available through a drinking spout.

On the fourth day, rats received a "sham" microinjection. Cages were set up identically for habituation and testing. On test days, 29 rats received DAMGO and vehicle counterbalanced across days with 48 h between each testing session. 13 rats received DPDPE, DAMGO, and vehicle, counterbalanced across days with 48 h between testing sessions. Rats were microinjected and then immediately placed into plastic tub cages, they were videotaped for 60 min, removed, and all food left in the cage was weighed. Behavioral video tapes were scored at a later date offline.

Taste Reactivity. Rats were habituated for 4 days to the taste reactivity testing apparatus and the food intake cages described above. On the fourth day rats received a "sham" intracranial injection of vehicle and a "sham" oral infusion of water (1ml/1min) to habituate them to the procedure. On test days rats received DAMGO or vehicle, counterbalanced across days. After a delay of 20 min (Pecina and Berridge 2005), 1% sucrose was delivered (1 mL/1 min) for 1 min into the mouth. Hedonic reactions were recorded from an angled mirror aimed up through the clear plastic floor of the testing apparatus. Videos were coded at a later date offline in slow-motion (ranging from frame-by-frame to $1/10^{th}$ normal speed) by an observer who was blind to the rat's DAMGO vs. vehicle condition. After completion of the oral infusion, 6 rats were transferred for 60 min to the food intake paradigm described above to measure food intake.

For solid M&M taste reactivity rats were habituated to the apparatus described above. On test days rats received either DAMGO or vehicle microinjections counter balanced across days. 20 minutes after the injection, rats were presented with a sequence of 0.2g fragments of M&M candy, which they were allowed to eat as the close-up camera recorded their orofacial reactions through the transparent floor. Each rat was only

allowed to consume a maximum of 8 M&M fragments to avoid influence of satiety. Immediately after consuming each fragment, rats typically persisted in emitting post-prandial hedonic reactions for about 6 sec. Hedonic reactions during the post-prandial 6-sec period were recorded and analyzed offline (Feurte, Nicolaidis et al. 2000).

Microdialysis. Rats were habituated for 3 days to a 15X15X12 inch Plexiglas enclosure containing ~3 cm of corn cob bedding, M&M candies, a drinking spout, and various "toys" (wood pellets, paper, and plastic weigh boats). On the third day, rats were connected to the tether line, but no probe was lowered, to allow them to habituate to the counter weight. Prior to testing, rats were restricted to 10 g of chow for the preceding light/dark cycle and M&M candies were removed from the testing apparatus. Testing occurred during the beginning of the dark cycle for the rats and the room was kept dim throughout collection as enkephalin levels are diurnally modulated and are highest at the start of the night period (Bayon and Anton 1986; Will, Vanderheyden et al. 2007) . At the start of each collection day the rat was handled, gently restrained, and a dummy probe inserted into the guide cannula. This probe was left in place for 5 min. A new probe (CMA-12 PAES, 2 mm) was flushed and inserted after the removal of the dummy. The rat was then attached to the tethering system and allowed to move freely about the apparatus. Perfusate was pumped at a rate of 1.5 µL/min for 1 h, at 1 µL/min and 0.6 µL/min for 30 min each during a 2 h washout period. Baseline collection then began. Sample bins of 20 min (equal to the dead volume) were collected at 0.6 µL/min while the animal moved freely and interacted with the "toys." After baseline, M&M candies were introduced to the chamber and animals were allowed to feed to satiety. M&M candies remained in the chamber until the end of the experiment. The entire experiment (baseline

and feeding) was videotaped and scored offline.

Behavioral Video Scoring

Microdialysis. Videos were scored by experimenters, offline. The following behaviors were recorded during baseline collections: rearing, locomotion, chewing on non-food objects, and drinking. During feeding collections all of the following were recorded as well as latency to first M&M consumption, time spent feeding, and number of M&Ms consumed.

Food Intake. Videos were scored by experimenters blind to the experimental condition of each rat. Seconds spent engaging in the following behaviors were recorded: eating M&Ms (actual chewing and consumption), eating chow, drinking, and chewing on non-food items. The following behaviors were recorded as a single event: sniffing M&Ms (anticipatory sniffs and approaches), sniffing chow, grooming, cage crossing, and rearing.

Taste Reactivity. Videos were scored at $1/25^{th}$ to $1/10^{th}$ speed using Observer software (Noldus, Netherlands). Total reactions were tallied using a previously described binning method (Berridge, Flynn et al. 1984; Berridge 2000). Hedonic reactions were rhythmic tongue protrusions, lateral tongue protrusions, and paw licking. Neutral reactions were neutral mouth movements and grooming behavior. Aversive reactions were rare and of those reactions only forelimb flails were observed, with only 4 rats total demonstrating forelimb flails in both conditions.

For solid chocolate M&M scoring of taste reactivity, the scoring procedure

subtracted any periods when a clear view of the rat's face and mouth was obscured by either paws or chocolate. The goal was to produce a hedonic reaction score per a total of 40 seconds of clear visibility. Hedonic reactions occurring during a 6 second post-prandial period when the mouth was unobstructed were scored. Rhythmic tongue protrusions, lateral tongue protrusions, and paw licking were observed and recorded (Feurte, Nicolaidis et al. 2000). Aversive reactions were not observed during chocolate eating at any point.

Dialysate Analysis

Met-ENK and leu-ENK were measured using a slight modification of a previously described capillary liquid chromatography-mass spectrometry (LC-MS) method (Li, Zubieta et al. 2009; Mabrouk, Li et al. 2011). Chromatography columns (50 μm i.d.) and electrospray ionization emitter tips (25 μm i.d.) were prepared in-house using 28 and 10 cm lengths of fused silica capillary, respectively. Columns were packed to 3 cm bed length with 5 μm Alltima C18 reversed-phase particles (Alltech, Deerfield, IL, USA). The column and emitter tip were coupled to a PV-550 nanospray ESI source (New Objective, Woburn, MA, USA) interfaced to a LTQ XL linear ion trap mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA). A +3.0 kV potential was applied to a liquid junction prior to the column for electrospray.

7.5 μL samples were injected using a WPS-3000TPL autosampler (Dionex, Sunnyvale, CA, USA), in partial loop injection mode (10 μL loop). Samples were loaded onto the column at 2 μL/min by an air-driven fluid pump (DHSF-151, Haskel Inc., Burbank, CA, USA). The column was rinsed and desalted with 0.15% formic acid in H_2O

for 2 min using the same pump and flow rate. Following loading and desalting, an injector valve was switched to put the micro HPLC pump (MicroPro, Eldex Laboratories, Napa, CA, USA) online for gradient elution at 200 nL/min. Mobile phase A was LC-MS grade H_2O with 0.15% formic acid and mobile phase B was LC-MS grade acetonitrile. The gradient program began with an isocratic step of 20% B for 1 min, then a linear increase to 95% over 2.5 min, followed by an isocratic step at 95% B for 0.5 min. All valve switching and runs were controlled with Xcalibur software (Thermo Fisher Scientific). The MS^3 m/z pathways for met-ENK and leu-ENK were: 574 → 397 → 278, 323, 380 and, 556 → 397 → 278, 323, and 380, respectively. The dynorphinA 1-8 fragment was measured and the MS^2 pathway for dynorphin was 491 →435.

Baseline met- and leu- enkephalin levels were 2.61±0.56 pM and 2.32±0.30 pM, respectively. Baseline dynorphin levels were 4.6±0.96 pM. According to our previous works, limits of detection for met-enkephalin were 1 pM and 0.5 pM for leu-enkephalin (Li, Zubieta et al. 2009). Therefore the baseline levels reported here are well above detection limits for the current LC-MS method.

Histology

Rats were sacrificed immediately after the final day of testing by administration of a sodium pentobarbital overdose. Rats were decapitated and the brains were extracted and fixed in 10% paraformaldehyde solution for 1-2 days followed by a 25% sucrose solution in 0.1 M sodium phosphate buffer for 2-3 days before slicing. 60 micron slices through the neostriatum were taken from each rat, mounted, dried, and stained with cresyl violet. Microinjection center was determined for each bilateral injection site for

microinjection experiments and probe center was determined for microdialysis studies. Slides were compared with the stereotaxic atlas (Paxinos and Watson 2007) to determine placement in the neostriatum.

Fos analysis for functional drug spread. Microinjection spread was assessed by "Fos plumes" surrounding drug microinjections. We have previously found that drug induction of Fos plumes is reduced after several microinjections (Berridge, Ho et al. 2011), and therefore we used a dedicated Fos group measured after a single microinjection to measure Fos radius under similar conditions in order to detect maximum spread of impact, as described previously (Pecina and Berridge 2005; Reynolds and Berridge 2008). Rats used for Fos analysis were anesthetized and transcardially perfused 75 min after bilateral microinjection of vehicle (n = 6), DAMGO (n = 10), or normal (n = 2). Brains were extracted, frozen and sliced at 40 μm. Slices were processed for c-Fos-like immunoreactivity using NDS, goat anti-cFos (Santa Cruz Biotechnology, Santa Cruz, CA) and donkey anti-goat Alexa Flour 488 (Invitrogen, Carlsbad, CA) (Reynolds and Berridge 2008; Berridge, Ho et al. 2011). Slices were mounted, air dried, and cover slipped with ProLong Gold antifade reagent (Invitrogen). Areas of c-Fos positive cells were mapped in "plumes" around microinjection sites, as described previously (Pecina and Berridge 2005; Reynolds and Berridge 2008; Berridge, Ho et al. 2011).

Statistical Analysis

All drug effects were compared using within subjects ANOVAs with Bonferroni corrected t-tests to examine group differences. Effects of placement were determined by designating placements as a between subjects factor. Met- and Leu- enkephalin levels

followed similar patterns and were combined for analysis as "Enkephalin." All enkephalin and dynorphin levels were normalized as percent baseline by computing the average peak area baseline levels and calculating a percentage of that score for each rat. Due to small sample size and nonparametric, normalized data, Freidman's test and Wilcoxon's signed rank test were used to assess within subjects differences across collection bin. Spearman's rho was calculated to test associations between latency to feed and enkephalin levels. To avoid the influence of outliers, we used Wilcox's skipped correlation to calculate spearman's rho without bivariate outliers using the R environment (Rousselet and Pernet 2012).Correlations calculated with and without the outlier are reported. All significant non-parametric tests were also significant using their parametric counterpart. Effect sizes were calculated using the equation for Cohen's d with the adjustment for repeated measures used where appropriate.

Results

Summary

In short, our microinjection results revealed that exogenous mu opioid stimulation by DAMGO microinjection into the anteromedial dorsal neostriatum potently enhanced eating of palatable M&M™ chocolates, more than doubling the total M&M™ intake. This hyperphagic effect was specifically localized within the anterior medial quadrant of the dorsal neostriatum (DAMGO = 251% average increase over vehicle levels). Accordingly, our microdialysis study of that same anteromedial quadrant of dorsal neostriatum found that endogenous enkephalin levels rose to 150% of baseline when rats

were suddenly allowed to eat chocolates. These endogenous and exogenous results are described in detail below.

Endogenous enkephalin release

Microdialysis probes implanted in anteromedial dorsal neostriatum measured extracellular levels of endogenous striatal opioid peptides: enkephalin (likely released from 'indirect path' neurons that also express dopamine D2 receptors), and dynorphin (likely released from 'direct path' neurons that express dopamine D1 receptors). Enkephalin and dynorphin were measured first during a normal quiet behavioral state in mildly hungry rats before any meal to establish a baseline, and next when a large quantity of palatable chocolate candies (M&Ms™) was suddenly presented. Opportunity to eat chocolate M&Ms™ evoked avid consumption, averaging 10 M&Ms™ in 20 min (≈10 g), and elicited an immediate rise in endogenous levels of met-enkephalin and leu-enkephalin, reaching an elevation of >150% over pre-meal baseline (Baseline: met-enkephalin = 2.61 ± SEM 0.56 pM, leu-enkephalin = 2.32 ± SEM 0.30 pM; Friedman's test, $p<0.01$; baseline = 100%; Figure 4.1 and Figure 4.2A). Enkephalin levels remained elevated throughout the roughly 20-40 min period that each rat continued to eat, and then began to decline as rats slowed and gradually ceased eating, typically returning fully back to baseline within the next 40 minutes (1^{st} baseline vs. last sample Wilcoxon's test, n.s.).

In contrast to enkephalin, dynorphin levels failed to rise during eating, and instead remained unchanged throughout the meal (Friedman's test, $p>0.1$; Figure 4.2B). Therefore, only enkephalin in the anteromedial quadrant of dorsal neostriatum became dynamically elevated during consumption of palatable food.

Enkephalin surges did not seem to be a consequence of mere motor activity. Enkephalin changes were measured during "motor" periods when the rat performed non-ingestive active movements, such as oromotor gnawing of plastic or wood objects, spontaneous body grooming, or locomotion (walking and/or rearing) in the absence of food (Figure 4.2C). During these non-ingestive activities, enkephalin levels never rose (Friedman's test, p=1, n.s.), suggesting that the rise described above in the same rats to eating chocolates was not due simply to the motoric production of active movements involved in eating. By contrast, enkephalin levels did rise when M&Ms™ were presented and eaten, compared to the previous periods when rats engaged in locomotor and other movements (Friedman's test, p=0.023), again suggesting that enkephalin reflected more than simply the occurrence of ongoing motor activities (Figure 4.2C). Therefore it appears that enkephalin rose specifically with onset of the reward experience of eating palatable chocolates, remained elevated during eating, and declined soon after.

Finally, we found that the magnitude of enkephalin surge in each rat correlated with that individual's speed or latency to begin consuming its first M&M (spearman's rho=-0.90, p=0.002, CI [-1,-.392]; Figure 4.1 inset): the faster a rat started eating, the higher its relative increase in enkephalin levels. This correlation raises the possibility that anteromedial dorsal neostriatum opioid levels might contribute a motivational "eat now" command. That causal hypothesis was tested further in the microinjection study below.

Exogenous mu stimulation of intense eating.

We found that DAMGO microinjection in dorsal neostriatum stimulated more intense eating of chocolates in non-deprived rats, but depending on precise site or quadrant. Sites within the anteromedial quadrant of dorsal neostriatum produced by far

the most intense increases of >250% in intake of M&Ms™ ($t_{(18)}$=5.1, p<.001, 95% CI [9.96, 4.15], Cohen's d= 1.217; compared to vehicle control intake levels by the same rats; Figure 3A). Anteromedial quadrant sites produced higher elevations of eating than all other quadrants of dorsal neostriatum (Anteromedial vs. other quadrants $F_{(1,36)}$=8.44, p=.006, 95%CI [136,235], Cohen's d=1.09). Localization of function was further determined by mapping the causal efficacy of neostriatal microinjection sites to stimulate eating, using symbols sized to the measured radius of Fos plumes surrounding DAMGO microinjections in dorsal neostriatum (Fos radius reflects the anatomical spread of drug impact, Fig 3). For sites that elevated eating >250%, at least 90% of the volume of DAMGO-induced local Fos plumes would have been contained entirely within the anteromedial quadrant of dorsal neostriatum. That is, DAMGO Fos plumes were measured to have a 0.18 mm total radius (0.02 mm^3 volume), containing an inhibited small center (0.15mm radius, volume=0.016mm^3 zone of halved Fos expression compared to vehicle baselines) surrounded by a larger excitatory Fos sphere (0.18mm radius, 0.02mm^3 volume; zone of doubled Fos expression over normal baseline; center/surround Fos opposition possibly reflects reciprocal local inhibitory connections between the two zones; Figure 4.2A inset). These plume measurements allow confidence that the intense over-consumption was generated by DAMGO stimulation of anteromedial dorsal neostriatum, rather than by diffusion to other regions of neostriatum, ventral striatum or nucleus accumbens.

For sites in the highly effective anteromedial quadrant of dorsal neostriatum, most rats ate over 17g of M&Ms™, equal to about 5% of their 300g body weight (Figure 4.3A). That level of elevated consumption (5% of body weight) is roughly proportional to

a 150lb human consuming about 8 lbs. of M&Ms™ in a single hour, thus clearly overriding normal satiety signals (Kelley, Baldo and Pratt 2005; Woolley, Lee et al. 2007).

DAMGO microinjection in this anteromedial quadrant also made rats *faster* to begin to eat (in addition to making them eat more): decreasing the latency to begin eating their first M&M of the day (Vehicle=55.4 ± SEM 10.4. DAMGO = 28.7 ± SEM 4.2; $t_{(25)}$=2.49, p=.019, 95% CI [4.69, 48.85], Cohen's d= 2.781). Faster speed to eat supports the hypothesis that mu opioid receptor stimulation in this neostriatum region provides a command to "eat now" as well as to "eat more."

In contrast to the anteromedial quadrant, as microinjection sites moved posteriorly in medial dorsal neostriatum the level of stimulated eating gradually declined. Strong elevation of eating was still produced at intermediate medial sites where the diameter of Fos plumes straddled the border between anteromedial and posteromedial quadrants of dorsal neostriatum (190% increase). No significant elevation was produced by more posterior sites fully contained within the posteromedial quadrant (i.e., where no part of a posterior site's Fos plume would contact the anteromedial border; average 118%, n.s.).Thus, overall for the entire posteromedial quadrant, intermediate 150% elevations of eating were found, due mostly to the medial sites that straddled the anterior/posterior border ($t_{(8)}$=2.52, p=.036, 95% CI [7.28, 0.33, Cohen's d= 0.939). Comparing anterior versus posterior directly as entire quadrants, DAMGO in the anteromedial quadrant produced a greater increase in intense eating than DAMGO in the posteromedial quadrant ($t_{(23)}$=2.21, p=.037, 95% CI [201.6, 6.96], Cohen's d= 0.85).

Eating elevations fell off to zero abruptly as microinjection sites moved laterally from the anteromedial quadrant. Anterolateral quadrant sites produced no increase at all over vehicle levels (i.e., outside and lateral to effective sites in dorsomedial neostriatum; only 103%; $t_{(9)}$=0.1, p=0.917; Fig 4.3A).

By contrast to mu stimulation of eating, delta opioid receptor stimulation by DPDPE microinjections failed to increase eating behavior or intake over vehicle control levels at all sites in dorsomedial neostriatum, even in the anteromedial quadrant ($F_{(1,12)}$=.4, p>0.1; Figure 4.4). Accordingly, M&M™ intake was much higher after mu agonist DAMGO microinjection than after delta agonist DPDPE microinjection at the same anteromedial dorsal neostriatum sites (DAMGO= 6.31 ± SEM 1.13, DPDPE= -0.46 ± SEM 0.68; $t_{(36)}$ = 5.10, p < 0.001, 95% CI [9.47, 4.09], Cohen's d = 1.65). That difference suggests that enkephalin may act primarily at mu receptors in anterior dorsomedial neostriatum to stimulate increases in consumption, rather than at delta receptors.

Further, DAMGO microinjections in all areas of neostriatum, including the anterior dorsomedial quadrant, failed to produce any general increases in locomotor or oromotor activity, measured by cage crosses, rearing, grooming or treading behaviors, chow consumption, or wooden block gnawing (behaviors $F_{(5,28)}$=0.151, n.s.; chow $F_{(15,90)}$=0.68, n.s.; gnawing $t_{(4)}$=2.1, n.s.).

Exogenous mu stimulation fails to alter hedonic 'liking' for sweetness. Finally, we used the affective taste reactivity test of orofacial 'liking' reactions to ask whether mu opioid enhancement of motivation to eat sweet food reflected purely generation of motivation to eat (similar to opioid effects in most of ventral striatum outside a cubic-millimeter hotspot and in central amygdala) or additionally involved any enhancement of

hedonic impact or 'liking' for the taste of sweet reward (typical only of restricted hotspots in ventral striatum, ventral pallidum, etc.)(Pecina and Berridge 2005; Smith and Berridge 2005; Mahler and Berridge 2011). This measure draws on rodent affective orofacial reactions (e.g. positive tongue protrusion and lip licking to sweetness versus aversive gapes to bitterness) that are homologous to human infant affective facial expressions elicited by tastes (Grill and Norgren 1978; Pecina and Berridge 2005; Smith and Berridge 2005). We tested for hedonic enhancement in a standard taste reactivity test using a sucrose solution infused directly in the mouth to control stimulus quality and duration, and separately for the taste of sweet/fatty chocolate as rats voluntarily ate 0.2 g fragments of M&Ms™, replicating the chocolate stimulus and conditions of eating enhancement (Feurte, Nicolaidis et al. 2000). Taste reactivity results of both tests showed that DAMGO microinjections in anterior dorsomedial neostriatum failed to enhance positive hedonic taste reactions at all, either to oral infusions of 1% sucrose solution ($F_{(3,9)}$=1.875, p=.204; Figure 4.3B) or to the chocolate taste of solid M&Ms™ fragments ($F_{(3,5)}$=0.175, p=0.91; Fig 4.3B). Although no increase in hedonic impact was observed, the DAMGO microinjections again increased motivation to eat in the voluntary intake test so that the rats doubled their consumption of chocolate over vehicle control levels ($t_{(5)}$=15.58, 95% CI [9.90, 7.09], Cohen's d= 2.3). Thus DAMGO in dorsomedial neostriatum appeared to make rats selectively 'want' to eat M&Ms™ more intensely, in the sense of enhanced eating behavior and intake, without making them 'like' sweetness any more, in the sense of hedonic reactions to sucrose or chocolate tastes. We note that similar dissociations involving selective increases in "wanting" without 'liking' are typical of opioid stimulation at many other brain sites, including ventral striatum (except

in the hotspot of nucleus accumbens shell) and striatal-related structures (Pecina and Berridge 2005; Smith and Berridge 2005; Mahler and Berridge 2011). This dissociation between "wanting" versus 'liking' suggests that mu opioid stimulation in anterior dorsomedial neostriatum may generate increases of motivation as a specific psychological mechanism to drive intense eating and consumption of food rewards.

Discussion

We acknowledge that our finding of intense over-consumption produced by mu opioid stimulation in the anteromedial dorsal neostriatum contrasts at first sight with previous reports of relative failure to observe any increase in eating after DAMGO microinjection in dorsal neostriatum (Bakshi and Kelley 1993a, b; Zhang and Kelley 2000). However, earlier studies never distinguished anatomically among the four quadrants of dorsal neostriatum as defined here, and typically used placements that as a whole were more centrally located in the dorsal half of neostriatum (i.e., more ventral and posterior to our eating hotspot). Our finding of eating localization in the anteromedial quadrant of the dorsal level may explain why studies that mixed together different subregions failed to find eating stimulation in dorsal neostriatum.

Other hints of dorsal neostriatum involvement in motivation to consume rewards have emerged in the past decade (Volkow, Wang, Fowler, Logan, Jayne, Franceschi et al. 2002; Volkow, Wang et al. 2006; Palmiter 2008a; Stice, Spoor et al. 2008; Wise 2009; Nummenmaa, Hirvonen et al. 2012). In humans, dorsal striatum activation has been reported to be elicited by palatable food and its cues in obese binge eaters, and by cocaine and its cues in drug addicts (Volkow, Wang, Fowler, Logan, Jayne, Wong et al. 2002; Volkow, Wang et al. 2006; Stice, Spoor et al. 2008; Nummenmaa, Hirvonen et al. 2012).

Such dorsal striatal activations have remained slightly ambiguous, as they could be viewed either as reward motivation or as hedonic impact, incipient movements, habits, cognitive processing, or learned predictions. Similarly, dorsal neostriatal neuronal activations elicited by rewards or cues are reported in monkeys and rodents, and often have been interpreted as reflecting learned predictions or teaching signals (e.g., prediction error model) (Apicella, Ljungberg et al. 1991; Haracz, Tschanz et al. 1993; Schultz and Dickinson 2000a; Suto, Wise et al. 2011). Our results more specifically indicate that dorsal striatal activation can participate in generating intense motivation to over-consume a reward. Thus the generative role shown here might link some functions of dorsal neostriatum more closely to the reward motivation functions of ventral striatum (nucleus accumbens) (Berridge and Robinson 1998; Carelli and Ijames 2001; Pecina and Berridge 2005; Will, Vanderheyden et al. 2007). This also seems consistent with reports that restoring synaptic function to a region of dorsal neostriatum can rescue eating in an aphagic mutant mouse model, and supports the interpretation that such neostriatum mediated rescues may involve a motivational component(Palmiter 2008b).

The hypothesis that opioid circuitry in dorsomedial neostriatum participates in generating motivation to over-consume a palatable food reward also seems concordant with its anatomical wiring from limbic prefrontal cortical inputs (Ragsdale and Graybiel 1988; Gerfen 1989; Ragsdale and Graybiel 1990; Eblen and Graybiel 1995; Kincaid and Wilson 1996; Levesque and Parent 1998) (Figure 4). For example, corticostriatal projections to the anteromedial region of dorsal neostriatum ("rostromedial sector of caudate-putamen"), similar to that studied here, originate from the prelimbic region of medial prefrontal cortex in rats (Levesque and Parent 1998). Corticostriatal projections

from “posterior [lateral] orbitofrontal/anterior insular cortex and the mediofrontal prelimbic/anterior cingulate cortex” similarly terminate in striosomes in the medial caudate in macaque primates, which probably overlaps with our eating site (Eblen and Graybiel 1995). It is also noteworthy that direct mu opioid stimulation of prefrontal cortex regions, via DAMGO microinjections in orbitofrontal and prelimbic/infralimbic (ventral anterior cingulate) cortex, can stimulate eating in rats, raising the possibility of a larger opioid-related corticostriatal circuit involved in eating and motivation (Mena, Sadeghian et al. 2011).

We speculate that opioid surges particularly within anteromedial patches of dorsal striatum might modulate presynaptic corticostriatal glutamate release or modulate postsynaptic activity of eating-related neurons in striosomes that contain mu opioid receptors (Jiang and North 1992; Wang and Pickel 1998). In addition, some D1 receptor-expressing neurons in striosomes may uniquely project directly to dopamine neurons in substantia nigra (Fujiyama, Sohn et al. 2011), which might facilitate modulation of dopamine systems to additionally help generate intense motivation. Beyond the neostriatum, the anterior dorsomedial neostriatal region described here likely interacts with other parts of the distributed mesocorticolimbic network involved in eating and intake, including hypothalamus, ventral striatum, limbic cortex, amygdala, and mesolimbic dopamine nuclei (Gosnell 1988; Kelley, Baldo and Pratt 2005; Will, Vanderheyden et al. 2007; Mena, Sadeghian et al. 2011)

In conclusion, our results provide novel evidence that enkephalin surges and mu opioid stimulation in the same anteromedial dorsal neostriatum region contribute to signaling the opportunity to eat a sensory reward and to causally generating increased

consumption of that reward. The neostriatum-generated increase in motivation can be powerful enough to more than double the amount of food a rat "wants" to eat, yet be functionally specific enough to the motivational component of reward, rather than the hedonic component, to not enhance "liking" for the same sweet chocolate treat. Opioid circuitry in anterior dorsomedial neostriatum could in this way participate in normal motivations, and perhaps even in generating intense pathological levels of motivation to over-consume rewards in binge eating disorders, drug addiction, and related compulsive pursuits.

Figures

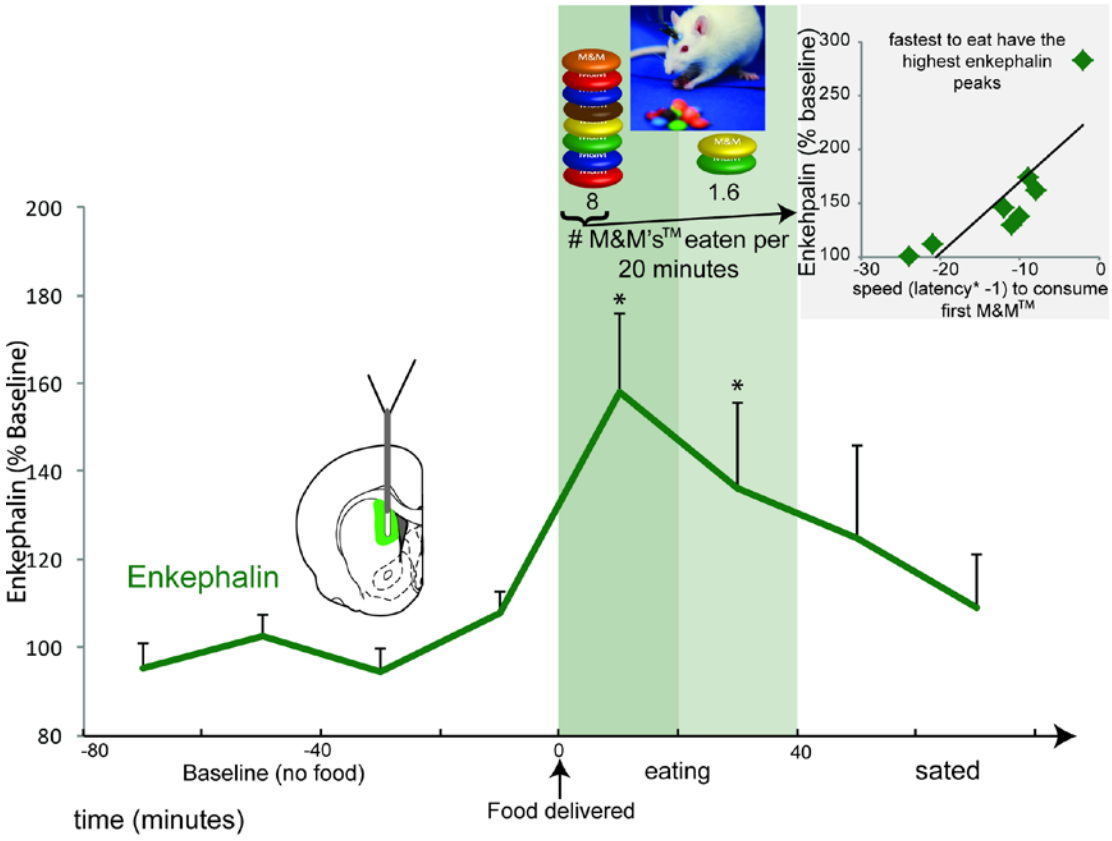


Figure 4.1. Endogenous extracellular opioid peptides in response to palatable food consumption. Extracellular enkephalin levels surged when rats began to eat milk chocolate M&Ms™. Onset of eating coincided with a robust increase in extracellular enkephalin (met and leu), which remained sustained during eating, and gradually tapered off as eating declined. The magnitude of the enkephalin rise in individuals correlated with their latency to eat their first M&M™: higher enkephalin rise for the fastest eaters. The correlation between faster speed to start eating and higher enkephalin also remains significant if the highest outlier individual (upper right of inset) is removed (spearman's rho=-0.85, p=0.013, 95% CI [-1,-0.4]). * indicates $p<0.05$. Error bars represent standard error of the mean.

Figure 4.2. Details of endogenous enkephalin surge. Absolute concentrations of met- enkephalin and leu- enkephalin are displayed in panel A. In contrast, dynorphin remained relatively stable throughout resting, eating and other behaviors (panel B). Enkephalin levels did not rise during non-ingestive activities involving forelimbs, body, or orofacial movements (walking, rearing, gnawing, grooming), here the "motor" period. Enkephalin levels as percent "quiet" are represented. Average percent of time the rats engage in each behavior is represented in the pie charts (panel C). * indicates $p<0.05$. Error bars represent standard error of the mean.

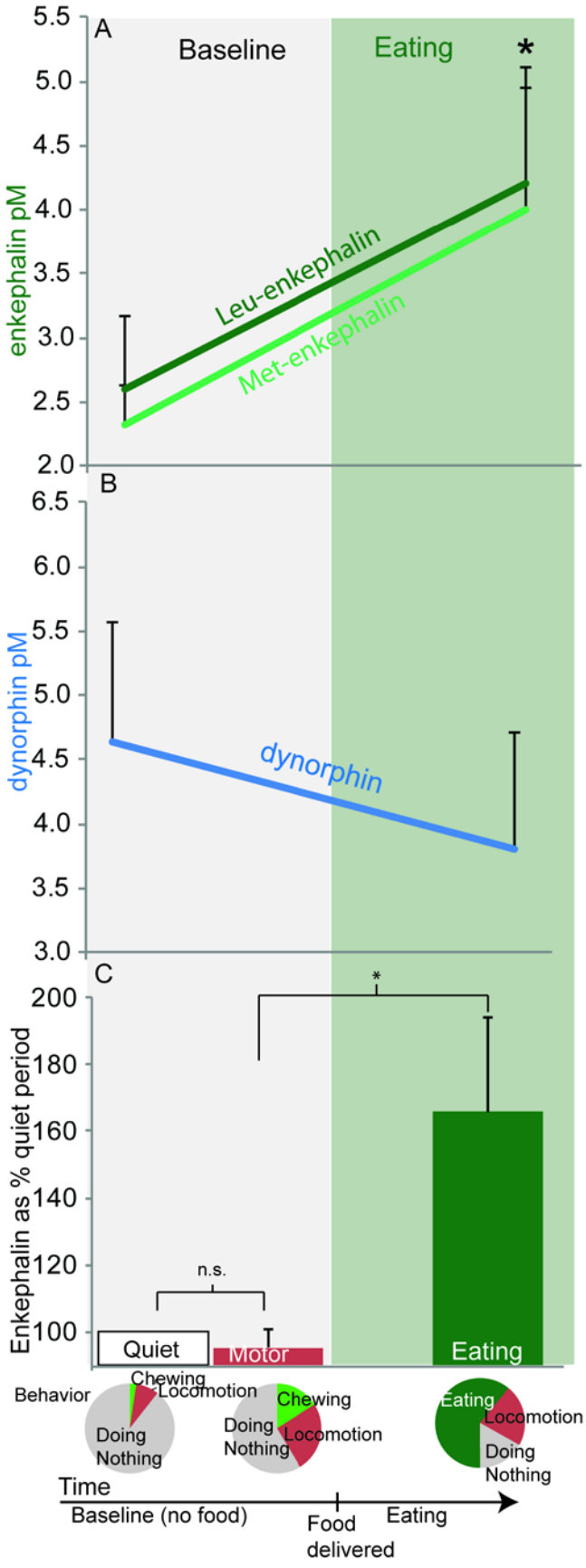
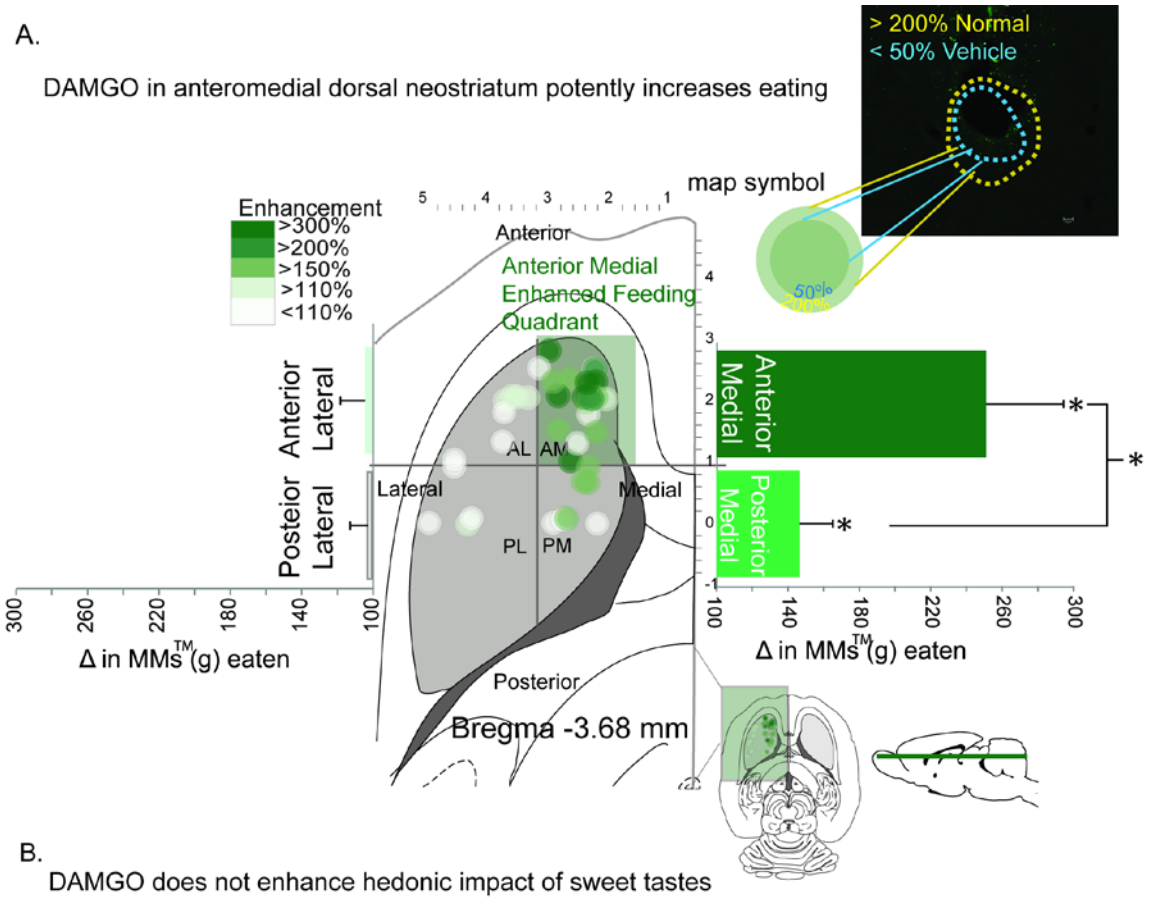

A
Baseline
Eating
*
enkephalin pM
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
Leu-enkephalin
Met-enkephalin
B
dynorphin pM
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
dynorphin
C
*
Enkephalin as % quiet period
200
180
160
140
120
100
n.s.
Quiet
Motor
Eating
Behavior
Chewing
Locomotion
Doing Nothing
Chewing
Doing Nothing
Locomotion
Eating
Locomotion
Doing Nothing
Time
Baseline (no food)
Food delivered
Eating

Figure 4.3. Map of microinjection causation of intense eating. DAMGO microinjection into anterior dorsomedial neostriatum potently enhanced intake of M&M™ chocolate candies (high-fat & high-sugar). All DAMGO-evoked eating behavior and intake changes are expressed as percent increases over vehicle-evoked control levels measured in the same rat (panel A). A two layer "Fos plume" shows the maximal spread of Fos locally surrounding DAMGO microinjections. This measured radius was used to assign the size of symbol to the microinjection site for each behaviorally-tested rat. The color of each symbol depicts the magnitude of eating behavior stimulated by DAMGO microinjection at that site (relative to vehicle control level of the same rat). The largest increases in eating were localized to the anteromedial quadrant of dorsal neostriatum. Taste reactivity results show that DAMGO injected into the same dorsomedial area did not increase hedonic impact of sucrose (during oral infusions) or M&M™ chocolates (during voluntary eating) (panel B). * indicates $p<0.05$. Error bars represent standard error of the mean.


A.
DAMGO in anteromedial dorsal neostriatum potently increases eating
> 200% Normal
< 50% Vehicle
map symbol
50%
Enhancement
>300%
>200%
>150%
>110%
<110%
Anterior
Anterior Medial
Enhanced Feeding
Quadrant
Anterior Lateral
Posteior Lateral
AL AM
PL PM
Lateral
Medial
Anterior Medial
Posterior Medial
Δ in MMs™ (g) eaten
Posterior
Bregma -3.68 mm
B.
DAMGO does not enhance hedonic impact of sweet tastes
Hedonic reactions to sucrose infused into the mouth
Sucrose
n=12
Total Number of Reactions
Veh
DAM
Lateral Tongue Protrusions
Rythmic Tongue
Paw Licking
Total Hedonic Reactions
Hedonic reactions to voluntary chocolate eating
n=8

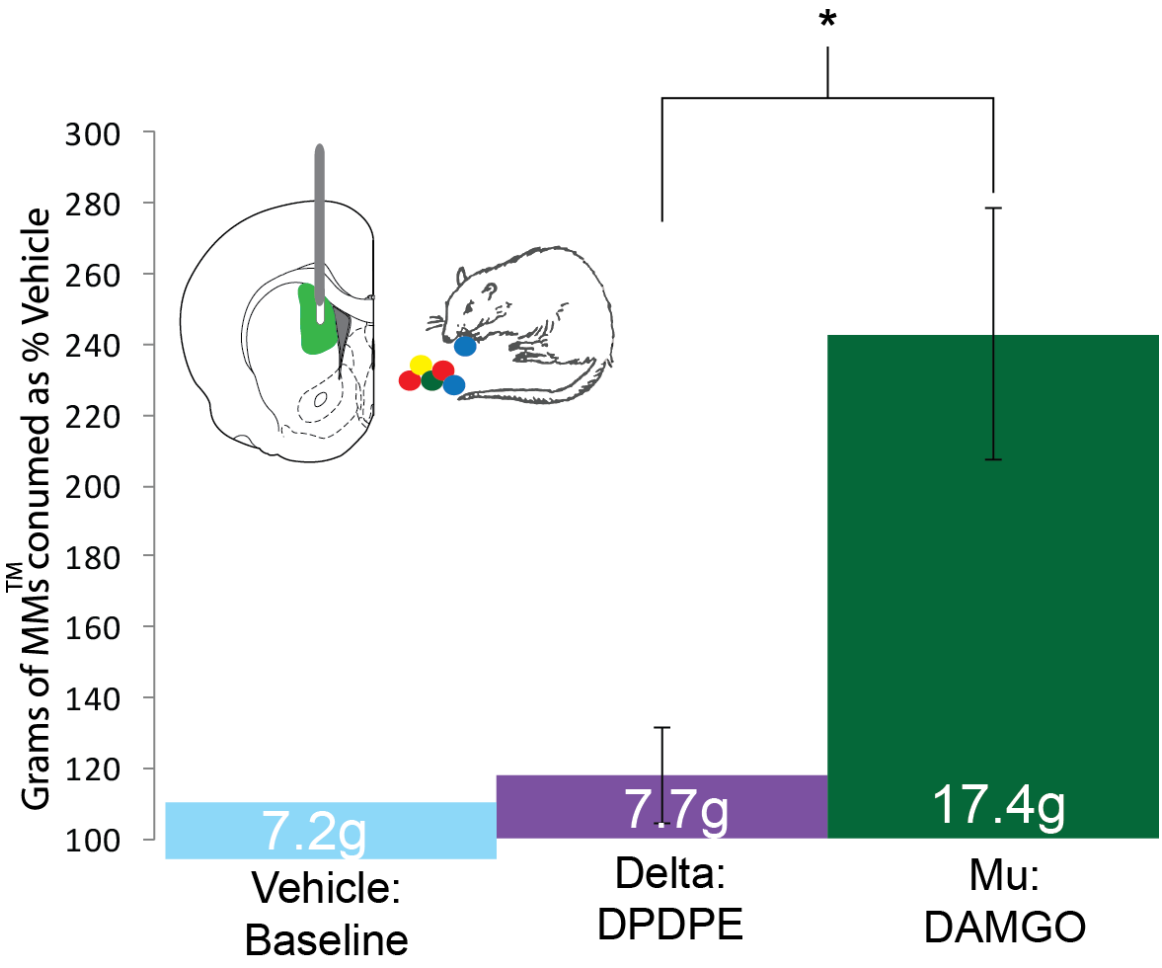


Figure 4.4.Mu but not delta. Mu- but not delta- opioid receptor activation enhances food intake. Microinjection of DAMGO potently enhanced the amount of M&M's consumed while DPDPE microinjection had no effect. Data is presented as percent of amount eaten on vehicle microinjection day, raw average amount eaten is written in bar. Error bars represent standard error of the mean, $p<.05$.

Chapter 5

Disruption of Sign-tracking and Enhancement of Goal-tracking after Mu Opioid Receptor Activation in Ventrolateral Neostriatum

Introduction

The ventrolateral (VLS) portion of striatum receives projections from forelimb and oromotor areas of sensory motor cortex and dopaminergic manipulation of this area often results in oromotor stereotypy (Kelley, Lang et al. 1988; Kelley, Gauthier et al. 1989b; Delfs and Kelley 1990; Dickson, Lang et al. 1994). However, opioid and some dopaminergic manipulations of VLS has been demonstrated to produce intense eating of palatable foods (Kelley, Gauthier et al. 1989a; Bakshi and Kelley 1993b, a; Zhang and Kelley 2000). One interpretation of this increase in consumption is that it is due to an increase in oromotor and forelimb movement that manifests as an increase in consumption (Salamone, Mahan et al. 1993; Cousins, Trevitt et al. 1999; Mayorga, Gianutsos et al. 1999). Another interpretation is that VLS is participating in a wider motivation generating circuitry (Kelley, Baldo and Pratt 2005). Here, I aim to use opioid manipulation of the VLS to test the role of the ventrolateral neostriatum in motivation for food and learned cues.

Often, manipulations that generate consumption of primary rewards also increase motivation for learned cues, but this is not always the case (Bakshi and Kelley 1993b, a; Pecina and Berridge 2000; Zhang and Kelley 2000; Jackson 2009). In previous chapters I have demonstrated that mu opioid receptor activation in dorsomedial neostriatum produces robust increases in eating with no change in motivation for learned cues. In contrast, mu opioid receptor activation in dorsolateral neostriatum produces an intense amplification and focusing of incentive salience on learned cues while producing no increase in motivation for primary rewards. Here we will test if mu opioid receptor activation in ventrolateral neostriatum produces intense motivation for learned cues using an autoshaping paradigm. I will also replicate previous findings that mu opioid receptor activation in this area increases consumption of palatable primary rewards.

Materials and Methods

Subjects.

Sprague Dawley rats (n=15 for autoshaping, n=6 for food intake) weighing 280-350 grams at the start of the experiment were pair housed on a reverse light/dark cycle. Water was provided *ad libitum*; food was provided *ad libitum* except during weeks containing autoshaping training or test sessions, when rats were restricted to 90% free feeding weight and fed about 14gs of standard laboratory chow daily after each training session. Before surgery, all rats received 2-4 10 minute sessions of experimenter handling to acclimate them to being held. All experiments were conducted in accordance with

protocols approved by the University of Michigan Committee on Use and Care of Animals (UCUCA).

Surgery.

All rats were anesthetized with ketamine (80 mg/kg), xylazine (7 mg/kg), and atropine (0.04 mg/kg). To prevent infection, chloramphenicol sodium succinate (60 mg/kg) was administered as well as carprofen (5 mg/kg) to provide pain relief. Carprofen and chloramphenicol were administered again 24 h post-surgery. All rats were allowed 5-7 days to recover from surgery before testing.

Chronic bilateral 14 mm (23 ga) guide cannulae aimed at ventrolateral neostriatum AP≈.6, ML≈3.6, DV≈7.5 based on Paxinos and Watson (Paxinos and Watson 2007). All guide cannula tips were implanted 2 mm above intended target injection site. Cannulae were anchored to the skull with bone screws and acrylic cement. Steel stylets were inserted into guide cannula to prevent occlusion.

Microinjections and drugs

Prior to all tests, steel stylets were removed and cleaned, and 16mm microinjectors were inserted into the guide cannula, pre-measured so that microinjector tips extended 2 mm below guides. Microinjections of [D-Ala^2, N-MePhe^4, Gly-ol]-enkephalin (DAMGO; Sigma) or vehicle (aCSF; Harvard Apparatus) were controlled by a syringe pump which delivered 0.5 µL over 120s. DAMGO was dissolved in aCSF at 973 µM concentration (0.25µg/0.5µL). Microinjector tips were left in cannulae for 1 min following the injection to allow diffusion away from microinjector tips. Each rat received

a “sham” injection 1 day prior to testing of vehicle to habituate them to the microinjection procedure.

Statistical Analysis.

Within subject repeated measures ANOVAs comparing drug and vehicle days were performed on data from autoshaping testing and food intake. All t-test presented were corrected for multiple comparisons using the Bonferroni correction.

Histology.

Rats were sacrificed immediately after the final day of testing by administration of a sodium pentobarbital overdose. Rats were decapitated and the brains were extracted and fixed in 10% paraformaldehyde solution for 1-2 days followed by a 25% sucrose solution in 0.1M NaPB for 2-3 days before slicing. 60 micron slices through the neostriatum were taken from each rat, mounted, dried, and stained with cresyl violet. Microinjection center was determined for each bilateral injection site and slides were compared with the stereotaxic atlas (Paxinos and Watson 2007) to determine placement within neostriatum.

Behavioral Autoshaping Training

All rats received the same autoshaping training procedures as previously described(Mahler and Berridge 2009; DiFeliceantonio and Berridge 2012). In brief, autoshaping training and testing for a particular rat was always carried out in one of eight operant chambers (Med Associates) controlled by Med PC software, containing two retractable levers on opposite sides of a food receptacle. Rats first received one session of magazine training consisting of 20 sucrose pellets being delivered into the food dish.

Pavlovian autoshaping training (CS+ paired with UCS) began started the second day. Training sessions began with illumination of the house lights, followed by insertion presentations of the CS+lever with a light emitting diode on its ventral surface and accompanied by an auditory 2.9KHz tone. Each CS+lever/tone presentation lasted 8s before the lever was retracted back through the wall, which was followed immediately by delivery of one sucrose pellet into the food dish (UCS; Test Diet). Twenty-five CS+ UCS pairs were presented on a 90s variable inter-trial interval schedule during the 40 minute session. A control lever was always present in the chamber.

Training sessions were repeated over 5 consecutive days. By the 3rd training day, every rat began to respond to the CS+ onset with an approach-consummatory CR predominantly focused toward either the CS+lever itself (in which case the rat was classified as a sign-tracker) or toward the CS_{dish} (in which case the rat was classified as a goal-tracker). All rats' prepotent and non-prepotent cues were discernible by day 3. Rats were formally defined as sign-trackers if they approached, nibbled, sniffed, grasped and bit the CS+ Lever at least 2.5 times more frequently than to the goal dish on day 5. Goal-trackers were classed if they made the same responses to the metal dish 2.5 times more frequently than to the lever. Therefore the CS+lever was the prepotent cue for sign-trackers (trigger and target of motivated CR) and temporal trigger for the goal-trackers, whereas the CS_{dish} was the prepotent target cue for goal-trackers (Boakes, Poli et al. 1978; Flagel, Watson et al. 2008; Mahler and Berridge 2009) .

Autoshaping Testing

Testing began on day 7 and continued day 9, when either DAMGO or vehicle was microinjected immediately prior to the autoshaping session (order counter-balanced

across rats; one microinjection per day). One camera was positioned under the transparent floor of the autoshaping chamber to provide a clear view of the rat's entire head and body wherever it was in the chamber. This allowed scoring of both sign-tracking approaches and goal-tracking approaches, as well as scoring of consummatory behaviors in sign-trackers. A second camera was directed from the side toward the inner surface of the CS_{dish} to provide a close up view of the rat's face and mouth movements when inside the dish. A test trial consisted of 25 CS-UCS autoshaping trials identical to trainings. Recordings were scored later offline by the experimenter blind to drug condition.

Behavioral Video Scoring: Autoshaping

Behavior of rats toward CS+ lever and dish were always video recorded from two angles simultaneously through two strategically positioned cameras. One camera was positioned under the transparent floor of the autoshaping chamber to provide a clear view of the rat's entire head and body movements wherever it was in the chamber. A second close-up camera was directed from the side toward the inner surface of the CS_{dish} to provide a detailed view of the rat's face and mouth movements when inside the metal dish. Both videos were analyzed off line in slow motion ($1/10^{th}$ to ½ actual speeds) by an observer blind to experimental conditions. For each trial, the 8 seconds before and 8 seconds during the 5^{th}, 10^{th}, 15^{th}, 20^{th}, and 25^{th} presentation of CS+ were selected for comparison (Mahler and Berridge 2009). Scored behaviors were *look* at the cue (orienting towards the cue by moving the head or forequarters toward it, without bodily approaching it), *approach* the cue, *sniff* the cue (contact of the nose and rhythmic nose flaring movements), and *nibble (contact of mouth or teeth* on lever or dish, combined

with rapid short (<0.5 sec) rhythmic 1-2 Hz bobbing movements of the head), and rhythmic opening and closing movements of jaw, tongue, and/or teeth similar to movements of normal eating of UCS) , and *bite* the cue (of jaw closing and contact by maxillary and mandibular incisors, often while grasping the object with one or both paws, similar to movements that bite the actual UCS sucrose pellet).

Behavioral testing: Food intake

Rats were habituated for 4 days to clear plastic tub cages with ~3 cm of corn cob bedding, 20 g of pre-weighed M&Ms, and 20 g of pre-weighed chow. Water was available through a drinking spout. On the fourth day, rats received a "sham" microinjection. Cages were set up identically for habituation and testing. On test days, rats received DAMGO and vehicle counterbalanced across days with 48 h between each testing session. Rats were microinjected and then immediately placed into plastic tub cages, they were videotaped for 60 min, removed, and all food left in the cage was weighed. Behavioral video tapes were scored at a later date offline.

Behavioral video scoring: Food intake.

Videos were scored by experimenters blind to the experimental condition of each rat. Seconds spent engaging in the following behaviors were recorded: eating M&Ms (actual chewing and consumption), eating chow, drinking, and chewing on non-food items. The following behaviors were recorded as a single event: sniffing M&Ms (anticipatory sniffs and approaches), sniffing chow, grooming, cage crossing, and rearing.

Results

Food intake: DAMGO microinjection potently consumption M&Ms

Consumption of highly palatable M&Ms was greatly increased after DAMGO microinjection. Under vehicle conditions, rats ate 6.5gs of M&Ms per hour session. After DAMGO injection, these same animals consumed around 10.6 grams of M&Ms ($F(1,5)=11.23$, $p=0.02$; Figure 5.1A). Rats also spent 50% more time eating after DAMGO injection ($F(1,5)=6.9$, $p=0.04$). Consumption of chow did not change across drug conditions, the increase in eating was specific to the highly palatable M&M, a replication of previous findings (Zhang and Kelley 2000).

It is important to note that no oral stereotypy was observed during the detailed analysis of the behavioral testing video. Cage crosses, a measure of general locomotor activation where the rat moves from one side of the tub to the other, did not increase (Vehicle = 37, DAMGO = 23; $F(1,5)=2.08$, $p=0.21$). Further, grooming chains, which involve forelimb and oromotor activity did not increase (Vehicle = 6, DDAMGO = 4; $F(1,5) = 2.27$, $p=0.16$)

Food intake: Anatomical specificity

Each placement was mapped onto a coronal slice and is shown on a representative slice here (Figure 5.1B). Placements contained within the ventrolateral striatum but above the accumbens shell were included in analysis. Based on previous fos plume sizes (0.2mm radius maximum) reported in this dissertation and previous reports, it is unlikely that the increase in consumption observed was due to drug diffusion to the accumbens (Zhang and Kelley 2000; DiFeliceantonio, Mabrouk et al. 2012).

Autoshaping: Summary of results

Sign- and goal-tracking responses can be broken down into two categories, approach to the cue (probability and latency) and the appetitive-consummatory actions directed at the cue when the animal arrives (grasps, nibbles and sniffs). After DAMGO microinjection, goal-trackers did not display any changes in either approach or appetitive actions. Sign-trackers on the other hand demonstrated increased goal-tracking approach and appetitive actions towards the CS_{dish} and a decrease in their approach and appetitive actions directed at their preferred cue, the CS+lever.

Goal-tracker's Autoshaping: DAMGO microinjection in VLS does not enhance goal-tracking.

Those animals classified as goal-trackers on the fifth and final day of training demonstrated a strong cue-locked increase in nibbles and sniffs directed at the CS_{dish} ($F(1,5) = 61.23$, $p=.001$). On average goal-trackers nibbled and sniffed their Cs_{dish} 1.9 times per 8 seconds in the absence of the CS+lever and 7.8 times in its presence. DAMGO microinjection, however, did not cause a significant increase in goal-tracking. Each rat's probability to approach the CS_{dish} remained stable at about 96% under both vehicle and drug conditions ($p>0.05$) and the probability to approach the CS+lever also remained stable across drug conditions at around 55% ($p>0.05$). In addition, the proportion of total responses directed at the CS_{dish} and CS+lever did not change after DAMGO microinjection. Under both DAMGO and vehicle conditions, rats directed 78.5% of appetitive consummatory behaviors at CS_{dish}, and 21.5% of appetitive consummatory behaviors at the CS+lever. There was a slight decrease in the latency to contact the CS_{dish}

after CS+lever presentation. Under vehicle conditions, rats were very fast, taking just 1.49 seconds to reach their dish, under DAMGO conditions, they were even faster, taking just 1.24 seconds to reach the dish ($p>0.05$).

Sign-tracker's Autoshaping: Sign-trackers shift to goal-tracking

The most dramatic effect of DAMGO microinjection was observed in sign-tracking animals. Sign-trackers showed a shift towards goal-tracing after DAMGO microinjection. Under vehicle conditions sign-trackers approached the CS_{dish} on 40% of trials, after DAMGO the percent of trials they approached the CS_{dish} doubled to 80% ($F(1,8)=12$, $p=0.009$). To confirm this enhancement in approach we used a method of calculation devised by Robinson and colleagues (Meyer, Lovic et al. 2012). This confirmed the approach enhancement as a doubling in probability to approach to the CS_{dish} after DAMGO microinjection (Vehicle = 0.40, DAMGO = 0.80; $F(1,8)= 12$, $p=0.009$). The CS+lever on the other hand, appeared to lose some of its attractive value. Sign-trackers decreased the proportion of approaches to the CS+lever from 100% to 95% ($p>0.05$) and approached the CS+lever slower (vehicle = 0.58s, DAMGO = 1.87s, $F(1,8)=5.95$, $p=.041$) after DAMGO microinjection.

Appetitive-consummatory behaviors directed at the CS_{dish} also increased dramatically after DAMGO microinjection. Under vehicle conditions the CS_{dish} was nearly ignored by sign-trackers during the lever presentation, with just 0.4 average nibbles and sniffs directed at it per 8s cue period. After DAMGO microinjection sign-trackers nearly quadrupled the number of nibbles and sniffs on the CS_{dish} to an average 1.5 per 8s cue period ($F(1,8)=7.85$, $p=0.02$). This increase in food dish nibbles and sniffs

was locked to the presentation of the CS+lever. Food dish nibbles and sniffs did not increase after DAMGO in the inter-trial interval (Vehicle = 0.92, DAMGO = 1.0, DRUG*CUE interaction $F(1,8)=7.85$, $p=0.02$). So, DAMGO microinjection was not in general making the food dish more attractive in a continuous fashion in the absence of the lever cue, but instead selectively enhancing goal-tracking behavior triggered by the cue during the CS period. To further analyze this shift to goal-tracking calculated what percent of total behaviors were made to each cue during the 8s period. After DAMGO microinjection the percent of total nibbles, sniffs, and bites on the dish increased from 4% for sign-trackers under vehicle conditions to 18%, an over fourfold increase ($F(1,8)=2.66$, $p=0.028$). DAMGO microinjection shifted the proportion of appetitive behaviors towards the $CS_{dish.}$ However, the same numbers show that sign-trackers still remained predominantly sign-tracking, showing a quantitative but not a qualitative shift to goal tracking. After DAMGO microinjection, sign-trackers still directed 82% of all appetitive-consummatory behaviors to their preferred CS+lever. Overall the effect was a biasing of behavior rather than transforming the phenotype of the sign-trackers to goal-trackers.

It was not the case that the decrease in sign-tracking reported was a result of an overall decreased all appetitive behaviors. Under vehicle conditions rats made about 8 appetitive consummatory responses per 8s CS period, after DAMGO rats made about 9 responses ($p>0.05$). So, although a decrease in sign-tracking may be interpreted by some as a decrease in overall incentive salience, this did not appear to be the case. Rats were still energized by the CS+lever, but they directed a portion of their responding to a new target, the CS_{dish}.

Autoshaping: Anatomical specificity

Each rat's microinjection placements were mapped on coronal slices and are shown here on a representative coronal slice (Figure 5.4). Placements for both sign- and goal-trackers were contained within the ventrolateral neostriatum. Each injection was within the ventral 30% of the neostriatum as a whole. This means the difference in the effects of DAMGO observed are not likely to be due to variation in microinjection site. Based on maximum Fos plume size for this same microinjection volume and concentration, it is unlikely that the results observed are due to diffusion to the accumbens shell (DiFeliceantonio, Mabrouk et al. 2012).

Discussion

Here, I have demonstrated that mu opioid activation in ventrolateral neostriatum potently enhances consumption of highly palatable foods, as previously reported (Zhang and Kelley 2000). The novel result of this study was that mu opioid receptor activation via DAMGO microinjection into the ventrolateral neostriatum shifted sign-tracking rat's behavior to display more goal-tracking behavior. Rats that already predominately goal-tracked did show significant changes in their behavior after microinjection. Therefore, mu opioid receptor activation in ventrolateral neostriatum seemed to impact the behavior of the sign-trackers exclusively.

Sign-trackers may be unique in attributing incentive salience primarily to the CS+lever. One possibility is that DAMGO reduces "wanting" and helps them revert to a more rational conditioned response (CR) elicited by a food pellet (Flagel, Watson et al. 2007; Flagel, Clark et al. 2011). However, mu opioid receptor activation enhanced food

consumption, suggesting at least a UCS enhancement of "wanting" if not a CS enhancement (Jackson 2009). An alternate explanation is that DAMGO microinjection has shifted the *target* of some of the incentive salience for sign trackers from the CS+lever to the CS_{dish}. As discussed in chapter 2, both sign- and goal-trackers can be interpreted as attributing incentive salience, just to different targets (DiFeliceantonio and Berridge 2012). For goal-trackers the target is the CS_{dish} and for sign-trackers the target is the CS+lever. Although the food cup is present at all times, it is not attributed with incentive salience until the *trigger* is present, the CS+lever. Both sign- and goal-trackers show phasic increases in incentive salience time locked to the presentation of the trigger. According to this hypothesis, mu opioid receptor activation in ventrolateral neostriatum enhanced the incentive salience of the CS_{dish} for sign-trackers, leading it to compete with the CS+lever.

Some evidence for this hypothesis is that overall levels of responding did not decrease for sign- or goal-trackers. If incentive salience was generally decreased, you may expect an overall decrease in approach and appetitive consummatory behaviors after DAMGO microinjection. These total numbers remained stable, but where they were directed was shifted towards the CS_{dish} for the sign-trackers. This shift was not absolute, as can be seen in figure 5.3, sign-trackers did not become solely goal-trackers. The amount of shift seems consistent with the idea that each cue has an amount of incentive salience attributed to it that is computed on line at each cue presentation (Zhang, Berridge et al. 2009). Here, DAMGO microinjection increased the amount of incentive salience attributed to the CS_{dish} allowing it to compete with the CS+lever and drawing appetitive consummatory behaviors away from the lever and to the CS_{dish}.

Other studies have reported a similar shift to goal-tracking after sensitization with drugs such as amphetamine and cocaine (Simon, Mendez et al. 2009; Holden and Peoples 2010) (but see also (Doremus-Fitzwater and Spear 2011)). Conceptually, sensitization should enhance incentive salience and accordingly the authors of these studies predicted sign-tracking would be increased. Instead, approach to the contiguous, proximal CS was enhanced, leading to the interpretation that contiguity could be important for incentive salience attributing.

Contiguity of CS with UCS may become even more important for incentive salience attribution when mesocorticolimbic circuits are pharmacologically stimulated (Tindell, Berridge et al. 2005; Smith, Berridge et al. 2011). For example, previous studies reported that opioid or dopamine stimulation of the nucleus accumbens selectively enhanced incentive salience of a UCS-contiguous CS2 stimulus, but not of a UCS-predictive CS1. Contiguity dominance applied to all rats when tested in a Pavlovian CS-CS-UCS series, in which a UCS-predictive CS1 was followed by a UCS-contiguous CS2, (Tindell, Berridge et al. 2005; Smith, Berridge et al. 2011).

Overall, these findings are not consistent with the view that enhancements in consumption after VLS manipulation are due to impairment of oromotor function (Salamone, Mahan et al. 1993; Cousins, Trevitt et al. 1999). A motor impairment hypothesis would predict overall deficits in responding in both sign-trackers and goal-trackers. Here, no overall changes in total amount of behavior were observed. In addition, no gross motor impairments were noted in a detailed analysis of video recordings for all tests. It appears instead that mu opioid receptor activation in VLS plays a specific role in

enhancing motivation for primary rewards and reward cues paired closest in time and space with those primary rewards.

This interpretation has interesting implications, because it means that mu opioid receptor activation in ventrolateral neostriatum selectively enhances the incentive salience of a proximal, contiguous cue over a distal, more predictive cue. CS-UCS contiguity has long been recognized as important to facilitating Pavlovian associations (Zener 1937; Mackintosh 1974; Rescorla and Cunningham 1979). Further, a proximal action is closely regulated by satiety state, but a more distal action is only changed after an experience with the sucrose reinforcer in a sated state, requiring a cognitive memory (Corbit and Balleine 2003). This means proximal, contiguous cues may gain incentive salience through a separable system that is closely tied to physiological state and may contain the ventrolateral neostriatum.

Figures

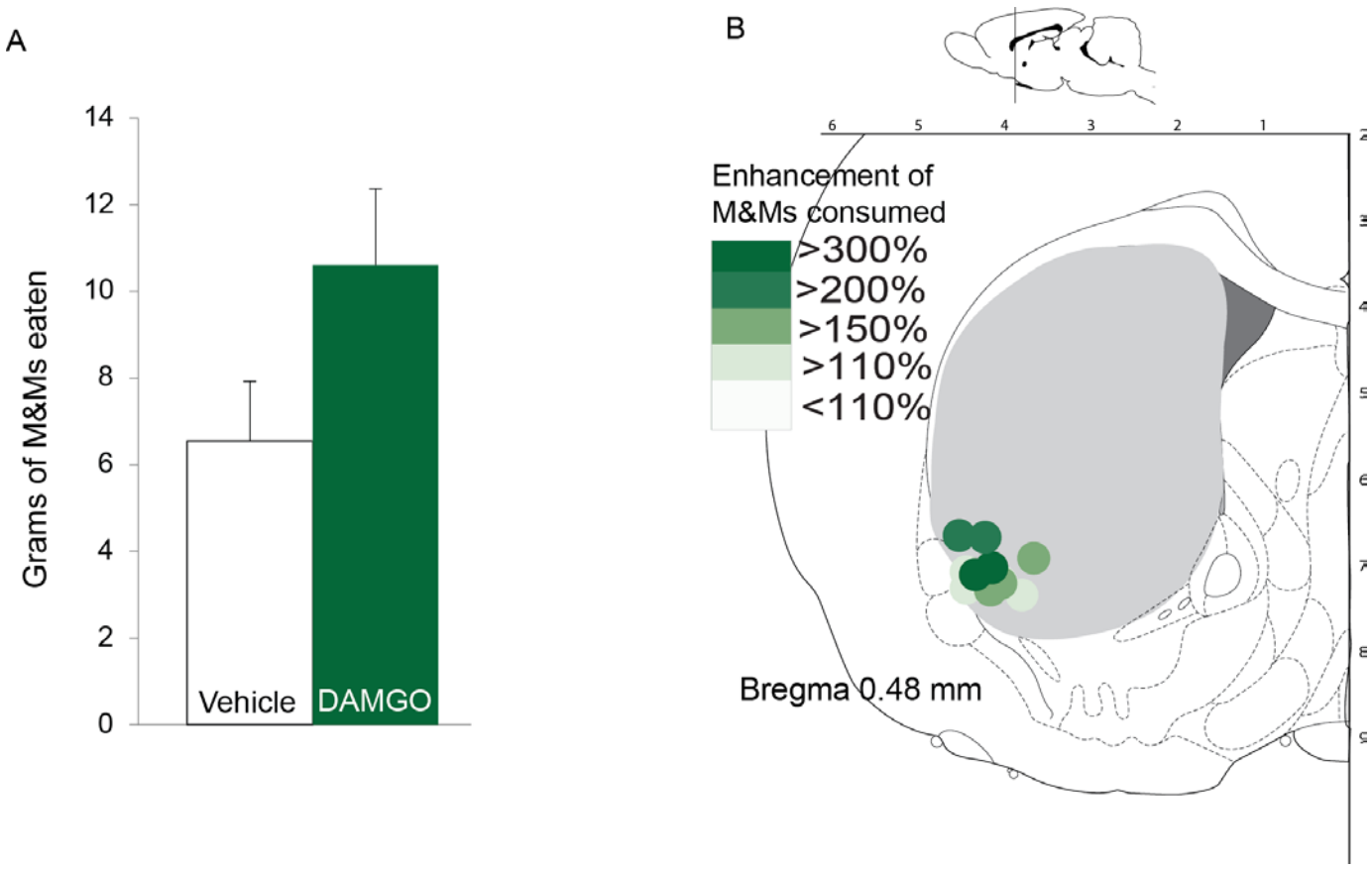


Figure 5.1. DAMGO potently enhances M&M consumption in ventrolateral neostriatum. DAMGO microinjection to ventrolateral neostriatum potently enhanced grams of M&Ms consumed (Panel A). Panel B displays the anatomical location of all rats. Intensity of eating enhancement is mapped as increasing intensity of green color.

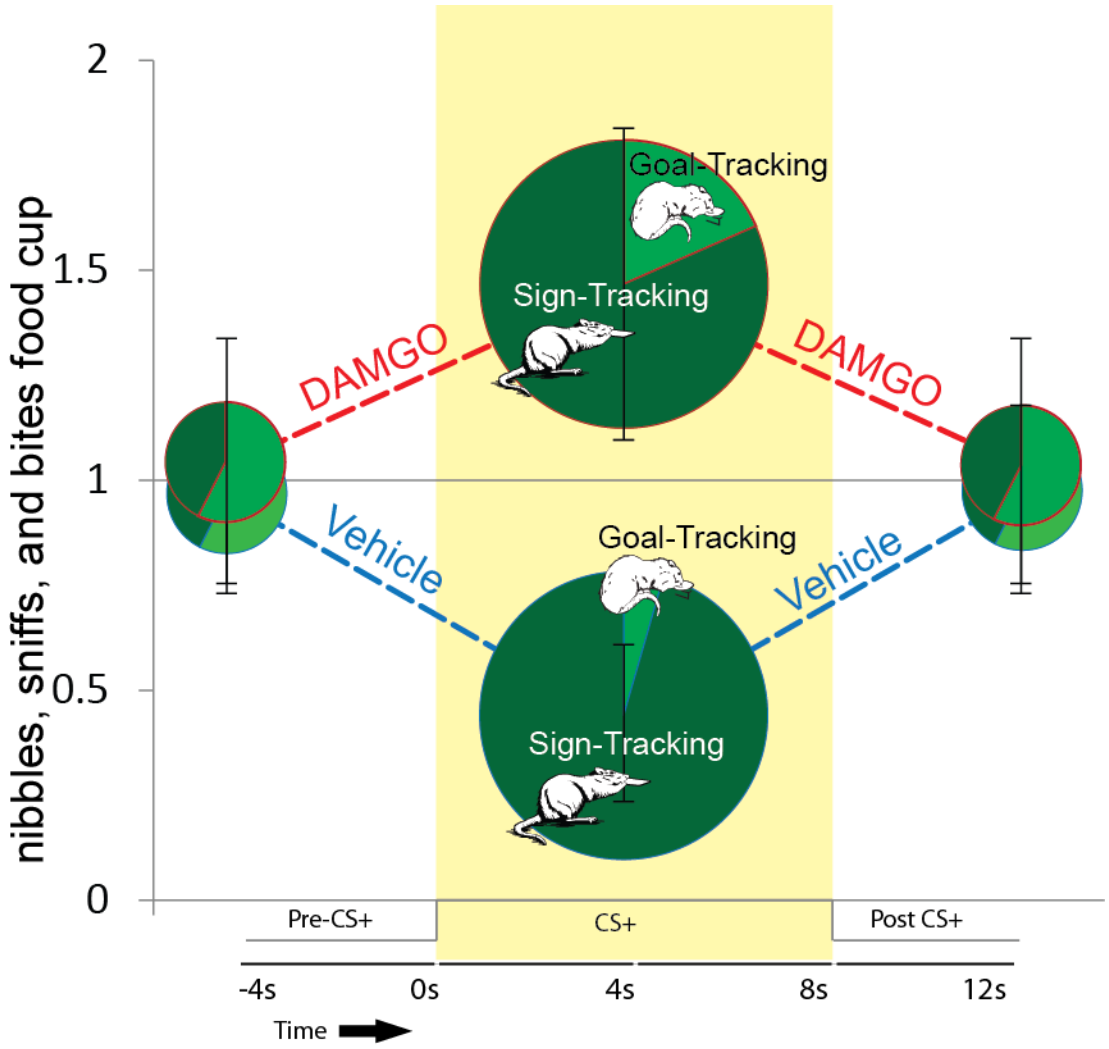


Figure 5.2. DAMGO enhances appetitive behaviors directed at the food cup in a time-locked manner. In the absence of the CS+lever, sign-tracking animals nibble, sniff, and bite the food cup at similar levels under both DAMGO and vehicle conditions. Under vehicle conditions and during the CS+ period, sign-trackers rarely interact with the food cup (line graph) and allocate a small percentage of their responding to the food cup (pie chart). After DAMGO injection and during the cue period, sign-trackers greatly increase the number of appetitive consummatory behaviors they direct at the food cup (line graph) and the proportion of overall appetitive behaviors directed at the food cup increases (pie chart).

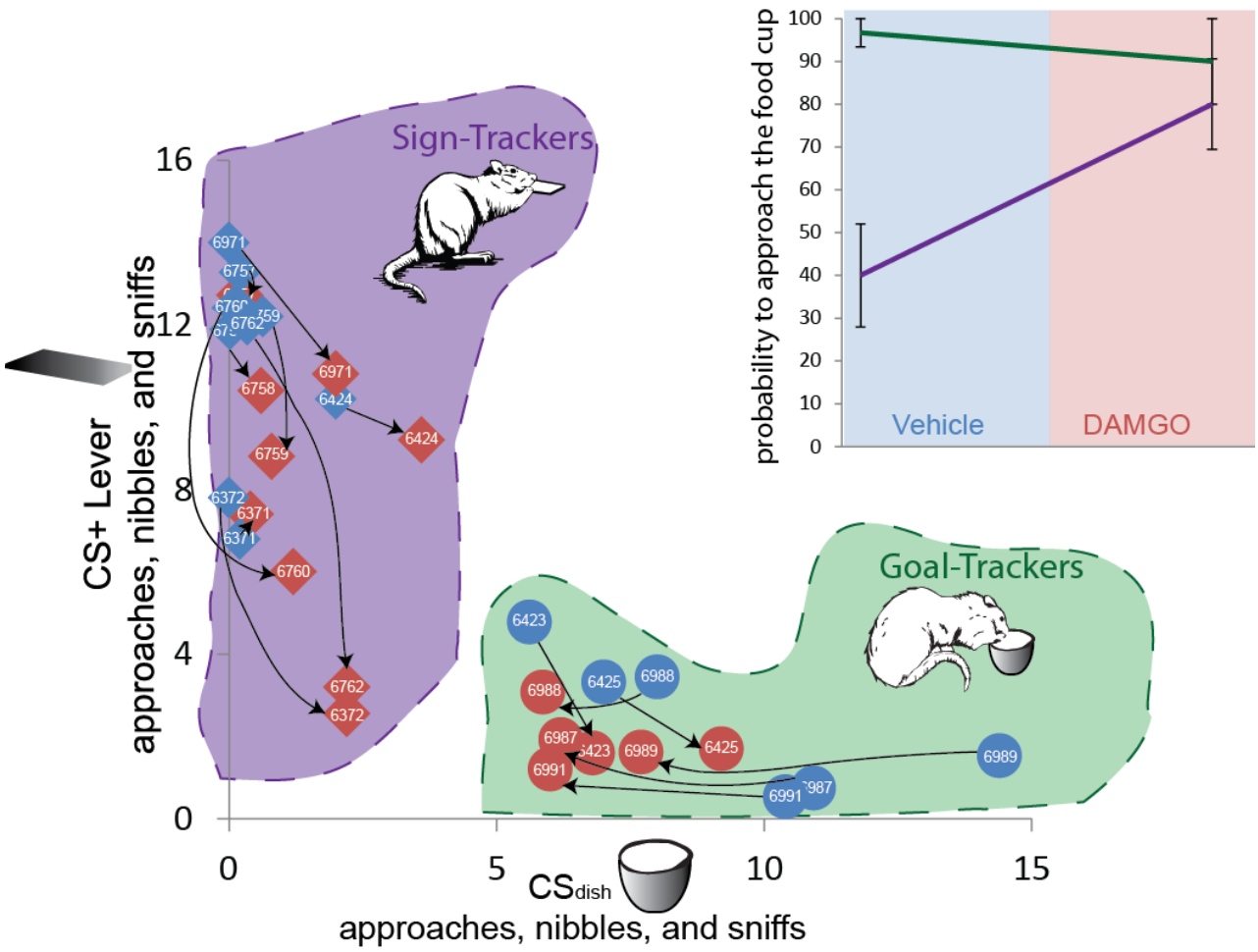


Figure 5.3. Individual Variation. DAMGO microinjection into the ventrolateral neostriatum shifts sign-trackers to engage in more goal-tracking. Sign-trackers (diamonds) show more approaches, nibbles, and sniffs to the $CS_{dish,}$ shown here as a rightward shift. Goal-trackers do not significantly shift behavior. Inset: DAMGO microinjection doubles the probability that sign-trackers will approach the CS_{dish}.

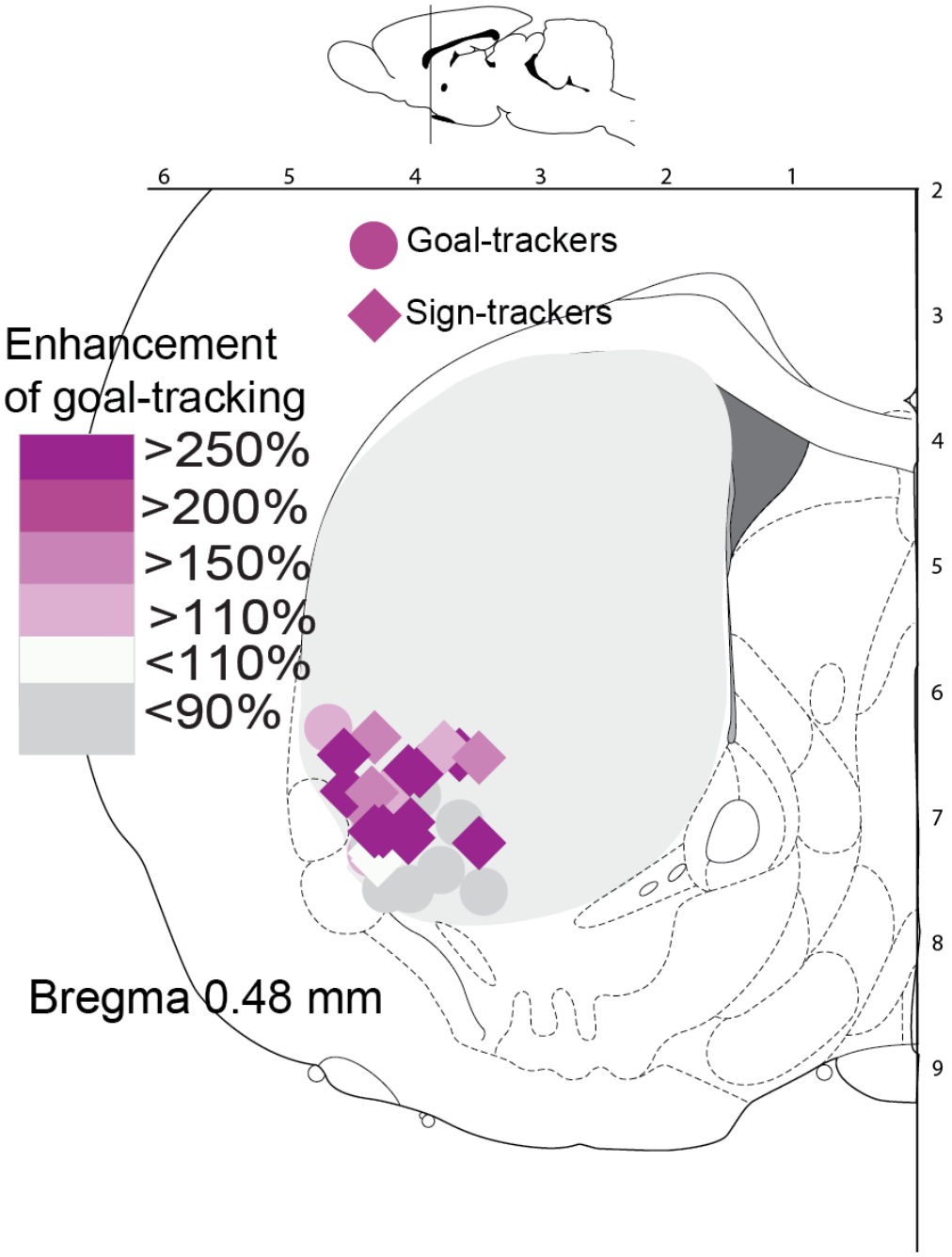


Figure 5.4. Microinjection sites within ventrolateral neostriatum. All microinjections included in the analysis were contained within the ventrolateral neostriatum Sign-trackers are represented as diamonds and goal-trackers as circles. Enhancement of goal-tracking by DAMGO microinjection is represented as percent vehicle and color coded with increasing pink hue indicating more goal-tracking.

Chapter 6

General Discussion

The experiments described in this dissertation demonstrate that central nucleus of the amygdala and dorsal neostriatum can amplify incentive motivation for natural rewards and learned cues.

General summary of findings

In chapter 2, I assessed whether central amygdala mu opioid receptor stimulation enhances the phasic incentive salience of the goal-cue for goal-trackers during moments of predictive cue presence (expressed in both approach and consummatory behaviors to goal cue), just as it enhances the attractiveness of the predictive cue target for sign-trackers. Using detailed video analysis I measured the approaches, nibbles, sniffs, and bites directed at their preferred target for both sign-trackers and goal-trackers. I reported here that DAMGO microinjections in central amygdala made goal-trackers, like sign-trackers, show phasic increases in appetitive nibbles and sniffs directed at the goal-cue expressed selectively whenever the predictive cue was present. This indicates enhancement of incentive salience attributed by both goal trackers and sign-trackers, but attributed in different directions: each to their own target cue. For both phenotypes, amygdala opioid stimulation makes the individual's prepotent cue into a stronger motivational magnet at phasic moments triggered by a CS that predicts the reward UCS.

Building on these findings in Chapter 3, I explored the function of the dorsolateral neostriatum (DLS), a neostriatal area functionally connected with the CeA (Gonzales and Chesselet 1990; Faure, Haberland et al. 2005; Lingawi and Balleine 2012). I microinjected the mu opioid receptor agonist DAMGO into DLS and reported an increase in intensity and focus of sign- and goal-tracking behavior. After DAMGO microinjection both sign- and goal-trackers approached their target cue more exclusively and expressed more intense appetitive behaviors.

To explore whether this enhancement was mediated by activation of habitual S-R response patterns, or whether it was due to the enhanced incentive motivation of the target cues, we altered 2 aspects of our paradigm known to reveal S-R responding, the environment and the value of the reinforcer (Carr and Watson 1908; Dickinson and Balleine 1990). When the CS+lever suddenly appeared out of the back wall of the operant chamber, a place it had never appeared before, rats abandoned their trained motor ritual and using a new motor pattern, followed the CS+lever to its new location. DAMGO microinjection did not inhibit this switch by enhancing the motor habit; in contrast DAMGO microinjection slightly facilitated this shift. To determine if following the lever to a location was in fact the S-R association (S = lever presentation, R = approach and follow), rather than the motor ritual, we devalued the sucrose pellet and presented the CS+lever in a new location. Rats that were fed to satiety on sucrose pellets before testing (in extinction) did not follow the CS+lever to its new location and rats that only received time in the operant chamber, but no food, followed the CS+lever (as was observed previously). So, the CS+lever's value and the value of the following response were dependent on the value of the UCS sucrose pellet, indicating the following response was

not habitual in nature. Thus, it can be concluded that the enhancement of sign- and goal-tracking by DAMGO was not due to activation of previous motor action patters or learned S-R associations, but rather to the dynamic enhancement of the incentive salience properties of the target cue.

Finally, we sought to test if DAMGO microinjection also enhanced the conditioned reinforcing properties of the CS+lever for sign-trackers. In this paradigm animals learned an entirely novel nose poke response. Responding in the active port was rewarded with a 2 second CS+lever presentation while responding in the inactive nose port produced nothing and was used as a motor control measure. After DAMGO microinjection, rats responded significantly more in the active port (responding on the inactive port did not change), this demonstrates that DAMGO activation of DLS increased the conditioned reinforcing properties of the CS+lever.

The enhancement of incentive salience attributed to reward cues after DAMGO microinjection in dorsolateral neostriatum was neuroanatomically unique within the neostriatum. Microinjections of DAMGO to the dorsomedial neostriatum did not enhance the incentive salience of learned cues, nor did it enhance the conditioned reinforcing properties of the CS+lever. This demonstrates a functional dichotomy between the two nearby regions where dorsomedial neostriatum is important for amplifying incentive salience for natural UCS rewards and dorsolateral neostriatum participates in amplifying incentive salience attributed to preferred learned CSs.

To explore the neurochemical specificity of this enhancement of incentive salience attributed to the preferred target cue, I injected a low dose of amphetamine into the DLS before autoshaping testing. The effects of this treatment were not similar to

those of DAMGO. After amphetamine microinjection, purely sign- and goal-tracking rats did not demonstrate an increase in responding. Those rats that were mainly sign-trackers, but showed some goal-tracking greatly increased the amount of goal-tracking they performed following amphetamine microinjection. These results are novel and indicate enhanced dopamine transmission in DLS may bias behavior towards more proximal, contiguous cues rather than more distal, predictive cues.

In chapter 3, I demonstrated that activation of mu opioid receptors within the dorsomedial neostriatum did not increase incentive motivation for learned cues. In chapter 4, I explored the role of this region in generation of motivation for primary rewards, namely palatable M&M candies. First, I investigated endogenous opioid release in dorsal neostriatum during spontaneous eating of a palatable sweet food using microdialysis. Samples were analyzed using liquid chromatography coupled with mass spectrometry (MS^3). I observed a robust increase in extracellular enkephalin, but not dynorphin, at the onset of the palatable meal, which decreased as rats stopped eating. This surge was unique to the period of time rats were consuming M&Ms. Periods of intense oromotor and forelimb movement did not produce the same enkephalin surge.

To determine if this enkephalin surge caused intense motivation to consume palatable foods, I stimulated mu opioid receptors via DAMGO microinjection in dorsomedial neostriatum and observed that rats doubled their intake of a sweet palatable food. This effect was confined anatomically to the anteromedial quadrant of dorsal neostriatum. Delta opioid receptor stimulation did not cause a similar effect. Using the taste reactivity test, I demonstrated that mu opioid receptor activation did not enhance the 'liking' or hedonic impact of the sweetness or the M&Ms themselves. Together these

results indicate a role for enkephalin and mu opioid receptors in dorsomedial neostriatum, in the generation of motivation to consume sensory rewards.

In chapter 5, I tested the ventrolateral neostriatum, an area that had previously been demonstrated to generate increases in consumption of palatable food, but is also implicated in forelimb motor function, making it difficult to separate motor function from motivation. In this chapter I replicated the findings that DAMGO microinjection to VLS enhances consumption of palatable foods. After DAMGO microinjection, rats ate about 180% vehicle levels of M&Ms. When DAMGO was injected in the autoshaping paradigm, it increased goal-tracking in sign-trackers. Sign-trackers doubled their approaches to the CS_{dish} and greatly increased the appetitive behaviors directed at the CS_{dish}. In contrast, goal-trackers did not shift to sign-trackers or goal-track more. It appears the DAMGO microinjection shifted the target of incentive salience from the distal, predictive lever, to the contiguous, proximal goal. This shift was not total, they did not become goal-trackers, but the CS_{dish} was now attributed with enough incentive salience to compete with the CS+lever.

General discussion of CS trigger versus CS target roles.

A continuing puzzle is why target and trigger are separate objects for goal-trackers, but not for sign-trackers. For sign-trackers, the situation is most intuitive. The CS+ Lever presentation is always the most *predictive* event for reward delivery, being correlated in event probability with the UCS. Each CS+ (lever insertion and sound) was followed 8 sec later by a UCS, and the UCS never occurred without being preceded by

the CS+. This predictive relationship makes the CS+lever the trigger (and at least for sign-trackers, also the target).

By comparison, the CS_{dish} was less informatively predictive, being always present and therefore associated both with UCS and with its absence. However, the CS_{dish} was the Pavlovian CS with closest spatial and temporal contiguity to the UCS. That is, the dish was always the last thing the rat saw or felt before tasting sucrose reward, because the rat's head was always inserted into that dish at the moment of pellet ingestion. This dish-in-the-face as stimulus complex was paired almost simultaneously with the hedonic taste of sucrose. CS-UCS contiguity has long been recognized as important to facilitating Pavlovian associations, and contiguity may remain important in controlling the target for goal-trackers even when contingency dominates the associative correlation that generates r_t as a phasic prediction from the trigger (Zener 1937; Mackintosh 1974; Rescorla and Cunningham 1979; Wassum, Cely et al. 2009).

Contiguity of CS with UCS may become even more important for incentive salience attribution when mesocorticolimbic circuits are pharmacologically stimulated (Tindell, Berridge et al. 2005; Smith, Berridge et al. 2011). For example, previous studies reported that opioid or dopamine stimulation of the nucleus accumbens selectively enhanced incentive salience of a UCS-contiguous CS2 stimulus, but not of a UCS-predictive CS1. Contiguity dominance applied to all rats when tested in a Pavlovian CS-CS-UCS series, in which a UCS-predictive CS1 was followed by a UCS-contiguous CS2, (Tindell, Berridge et al. 2005; Smith, Berridge et al. 2011). Here, DAMGO in CeA and DLS of goal-tracking individuals selectively enhanced attribution of incentive salience to

that contiguous CS_{dish}, target alone, just as it enhanced the attributions by sign-trackers to their own prepotent cue, the predictive CS+ Lever.

In contrast, activation of dopamine receptors indirectly through microinjection of amphetamine only enhanced the incentive salience to the contiguous CS_{dish}. Previous studies using amphetamine or cocaine systemically have demonstrated a shift from sign- to goal-tracking after injection (Simon, Mendez et al. 2009; Holden and Peoples 2010). This is not exactly what was observed in the present study, however. Those rats that did not goal-track at all during training were not transformed into goal-trackers. Only those rats that had attributed some incentive salience to the CS_{dish} during training demonstrated an enhancement in goal-tracking after amphetamine injection. So, it appears that amphetamine microinjection to DLS is not enough to entirely shift the target of incentive salience to the CS_{dish}, but instead is able to enhance the attractive power of the contiguous CS_{dish} only if it had any to begin with.

Anatomical connectivity of extended amygdala with neostriatum

How can manipulations of activity in the central nucleus of the amygdala and the dorsolateral striatum produce similar enhancements in focus and selectivity? More generally how does the extended amygdala interact anatomically with the striatum?

CeA has been suggested to be embedded within the larger extended amygdala macrosystem (Swanson and Petrovich 1998; de Olmos and Heimer 1999; Alheid 2003; Heimer, Van Hosen et al. 2008), the lateral (or central) division of which begins in CeA and connects to the bed nucleus of stria terminalis (BNST), sublenticular extended amygdala (SLEA) and interstitial posterior limb of the anterior commissure (IPAC) (Zahm 2006). The extended amygdala system shares special features with caudal portions

of the medial shell of the nucleus accumbens (Reynolds and Zahm 2005; Heimer, Van Hosen et al. 2008). The CeA also can be viewed in light of macrocircuit concepts described by Swanson (Swanson 2005; Heimer, Van Hosen et al. 2008), in which CeA is a striatal-level component (GABAergic), receiving inputs from the basolateral nucleus of amygdala (BLA) as a cortical-level component (glutamatergic), and sending outputs to BNST, SLEA and IPAC as pallidal-level components (GABAergic). This striatal-level status may be especially noteworthy for CeA's status as an incentive salience generator, in that several other striatal-level structures also can generate intense enhancements of incentive salience when neurochemically stimulated, including the ventral striatum and portions of the neostriatum as described here (Wyvell and Berridge 2000; Pecina and Berridge 2008; DiFeliceantonio, Mabrouk et al. 2012). This allows us to group these structures anatomically and psychologically as striatal incentive salience generators.

Interestingly, the CeA can also interact with the DLS through the substantia nigra. The CeA is functionally and anatomically connected to the substantia nigra pars compacta (Gonzales and Chesselet 1990; Rouillard and Freeman 1995). Specifically, the CeA projects to the same region of the compacta that then projects to the dorsolateral neostriatum (Gonzales and Chesselet 1990; Faure, Haberland et al. 2005; Lingawi and Balleine 2012). So, change in activity at either end (CeA or DLS) could be tuning part of the same circuit that directs and amplifies incentive salience.

The major input structure to the CeA within the extended amygdala construct is the BLA (Alheid 2003). The BLA itself receives cortical input from prelimbic, infralimbic, and orbitofrontal cortices (Grace and Rosenkranz 2002; Rosenkranz and Grace 2002; Wassum, Tolosa et al. 2012). So, through the BLA, the CeA receives similar

cortical information as does the striosome/patch compartment within the dorsal neostriatum (Figure 6.1).

Opioid neurotransmission in CeA and dorsal neostriatum appears to be especially important to dynamic amplification and focusing of incentive salience that makes a Pavlovian cue into a motivational magnet. Endogenously, CeA neurons receive mu opioid stimulation from local enkephalin neurons of amygdala and from β-endorphin axons projecting from the hypothalamic arcuate nucleus (Jackson and Berridge 2008; Poulin, Castonguay-Lebel et al. 2008; Le Merrer, Becker et al. 2009). DAMGO microinjection in CeA may mimic such endogenous opioid sources, increasing FOS gene transcription in CeA neurons (Mahler and Berridge 2009). Opioid stimulation may promote GABAergic disinhibition of output structures (Morris and Dolan 2001; Zhu and Pan 2004), to modulate and stimulate mesocorticolimbic dopamine circuits, via indirect projections such as to the lateral hypothalamus and peduncular pontine nucleus which in turn project to VTA (Gonzales and Chesselet 1990; Gauthier, Parent et al. 1999; Zahm 1999; Heimer, Van Hosen et al. 2008; Day, Jones et al.). Here, DAMGO microinjections into CeA may well have potentiated mesolimbic dopamine circuits to nucleus accumbens as a step in amplifying the intense bouts of incentive salience observed in appetitive-consummatory behavior (Wyvell and Berridge 2000; Jackson 2009; Smith, Berridge et al. 2011).

Within the dorsal neostriatum, enkephalin in released from dopamine D2 receptor expressing neurons in the matrix. In dorsal neostriatum, mu opioid receptors are localized mainly in “patch” or “striosome” compartments (Pert, Kuhar et al. 1976; Herkenham and Pert 1980; Gerfen 1984; Crittenden and Graybiel 2011). Patches or striosomes in

neostriatum receive converging inputs from limbic regions of prefrontal cortex, including from orbitofrontal, prelimbic, and anterior cingulate regions (Ragsdale and Graybiel 1988, 1990; Eblen and Graybiel 1995; Kincaid and Wilson 1996; Levesque and Parent 1998; Graybiel 2008; Crittenden and Graybiel 2011), again similar to those that converge on the BLA and in turn the CeA (Figure 6.1).

Cortical inputs are also compartmentalized according to layer, with "deep" layers projecting preferentially to patch and superficial layers projecting preferentially to matrix (Wilson 1987; Gerfen 1989). Further, although these distinctions hold true for dorsal neostriatum, they start to break down along the dorsoventral axis where areas that did project solely to patch begin to project also to the matrix (Gerfen 1984; Eblen and Graybiel 1995; Kincaid and Wilson 1996; Levesque and Parent 1998).

So, cortical afferents seem to project preferentially to one compartment over the other, at least most clearly in dorsal neostriatum. Cortical projecting neurons can also be categorized into one of two projection types: 1) intra-telencephalic-projecting (IT-type) and 2) pyramidal tract-projecting (PT-type) (Reiner, Jiao et al. 2003). IT-type neurons project to both hemispheres and synapse preferentially on D1 receptor containing neurons (direct pathway). PT-type neurons on the other hand synapse preferentially on D2 receptor containing indirect pathway neurons. IT-type neurons are enriched in upper layers while PT-type neurons are enriched in deep layers. There is some debate as to whether these projection types show compartment specificity and what the functional role of this specificity would be (Reiner, Hart et al. 2010; Crittenden and Graybiel 2011). Reiner et al (2010), suggest that both IT-type and PT-type neurons synapse on direct and indirect pathway neurons respectively in the matrix, but only PT-type projections are

found in the patch. The authors do not posit a functional role for this compartmental specificity, but rather for the general direct or indirect pathway systems, irrespective of compartment. Based on their model, however, this would mean activity in patch is related to suppression of movements and disruption of this inhibition through mu opioid receptor activation by enkephalin should lead to an increase in a selected behavior (Graybiel 2005; Reiner, Hart et al. 2010).

Other roles for the patch and matrix

Another division of function for patch and matrix comes from a model of learning known as the actor-critic model. In this model there is an adaptive critic that computes the likelihood and magnitude of reward for different actions and an actor that carries out these actions by referring to saved action values (Joel, Niv et al. 2002). Houk and colleagues (1995) proposed that within the striatum, the patch compartment acts as the critic, while the matrix acts as the actor. In this model the patch critic signals the value of an action or a stimulus by chronically inhibiting DA neuron firing and allowing bursts of phasic excitation (Houk, Adams et al. 1995). Altering activity of the critic through pharmacology could lead to a specific action gaining exaggerated value. Specifically activation of mu opioid receptors could lead to the critic assigning more value to approach and consumption of the CS+lever or CS_{dish}. This model would either require incremental increases in approach and appetitive-consummatory behaviors to the preferred cue reflecting incremental increases in the feedback from the critic to the actor or a single feedback event that brings the action value of approach to the preferred cue to asymptote. Therefore, the results reported here are congruent with an actor/critic model of patch/matrix function. Some evidence for this hypothesis is that rats will more readily

administer intracranial self-stimulation if the electrodes are placed in the patch, rather than the matrix. This could be due to stimulation of the critic which then provides exaggerated feedback to the actor, greatly increasing the action value of lever pressing (White and Hiroi 1998).

This raises the further question of the division of function between dorsomedial and dorsolateral neostriatum. Presumably, DAMGO microinjection is affecting the patch/critic similarly regardless of area within the neostriatum. This begs the question of where the regional specificity arises. One option is which action value that is enhanced by the critic would logically differ according to which actions are encoded by nearby ensembles in the matrix actor. If values of approach and appetitive behaviors directed at cues were encoded in dorsolateral neostriatum and values of more proximal primary consumption actions were held in the dorsomedial neostriatum, the results observed here would be expected.

Other roles for the dorsolateral neostriatum

The dorsolateral or sensorimotor region of neostriatum is known to play roles in serial movement patterns and S-R habits. One line of evidence for DLS in habits has been that DLS apparently becomes increasingly recruited over time as learned actions become more over trained or habitual. For example, DLS lesions are reported not to disrupt cocaine self-administration early in training, but do produce disruptions of self-administration if made later after additional training (Murray, Belin et al. 2012). This and similar evidence has led to the hypothesis that dorsolateral neostriatum mediates habitual action and is especially important for pathologies such as addiction (Everitt and Robbins

2005; Everitt, Belin et al. 2008). Interestingly, many of these studies use a conditioned reinforcer during the test phase (Vanderschuren, Di Ciano et al. 2005; Belin and Everitt 2008b; Murray, Belin et al. 2012). Here, I have demonstrated that opioid receptor activation in dorsolateral neostriatum enhances the conditioned reinforcing properties of cues. It is possible that, rather than controlling an established action pattern solely; dorsolateral neostriatum can also maintain and amplify cue values. This interpretation does not detract from the work of Everitt and colleagues but generates new questions that can be answered through experimental techniques that explicitly test action versus cue value such as the ones used here in Chapter 3.

A related line of evidence is that learned reward-seeking actions become increasingly independent of outcome as overtraining proceeds, so that over trained learned behaviors perseverate even if the reward has been devalued by satiety or if the act-outcome contingency is diluted by free rewards (Dickinson and Balleine 1990; Balleine and O'Doherty 2010). Lesions of the DLS reduce such perseveration, so that learned responses decline again after reward devaluation or contingency dilution (Yin, Knowlton et al. 2004, 2006). A third line of evidence for DLS involvement in habits is serial ritualization into stereotyped patterns that characterize habits (Graybiel 2008). Habitual actions can be computationally generated by a prediction error mechanism that once trained operates inflexibly (Daw, Niv et al. 2005). Rigid serial patterns of action are disrupted by lesions of the dorsolateral neostriatum, both for learned serial rituals (Yin 2010) and for instinctive rituals of serial actions (Cromwell and Berridge 1996). Further, neurons within the dorsolateral neostriatum track the chunking of serial actions into ritualized patterns, both for learned rituals (Barnes, Kubota et al. 2005) and instinctive

serial rituals (Aldridge and Berridge 1998). In short, "habits are sequential, repetitive, motor, or cognitive behaviors elicited by external or internal triggers that, once released, can go to completion without constant conscious oversight" (Graybiel 2008), in which DLS plays an important role.

DLS opioid enhancement of CS attraction: "wanting" motivation or S-R habit?

With the above points in mind, it is crucial to ask whether DLS microinjections of DAMGO strengthened an S-R habit ritual to enhance approach and consummatory responses to a prepotent CS. I believe the evidence clearly shows the answer to be 'no'. First, there was no 'who moved my cheese?' perseveration of sign-trackers' well-established approach ritual to familiar-location when their CS+lever was suddenly moved to a new location. Instead, rats almost immediately abandoned their old ritual within a second or two, and switched to the new location using a new movement sequence of opposite-direction turn and longer strides. Rats switched even faster after DAMGO and reached the new location with even greater alacrity, suggesting the rats more intensely 'wanted' the moved cue and were willing to flexibly follow it. Second, DLS microinjections of DAMGO also made rats 'want' the CS+lever cue more in the sense of being willing to learn an entirely new movement response to earn it. Rats learned a new nose-poke response, and performed at higher levels to earn CS+lever insertions as an instrumental conditioned reinforcer after DAMGO microinjection in DLS than after vehicle microinjections. Although conditioned reinforcement can be explained in several ways, the enhancement is certainly consistent with the notion that DLS opioid stimulation made rats 'want' the Pavlovian cue more intensely. Finally, sign-tracking itself as a behavioral response never quite became habitual in the accepted sense of persisting after

UCS devaluation. Instead, sign-tracking immediately reduced after UCS devaluation by satiety induction. Devaluation sensitivity suggests that sign-tracking always remained a motivated response, integrating current biological state with learned Pavlovian information as incentive salience computation typically does (Zhang, Berridge et al. 2009; Berridge 2012; Robinson and Berridge 2013).

Dopamine stimulation in DLS by amphetamine microinjections also may have produced a motivational enhancement but of a different type. Amphetamine microinjection more selectively enhanced goal-tracking alone, and only in 'mixed' individuals that originally both sign-tracked and goal-tracked to comparable degrees. It is difficult to view this as S-R habit enhancement, since there was no enhancement in individuals that showed the strongest patterns to begin with, i.e., sign-trackers

Other roles for the dorsomedial neostriatum

In contrast to the dorsolateral neostriatum, the dorsomedial neostriatum has been implicated flexible act-outcome associations rather than rigid S-R associations. Inactivation of this area prevents animals from updating behavior after changes in reward value (Yin, Knowlton et al. 2005; Yin, Ostlund et al. 2005). If dorsomedial neostriatum is important for encoding current reward value, the increase in food consumption reported here after DAMGO microinjection could be due to an increase in primary reward value.

Dorsomedial neostriatum is also involved in reversal learning and task switching (Pisa and Cyr 1990; Ragozzino 2003; Kehagia, Murray et al. 2010). Therefore, dorsomedial neostriatum along with its cortical affects from the prefrontal cortex and the orbitofrontal cortex has been hypothesized to be critical for "cognitive" flexible behaviors (Arana, Parkinson et al. 2003; Coutureau and Killcross 2003; Pickens, Saddoris

et al. 2003; Grahn, Parkinson et al. 2008). Here, activation of mu opioid receptors potently enhanced consumption of palatable rewards, but did not enhance approach to reward paired cues or the conditioned reinforcing value of those cues. We also demonstrated that there is an endogenous enkephalin code of consumption of palatable foods in dorsomedial neostriatum, so the eating observed was not due to an artificial disruption of the system. How do we reconcile the role of the dorsomedial neostriatum in simple eating with its established role in "cognitive" processes? Grahn and colleagues (2008) suggest the "cognitive" function of medial neostriatum is that it works to promote an appropriate behavioral strategy. In the presence of a calorically dense resource, the most appropriate behavioral strategy is to engage in intense eating. This hypothesis requires further testing, however, perhaps by measuring enkephalin levels in task switching and reinforcer devaluation paradigms.

Previous work on the function of dorsolateral and dorsomedial neostriatum has relied mainly on lesion and inactivation studies (Yin, Knowlton et al. 2004; Vanderschuren, Di Ciano et al. 2005; Yin, Knowlton et al. 2006; Belin and Everitt 2008a). Lesions or mixtures of GABA and glutamate acting drugs would affect both patch and matrix similarly. This could be why the studies described here reveal motivational processes of the dorsal neostriatum while others do not. Here, I've preferentially engaged the patch with a mu opioid receptor agonist, without engaging the surrounding matrix. It is possible that the patch acts as a distributed motivational network throughout dorsal neostriatum, allowing integration of motivational and motor information. Lesions and inactivations would not reveal these effects, but would rather show the effect of disrupting the network as a whole.

Other roles for the ventrolateral neostriatum

Some researchers have suggested that the increase in eating observed after manipulation of ventrolateral neostriatum is due to activation of oromotor and forelimb motor responses or motor impairment (Salamone, Mahan et al. 1993; Cousins, Trevitt et al. 1999). Here, I sought to better understand the role of ventrolateral neostriatum in generating motivation by also testing a manipulation that produces eating in an autoshaping paradigm. A motor impairment hypothesis would predict overall deficits in responding in both sign-trackers and goal-trackers. Here, no overall changes in total amount of behavior were observed. In addition, no gross motor impairments were noted in a detailed analysis of video recordings for all tests. It appears instead that mu opioid receptor activation in VLS plays a specific role in enhancing motivation for primary rewards and reward cues paired closest in time and space with those primary rewards.

Other roles for central amygdala

CeA has traditionally been thought of as part of circuitry responsible for conditioned fear (LeDoux 2000), but more recently the CeA has been shown to be involved in translating learned information into motivation regardless of affective valence (Hollerman and Schultz 1998; Everitt, Parkinson et al. 1999; Hall, Parkinson et al. 2001; Holland and Gallagher 2003; Corbit and Balleine 2005). Lesions of CeA impair conditioned orienting to a discreet appetitive CS, establishing a role for CeA in appetitive conditioning (Gallagher, Graham et al. 1990). Further, CeA is needed for the general activating effects of Pavlovian to Instrumental transfer, as is the DLS (Corbit and

Balleine 2005; Corbit and Janak 2007), demonstrating that CeA is needed to translate appetitive cues into motivated action. The results presented here do not conflict with previous findings on CeA. Rather, they extend these findings to demonstrate that mu opioid receptor activation of this area enhances approach and consummatory behaviors directed at a preferred appetitive CS.

Future directions

Optogenetics is a promising tool to begin to answer some of the lingering questions this dissertation leaves. In CeA, CAG-ArchT or CAG-ChR2 can be used to generally inhibit or excite the neuronal population (Huff, Miller et al. 2013). If DAMGO microinjection is causing a general increase in neuronal activity in CeA, I expect those animals with CAG-ChR2 viral infusions will show behaviors similar to DAMGO microinjection.

In addition, a promising avenue to understand how enkephalin release is controlled in the striatum is to combine optogenetics and microdialysis. We could take advantage of mice that express that uniquely express Cre recombinase in D2 containing striatal neurons (Kravitz, Freeze et al. 2010). Using Cre dependent channelrhodopsin, we could preferentially drive D2 neuron firing with laser stimulation and measure extra-cellular enkephalin concurrently. For example, in dorsomedial neostriatum, we would expect optogenetic excitation of the D2 pathway would drive enkephalin release and increase eating.

Conclusion

In this dissertation I sought to expand our understanding of motivational circuitry to areas outside the traditional "reward pathway." Through the course of this work I have confirmed that central amygdala plays a role in generating intense motivation. I have demonstrated that dorsomedial neostriatum and dorsolateral neostriatum generate motivation for unconditioned and conditioned rewards, respectively. Finally, I have expanded our understanding of the role of the ventrolateral neostriatum in generating motivation. As a whole, this work begins to identify a distributed motivational network throughout the brain, not just in traditional "reward structures." It underscores the importance of continued testing and questioning of multiple brain areas through multiple theoretical viewpoints.

Figures

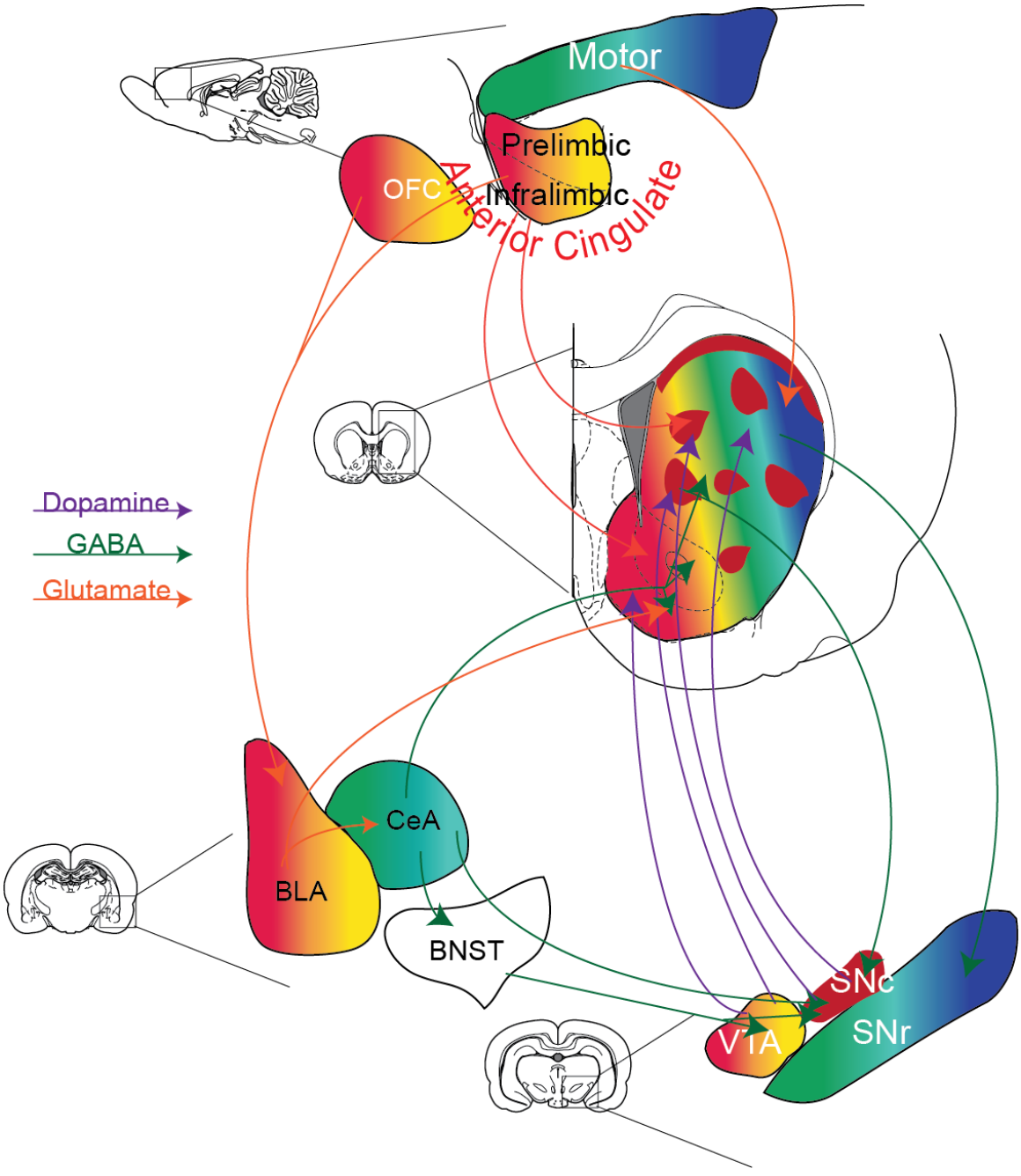


Figure 6.1. Anatomical connectivity of the extended amygdala, midbrain dopamine nuclei, striatum, and cortex. Structures are color coded according to where they project to within the striatum. Lines are color coded according to major neurotransmitter content.

References

Akil, H., S. J. Watson, et al. (1984). "Endogenous opioids: Biology and function." Annu Rev Neurosci **7**: 223-255.

Aldridge, J. W. and K. C. Berridge (1998). "Coding of serial order by neostriatal neurons: A "natural action" approach to movement sequence." J Neurosci **18**(7): 2777-2787.

Alheid, G. F. (2003). "Extended amygdala and basal forebrain." Ann N Y Acad Sci **985**: 185-205.

Anselme, P. (2010). "The uncertainty processing theory of motivation." Behav Brain Res **208**(2): 291-310.

Apicella, P., T. Ljungberg, et al. (1991). "Responses to reward in monkey dorsal and ventral striatum." Exp Brain Res **85**(3): 491-500.

Arana, F. S., J. A. Parkinson, et al. (2003). "Dissociable contributions of the human amygdala and orbitofrontal cortex to incentive motivation and goal selection." J Neurosci **23**(29): 9632-9638.

Bakshi, V. P. and A. E. Kelley (1993a). "Feeding induced by opioid stimulation of the ventral striatum: Role of opiate receptor subtypes." J Pharmacol Exp Ther **265**(3): 1253-1260.

Bakshi, V. P. and A. E. Kelley (1993b). "Striatal regulation of morphine-induced hyperphagia: An anatomical mapping study." Psychopharmacology (Berl) **111**(2): 207-214.

Baldo, B. A. and A. E. Kelley (2007). "Discrete neurochemical coding of distinguishable motivational processes: Insights from nucleus accumbens control of feeding." Psychopharmacology (Berl) **191**(3): 439-459.

Balleine, B. and A. Dickinson (1992). "Signalling and incentive processes in instrumental reinforcer devaluation." Q J Exp Psychol B **45**(4): 285-301.

Balleine, B. W. and J. P. O'Doherty (2010). "Human and rodent homologies in action control: Corticostriatal determinants of goal-directed and habitual action." Neuropsychopharmacology **35**(1): 48-69.

Balleine, B. W. and S. B. Ostlund (2007). "Still at the choice-point: Action selection and initiation in instrumental conditioning." Ann N Y Acad Sci **1104**: 147-171.

Barnes, T. D., Y. Kubota, et al. (2005). "Activity of striatal neurons reflects dynamic encoding and recoding of procedural memories." Nature **437**(7062): 1158-1161.

Bayon, A. and B. Anton (1986). "Diurnal rhythm of the in vivo release of enkephalin from the globus pallidus of the rat." Regul Pept **15**(1): 63-70.

Belin, D. and B. J. Everitt (2008a). "Cocaine seeking habits depend upon dopamine-dependent serial connectivity linking the ventral with the dorsal striatum." Neuron **57**(3): 432-441.

Belin, D. and B. J. Everitt (2008b). "Cocaine seeking habits depend upon doparnine-dependent serial connectivity linking the ventral with the dorsal striatum." Neuron **57**(3): 432-441.

Berridge, K. C. (2000). "Measuring hedonic impact in animals and infants: Microstructure of affective taste reactivity patterns." Neurosci Biobehav Rev **24**(2): 173-198.

Berridge, K. C. (2012). "From prediction error to incentive salience: Mesolimbic computation of reward motivation." Eur J Neurosci **35**(7): 1124-1143.

Berridge, K. C., F. W. Flynn, et al. (1984). "Sodium depletion enhances salt palatability in rats." Behav Neurosci **98**(4): 652-660.

Berridge, K. C., C. Y. Ho, et al. (2011). "The tempted brain eats: Pleasure and desire circuits in obesity and eating disorders." Brain Res **1350**: 43-64.

Berridge, K. C. and T. E. Robinson (1998). "What is the role of dopamine in reward: Hedonic impact, reward learning, or incentive salience?" Brain Res Brain Res Rev **28**(3): 309-369.

Berridge, K. C. and J. Schulkin (1989). "Palatability shift of a salt-associated incentive during sodium depletion." Q J Exp Psychol B **41**(2): 121-138.

Berthoud, H. R. and C. Morrison (2008). "The brain, appetite, and obesity." Annu Rev Psychol **59**: 55-92.

Bindra, D. (1978). "How adaptive behavior is produced -perceptual-motivational alternative to response-reinforcements." Behavioral and Brain Sciences **1**(1): 41-52.

Boakes, R. A., M. Poli, et al. (1978). "A study of misbehavior: Token reinforcement in the rat." J Exp Anal Behav **29**(1): 115-134.

Bornstein, A. M. and N. D. Daw (2011). "Multiplicity of control in the basal ganglia: Computational roles of striatal subregions." Curr Opin Neurobiol **21**(3): 374-380.

Breland, K. and M. Breland (1961). "The misbehavior of organisms." American Psychologist **16**(11): 681-684.

Carelli, R. M. and S. G. Ijames (2001). "Selective activation of accumbens neurons by cocaine-associated stimuli during a water/cocaine multiple schedule." Brain Res **907**(1-2): 156-161.

Carr, H. and J. B. Watson (1908). "Orientation in the white rat." Journal of Comparative Neurology and Psychology **18**(1): 27-44.

Cetinkaya, H. and M. Domjan (2006). "Sexual fetishism in a quail (coturnix japonica) model system: Test of reproductive success." J Comp Psychol **120**(4): 427-432.

Colwill, R. M. and R. A. Rescorla (1990). "Effect of reinforcer devaluation on discriminative control of instrumental behavior." J Exp Psychol Anim Behav Process **16**(1): 40-47.

Corbit, L. H. and B. W. Balleine (2003). "Instrumental and pavlovian incentive processes have dissociable effects on components of a heterogeneous instrumental chain." J Exp Psychol Anim Behav Process **29**(2): 99-106.

Corbit, L. H. and B. W. Balleine (2005). "Double dissociation of basolateral and central amygdala lesions on the general and outcome-specific forms of pavlovian-instrumental transfer." J Neurosci **25**(4): 962-970.

Corbit, L. H. and P. H. Janak (2007). "Inactivation of the lateral but not medial dorsal striatum eliminates the excitatory impact of pavlovian stimuli on instrumental responding." J Neurosci **27**(51): 13977-13981.

Cousins, M. S., J. Trevitt, et al. (1999). "Different behavioral functions of dopamine in the nucleus accumbens and ventrolateral striatum: A microdialysis and behavioral investigation." Neuroscience **91**(3): 925-934.

Coutureau, E. and S. Killcross (2003). "Inactivation of the infralimbic prefrontal cortex reinstates goal-directed responding in overtrained rats." Behav Brain Res **146**(1-2): 167-174.

Cox, S. M., C. Benkelfat, et al. (2009). "Striatal dopamine responses to intranasal cocaine self-administration in humans." Biol Psychiatry **65**(10): 846-850.

Crittenden, J. R. and A. M. Graybiel (2011). "Basal ganglia disorders associated with imbalances in the striatal striosome and matrix compartments." Front Neuroanat **5**: 59.

Cromwell, H. C. and K. C. Berridge (1996). "Implementation of action sequences by a neostriatal site: A lesion mapping study of grooming syntax." J Neurosci **16**(10): 3444-3458.

Cunningham, S. T. and A. E. Kelley (1992). "Opiate infusion into nucleus accumbens: Contrasting effects on motor activity and responding for conditioned reward." Brain Res **588**(1): 104-114.

Daw, N. D., Y. Niv, et al. (2005). "Uncertainty-based competition between prefrontal and dorsolateral striatal systems for behavioral control." Nat Neurosci **8**(12): 1704-1711.

Day, J. J., J. L. Jones, et al. (2010). "Phasic nucleus accumbens dopamine release encodes effort- and delay-related costs." Biol Psychiatry **68**(3): 306-309.

de Borchgrave, R., J. N. Rawlins, et al. (2002). "Effects of cytotoxic nucleus accumbens lesions on instrumental conditioning in rats." Exp Brain Res **144**(1): 50-68.

de Olmos, J. S. and L. Heimer (1999). "The concepts of the ventral striatopallidal system and extended amygdala." Ann N Y Acad Sci **877**: 1-32.

Delamater, A. R. and P. C. Holland (2008). "The influence of cs-us interval on several different indices of learning in appetitive conditioning." J Exp Psychol Anim Behav Process **34**(2): 202-222.

Delfs, J. M. and A. E. Kelley (1990). "The role of d1 and d2 dopamine receptors in oral stereotypy induced by dopaminergic stimulation of the ventrolateral striatum." Neuroscience **39**(1): 59-67.

Dickinson, A. and B. Balleine (1990). "Motivational control of instrumental performance following a shift from thirst to hunger." Q J Exp Psychol B **42**(4): 413-431.

Dickinson, A., J. Campos, et al. (1996). "Bidirectional instrumental conditioning." Q J Exp Psychol B **49**(4): 289-306.

Dickinson, A., J. Smith, et al. (2000). "Dissociation of pavlovian and instrumental incentive learning under dopamine antagonists." Behav Neurosci **114**(3): 468-483.

Dickson, P. R., C. G. Lang, et al. (1994). "Oral stereotypy induced by amphetamine microinjection into striatum: An anatomical mapping study." Neuroscience **61**(1): 81-91.

DiFeliceantonio, A. G. and K. C. Berridge (2012). "Which cue to 'want'? Opioid stimulation of central amygdala makes goal-trackers show stronger goal-tracking, just as sign-trackers show stronger sign-tracking." Behav Brain Res **230**(2): 399-408.

DiFeliceantonio, A. G., O. S. Mabrouk, et al. (2012). "Enkephalin surges in dorsal neostriatum as a signal to eat." Current Biology **22**(20): 1918-1924.

Doremus-Fitzwater, T. L. and L. P. Spear (2011). "Amphetamine-induced incentive sensitization of sign-tracking behavior in adolescent and adult female rats." Behav Neurosci.

Dores, R. M., H. Akil, et al. (1984). "Strategies for studying opioid peptide regulation at the gene, message and protein levels." Peptides **5 Suppl 1**: 9-17.

Eblen, F. and A. M. Graybiel (1995). "Highly restricted origin of prefrontal cortical inputs to striosomes in the macaque monkey." J Neurosci **15**(9): 5999-6013.

Everitt, B. J., D. Belin, et al. (2008). "Review. Neural mechanisms underlying the vulnerability to develop compulsive drug-seeking habits and addiction." Philos Trans R Soc Lond B Biol Sci **363**(1507): 3125-3135.

Everitt, B. J., J. A. Parkinson, et al. (1999). "Associative processes in addiction and reward - the role of amygdala-ventral striatal subsystems." Advancing from the Ventral Striatum to the Extended Amygdala **877**: 412-438.

Everitt, B. J. and T. W. Robbins (2005). "Neural systems of reinforcement for drug addiction: From actions to habits to compulsion." Nat Neurosci **8**(11): 1481-1489.

Faure, A., U. Haberland, et al. (2005). "Lesion to the nigrostriatal dopamine system disrupts stimulus-response habit formation." J Neurosci **25**(11): 2771-2780.

Feurte, S., S. Nicolaidis, et al. (2000). "Conditioned taste aversion in rats for a threonine-deficient diet: Demonstration by the taste reactivity test." Physiol Behav **68**(3): 423-429.

Flagel, S. B., H. Akil, et al. (2009). "Individual differences in the attribution of incentive salience to reward-related cues: Implications for addiction." Neuropharmacology **56 Suppl 1**: 139-148.

Flagel, S. B., J. J. Clark, et al. (2011). "A selective role for dopamine in stimulus-reward learning." Nature **469**(7328): 53-57.

Flagel, S. B., S. J. Watson, et al. (2008). "Individual differences in the attribution of incentive salience to a reward-related cue: Influence on cocaine sensitization." Behav Brain Res **186**(1): 48-56.

Flagel, S. B., S. J. Watson, et al. (2007). "Individual differences in the propensity to approach signals vs goals promote different adaptations in the dopamine system of rats." Psychopharmacology (Berl) **191**(3): 599-607.

Fudge, J. L. and A. B. Emiliano (2003). "The extended amygdala and the dopamine system: Another piece of the dopamine puzzle." J Neuropsychiatry Clin Neurosci **15**(3): 306-316.

Fujiyama, F., J. Sohn, et al. (2011). "Exclusive and common targets of neostriatofugal projections of rat striosome neurons: A single neuron-tracing study using a viral vector." Eur J Neurosci **33**(4): 668-677.

Gallagher, M., P. W. Graham, et al. (1990). "The amygdala central nucleus and appetitive pavlovian conditioning: Lesions impair one class of conditioned behavior." J Neurosci **10**(6): 1906-1911.

Gauthier, J., M. Parent, et al. (1999). "The axonal arborization of single nigrostriatal neurons in rats." Brain Res **834**(1-2): 228-232.

Gerfen, C. R. (1984). "The neostriatal mosaic: Compartmentalization of corticostriatal input and striatonigral output systems." Nature **311**(5985): 461-464.

Gerfen, C. R. (1989). "The neostriatal mosaic: Striatal patch-matrix organization is related to cortical lamination." Science **246**(4928): 385-388.

Gerfen, C. R., M. Herkenham, et al. (1987). "The neostriatal mosaic: Ii. Patch- and matrix-directed mesostriatal dopaminergic and non-dopaminergic systems." J Neurosci **7**(12): 3915-3934.

Gonzales, C. and M. F. Chesselet (1990). "Amygdalonigral pathway: An anterograde study in the rat with phaseolus vulgaris leucoagglutinin (pha-l)." J Comp Neurol **297**(2): 182-200.

Gosnell, B. A. (1988). "Involvement of mu opioid receptors in the amygdala in the control of feeding." Neuropharmacology **27**(3): 319-326.

Grace, A. A. and J. A. Rosenkranz (2002). "Regulation of conditioned responses of basolateral amygdala neurons." Physiol Behav **77**(4-5): 489-493.

Grahn, J. A., J. A. Parkinson, et al. (2008). "The cognitive functions of the caudate nucleus." Prog Neurobiol **86**(3): 141-155.

Graybiel, A. M. (2005). "The basal ganglia: Learning new tricks and loving it." Curr Opin Neurobiol **15**(6): 638-644.

Graybiel, A. M. (2008). "Habits, rituals, and the evaluative brain." Annu Rev Neurosci **31**: 359-387.

Grill, H. J. and R. Norgren (1978). "The taste reactivity test. I. Mimetic responses to gustatory stimuli in neurologically normal rats." Brain Res **143**(2): 263-279.

Haber, S. N., K. S. Kim, et al. (2006). "Reward-related cortical inputs define a large striatal region in primates that interface with associative cortical connections, providing a substrate for incentive-based learning." J Neurosci **26**(32): 8368-8376.

Hall, J., J. A. Parkinson, et al. (2001). "Involvement of the central nucleus of the amygdala and nucleus accumbens core in mediating pavlovian influences on instrumental behaviour." Eur J Neurosci **13**(10): 1984-1992.

Haracz, J. L., J. T. Tschanz, et al. (1993). "Striatal single-unit responses to amphetamine and neuroleptics in freely moving rats." Neurosci Biobehav Rev **17**(1): 1-12.

Heimer, L., G. Van Hosen, et al. (2008). Anatomy of neuropsychiatry, Academic Press.

Herkenham, M. and C. B. Pert (1980). "In vitro autoradiography of opiate receptors in rat brain suggests loci of "opiatergic" pathways." Proc Natl Acad Sci U S A **77**(9): 5532-5536.

Holden, J. M. and L. L. Peoples (2010). "Effects of acute amphetamine exposure on two kinds of pavlovian approach behavior." Behav Brain Res **208**(1): 270-273.

Holland, P. C. and M. Gallagher (2003). "Double dissociation of the effects of lesions of basolateral and central amygdala on conditioned stimulus-potentiated feeding and pavlovian-instrumental transfer." Eur J Neurosci **17**(8): 1680-1694.

Hollerman, J. R. and W. Schultz (1998). "Dopamine neurons report an error in the temporal prediction of reward during learning." Nat Nuerosci **1**(4): 304-309.

Hollmann, M., L. Hellrung, et al. (2012). "Neural correlates of the volitional regulation of the desire for food." Int J Obes (Lond) **36**(5): 648-655.

Houk, J. C., J. L. Adams, et al., Eds. (1995). A model of how the basal ganglia generate and use reward signals that predict reinforcement. Models of information processing in the basal ganlia. Cambridge, MIT Press.

Huff, M. L., R. L. Miller, et al. (2013). "Posttraining optogenetic manipulations of basolateral amygdala activity modulate consolidation of inhibitory avoidance memory in rats." Proc Natl Acad Sci U S A **110**(9): 3597-3602.

Ito, R., J. W. Dalley, et al. (2002). "Dopamine release in the dorsal striatum during cocaine-seeking behavior under the control of a drug-associated cue." J Neurosci **22**(14): 6247-6253.

Jackson, E. D. (2009). The extended amygdala in appetitive motivation for reward: Role of the bed nucleus of the stria terminalis.

Jackson, E. D. and K. C. Berridge (2008). "Accumbens shell opioids enhance 'wanting' for preferred and non-preferred reward cues. ." Neuroscience Meeting Planner **298.13/TT46**.

Jenkins, H. M. and B. R. Moore (1973). "Form of auto-shaped response with food or water reinforcers." Journal of the Experimental Analysis of Behavior **20**(2): 163-181.

Jiang, Z. G. and R. A. North (1992). "Pre- and postsynaptic inhibition by opioids in rat striatum." J Neurosci **12**(1): 356-361.

Joel, D., Y. Niv, et al. (2002). "Actor-critic models of the basal ganglia: New anatomical and computational perspectives." Neural Netw **15**(4-6): 535-547.

Kehagia, A. A., G. K. Murray, et al. (2010). "Learning and cognitive flexibility: Frontostriatal function and monoaminergic modulation." Curr Opin Neurobiol **20**(2): 199-204.

Kelley, A. E., B. A. Baldo, et al. (2005). "A proposed hypothalamic-thalamic-striatal axis for the integration of energy balance, arousal, and food reward." Journal of Comparative Neurology **493**(1): 72-85.

Kelley, A. E., B. A. Baldo, et al. (2005). "Corticostriatal-hypothalamic circuitry and food motivation: Integration of energy, action and reward." Physiol Behav **86**(5): 773-795.

Kelley, A. E. and J. M. Delfs (1991). "Dopamine and conditioned reinforcement. I. Differential effects of amphetamine microinjections into striatal subregions." Psychopharmacology (Berl) **103**(2): 187-196.

Kelley, A. E., A. M. Gauthier, et al. (1989a). "Amphetamine microinjections into distinct striatal subregions cause dissociable effects on motor and ingestive behavior." Behav Brain Res **35**(1): 27-39.

Kelley, A. E., A. M. Gauthier, et al. (1989b). "Amphetamine microinjections into distinct striatal subregions cuase dissociable effects on motor and ingestive behavior." Behav Brain Res **35**(1): 27-39.

Kelley, A. E., C. G. Lang, et al. (1988). "Induction of oral stereotypy following amphetamine microinjection into a discrete subregion of the striatum." Psychopharmacology (Berl) **95**(4): 556-559.

Kessler, D. A. (2009). The end of overeating : Taking control of the insatiable american appetite. New York, Rodale.

Killcross, S., T. W. Robbins, et al. (1997). "Different types of fear-conditioned behaviour mediated by separate nuclei within amygdala." Nature **388**(6640): 377-380.

Kincaid, A. E. and C. J. Wilson (1996). "Corticostriatal innervation of the patch and matrix in the rat neostriatum." J Comp Neurol **374**(4): 578-592.

Kravitz, A. V., B. S. Freeze, et al. (2010). "Regulation of parkinsonian motor behaviours by optogenetic control of basal ganglia circuitry." Nature **466**(7306): 622-626.

Kubota, Y., J. Liu, et al. (2009). "Stable encoding of task structure coexists with flexible coding of task events in sensorimotor striatum." J Neurophysiol **102**(4): 2142-2160.

Le Merrer, J., J. A. Becker, et al. (2009). "Reward processing by the opioid system in the brain." Physiol Rev **89**(4): 1379-1412.

LeDoux, J. E. (2000). "Emotion circuits in the brain." Annu Rev Neurosci **23**: 155-184.

Lee, H. J., M. Gallagher, et al. (2010). "The central amygdala projection to the substantia nigra reflects prediction error information in appetitive conditioning." Learn Mem **17**(10): 531-538.

Levesque, M. and A. Parent (1998). "Axonal arborization of corticostriatal and corticothalamic fibers arising from prelimbic cortex in the rat." Cereb Cortex **8**(7): 602-613.

Li, Q., J. K. Zubieta, et al. (2009). "Practical aspects of in vivo detection of neuropeptides by microdialysis coupled off-line to capillary lc with multistage ms." Anal Chem **81**(6): 2242-2250.

Lingawi, N. W. and B. W. Balleine (2012). "Amygdala central nucleus interacts with dorsolateral striatum to regulate the acquisition of habits." J Neurosci **32**(3): 1073-1081.

Lolordo, V. M. and R. A. Rescorla (1964). "Effects of a stimulus previously contrasted with shock upon ongoing avoidance responding." American Psychologist **19**(7): 520-520.

Lundy, R. F. (2001). "Pontine gustatory activity is altered by electrical stimulation in the central nucleus of the amygdala." Journal of neurophysiology **85**(2): 770-783.

Mabrouk, O. S., Q. Li, et al. (2011). "Microdialysis and mass spectrometric monitoring of dopamine and enkephalins in the globus pallidus reveal reciprocal interactions that regulate movement." J Neurochem **118**(1): 24-33.

Mackintosh, N. J. (1974). The psychology of animal learning. London; New York, Academic Press.

Mahler, S. V. and K. C. Berridge (2009). "Which cue to "want?" Central amygdala opioid activation enhances and focuses incentive salience on a prepotent reward cue." J Neurosci **29**(20): 6500-6513.

Mahler, S. V. and K. C. Berridge (2011). "What and when to "want"? Amygdala-based focusing of incentive salience upon sugar and sex." Psychopharmacology (Berl).

Mansour, A., C. A. Fox, et al. (1994). "Mu, delta, and kappa opioid receptor mrna expression in the rat cns: An in situ hybridization study." J Comp Neurol **350**(3): 412-438.

Mayorga, A. J., G. Gianutsos, et al. (1999). "Effects of striatal injections of 8-bromo-cyclic-amp on pilocarpine-induced tremulous jaw movements in rats." Brain Res **829**(1-2): 180-184.

McGinty, V. B., B. Y. Hayden, et al. (2011). "Emerging, reemerging, and forgotten brain areas of the reward circuit: Notes from the 2010 motivational neural networks conference." Behav Brain Res **225**(1): 348-357.

Mena, J. D., K. Sadeghian, et al. (2011). "Induction of hyperphagia and carbohydrate intake by mu-opioid receptor stimulation in circumscribed regions of frontal cortex." J Neurosci **31**(9): 3249-3260.

Meyer, P. J., V. Lovic, et al. (2012). "Quantifying individual variation in the propensity to attribute incentive salience to reward cues." PLoS One **7**(6): e38987.

Mogenson, G. J., D. L. Jones, et al. (1980). "From motivation to action - functional interface between the limbic and the motor system." Progress in Neurobiology **14**(2-3): 69-97.

Morris, J. and R. Dolan (2001). "Interaction of amygdala and orbitofrontal cortex responses during hunger-enhanced memory for food stimuli." Neuroimage **13**(6): S449-S449.

Murray, J. E., D. Belin, et al. (2012). "Double dissociation of the dorsomedial and dorsolateral striatal control over the acquisition and performance of cocaine seeking." Neuropsychopharmacology **37**(11): 2456-2466.

Nilsson, J., T. S. Kristiansen, et al. (2008). "Sign- and goal-tracking in atlantic cod (gadus morhua)." Anim Cogn **11**(4): 651-659.

Nummenmaa, L., J. Hirvonen, et al. (2012). "Dorsal striatum and its limbic connectivity mediate abnormal anticipatory reward processing in obesity." PLoS One **7**(2): e31089.

Packard, M. G. and B. J. Knowlton (2002a). "Learning and memory functions of the basal ganglia." Annu Rev Neurosci **25**: 563-593.

Packard, M. G. and B. J. Knowlton (2002b). "Learning and memory functions of the basal ganglia." Annu Rev Neurosci **25**: 563-593.

Palmiter, R. D. (2008a). "Dopamine signaling in the dorsal striatum is essential for motivated behaviors - lessons from dopamine-deficient mice." Molecular and Biophysical Mechanisms of Arousal, Alertness, and Attention **1129**: 35-46.

Palmiter, R. D. (2008b). "Dopamine signaling in the dorsal striatum is essential for motivated behaviors: Lessons from dopamine-deficient mice." Ann N Y Acad Sci **1129**: 35-46.

Pare, D., G. J. Quirk, et al. (2004). "New vistas on amygdala networks in conditioned fear." J Neurophysiol **92**(1): 1-9.

Parkinson, J. A., R. N. Cardinal, et al. (2000). "Limbic cortical-ventral striatal systems underlying appetitive conditioning." Prog Brain Res **126**: 263-285.

Pavlov, I. P. and G. V. Anrep (1927). Conditioned reflexes; an investigation of the physiological activity of the cerebral cortex. London, Oxford University Press: Humphrey Milford.

Paxinos, G. and C. Watson (2007). The rat brain in stereotaxic coordinates, New York: Academic.

Pecina, S. and K. C. Berridge (2000). "Opioid site in nucleus accumbens shell mediates eating and hedonic 'liking' for food: Map based on microinjection fos plumes." Brain Res **863**(1-2): 71-86.

Pecina, S. and K. C. Berridge (2005). "Hedonic hot spot in nucleus accumbens shell: Where do mu-opioids cause increased hedonic impact of sweetness?" J Neurosci **25**(50): 11777-11786.

Pecina, S. and K. C. Berridge (2008). Incentive salience mediation by opioid versus dopamine in nucleus accumbens shell and core: Amplified cue-triggered 'wanting' for reward. Society for Neuroscience, Washington, D.C.

Pecina, S., J. Schulkin, et al. (2006). "Nucleus accumbens corticotropin-releasing factor increases cue-triggered motivation for sucrose reward: Paradoxical positive incentive effects in stress?" BMC Biol **4**: 8.

Pert, C. B., M. J. Kuhar, et al. (1976). "Opiate receptor: Autoradiographic localization in rat brain." Proc Natl Acad Sci U S A **73**(10): 3729-3733.

Peterson, G. B., G. P. Frommer, et al. (1972). "Conditioned approach and contact behavior toward signals for food or brain-stimulation reinforcement " Science **177**(4053): 1009-&.

Phelps, E. A. and J. E. LeDoux (2005). "Contributions of the amygdala to emotion processing: From animal models to human behavior." Neuron **48**(2): 175-187.

Phillips, A. G., G. Vacca, et al. (2008). "A top-down perspective on dopamine, motivation and memory." Pharmacol Biochem Behav **90**(2): 236-249.

Phillips, G. D. and P. K. Hitchcott (2009). "Blockade of the acquisition, but not expression, of associative learning by pre-session intra-amygdala r(+) 7-oh-dpat." Psychopharmacology (Berl) **203**(1): 161-173.

Pickens, C. L., M. P. Saddoris, et al. (2003). "Different roles for orbitofrontal cortex and basolateral amygdala in a reinforcer devaluation task." J Neurosci **23**(35): 11078-11084.

Pisa, M. and J. Cyr (1990). "Regionally selective roles of the rat's striatum in modality-specific discrimination learning and forelimb reaching." Behav Brain Res **37**(3): 281-292.

Poulin, J. F., Z. Castonguay-Lebel, et al. (2008). "Enkephalin co-expression with classic neurotransmitters in the amygdaloid complex of the rat." J Comp Neurol **506**(6): 943-959.

Pryor, K. W., R. Haag, et al. (1969). "The creative porpoise: Training for novel behavior." J Exp Anal Behav **12**(4): 653-661.

Ragozzino, M. E. (2003). "Acetylcholine actions in the dorsomedial striatum support the flexible shifting of response patterns." Neurobiol Learn Mem **80**(3): 257-267.

Ragsdale, C. W., Jr. and A. M. Graybiel (1988). "Fibers from the basolateral nucleus of the amygdala selectively innervate striosomes in the caudate nucleus of the cat." J Comp Neurol **269**(4): 506-522.

Ragsdale, C. W., Jr. and A. M. Graybiel (1990). "A simple ordering of neocortical areas established by the compartmental organization of their striatal projections." Proc Natl Acad Sci U S A **87**(16): 6196-6199.

Reiner, A., N. M. Hart, et al. (2010). "Corticostriatal projection neurons - dichotomous types and dichotomous functions." Front Neuroanat **4**: 142.

Reiner, A., Y. Jiao, et al. (2003). "Differential morphology of pyramidal tract-type and intratelencephalically projecting-type corticostriatal neurons and their intrastriatal terminals in rats." J Comp Neurol **457**(4): 420-440.

Rescorla, R. A. and C. L. Cunningham (1979). "Spatial contiguity facilitates pavlovian second-order conditioning." J Exp Psychol Anim Behav Process **5**(2): 152-161.

Rescorla, R. A. and A. R. Wagner, Eds. (1972). A theory of pavlovian conditioning: Variations in the effectiveness of reinforcement and nonreinfrocement. Classical conditioning ii. New York, Appleton-Century-Crofts.

Reynolds, S. M. and K. C. Berridge (2008). "Emotional environments retune the valence of appetitive versus fearful functions in nucleus accumbens." Nat Neurosci **11**(4): 423-425.

Reynolds, S. M. and D. S. Zahm (2005). "Specificity in the projections of prefrontal and insular cortex to ventral striatopallidum and the extended amygdala." J Neurosci **25**(50): 11757-11767.

Richard, J. M. and K. C. Berridge (2011). "Nucleus accumbens dopamine/glutamate interaction switches modes to generate desire versus dread: D(1) alone for appetitive eating but d(1) and d(2) together for fear." J Neurosci **31**(36): 12866-12879.

Robbins, T. W. and B. J. Everitt (2002). "Limbic-striatal memory systems and drug addiction." Neurobiol Learn Mem **78**(3): 625-636.

Robinson, M. J. and K. C. Berridge (2013). "Instant transformation of learned repulsion into motivational "wanting"." Curr Biol **23**(4): 282-289.

Robinson, M. J. F. and K. C. Berridge (2010). "Instant incentive salience: Dynamic transformation of an aversive salt cue into a 'wanted' motivational magnet." Society for Neuroscience Abstract Viewer and Itinerary Planner: 40.

Robinson, T. E. and K. C. Berridge (1993). "The neural basis of drug craving: An incentive-sensitization theory of addiction." Brain Res Brain Res Rev **18**(3): 247-291.

Robinson, T. E. and S. B. Flagel (2009). "Dissociating the predictive and incentive motivational properties of reward-related cues through the study of individual differences." Biol Psychiatry **65**(10): 869-873.

Rosenkranz, J. A. and A. A. Grace (2002). "Cellular mechanisms of infralimbic and prelimbic prefrontal cortical inhibition and dopaminergic modulation of basolateral amygdala neurons in vivo." J Neurosci **22**(1): 324-337.

Rosse, R. B., M. Fay-McCarthy, et al. (1993). "Transient compulsive foraging behavior associated with crack cocaine use." Am J Psychiatry **150**(1): 155-156.

Rouillard, C. and A. S. Freeman (1995). "Effects of electrical stimulation of the central nucleus of the amygdala on the in vivo electrophysiological activity of rat nigral dopaminergic neurons." Synapse **21**(4): 348-356.

Rousselet, G. A. and C. R. Pernet (2012). "Improving standards in brain-behavior correlation analyses." Front Hum Neurosci **6**: 119.

Salamone, J. D., K. Mahan, et al. (1993). "Ventrolateral striatal dopamine depletions impair feeding and food handling in rats." Pharmacol Biochem Behav **44**(3): 605-610.

Sato, K., C. Sumi-Ichinose, et al. (2008). "Differential involvement of striosome and matrix dopamine systems in a transgenic model of dopa-responsive dystonia." Proc Natl Acad Sci U S A **105**(34): 12551-12556.

Saunders, B. T. and T. E. Robinson (2010). "A cocaine cue acts as an incentive stimulus in some but not others: Implications for addiction." Biological Psychiatry **67**(8): 730-736.

Saunders, B. T. and T. E. Robinson (2011). "Individual variation in the motivational properties of cocaine." Neuropsychopharmacology **36**(8): 1668-1676.

Saunders, B. T. and T. E. Robinson (2012). "The role of dopamine in the accumbens core in the expression of pavlovian-conditioned responses." Eur J Neurosci **36**(4): 2521-2532.

Schneck, N. and P. Vezina (2012). "Enhanced dorsolateral striatal activity in drug use: The role of outcome in stimulus-response associations." Behav Brain Res **235**(2): 136-142.

Schultz, W. and A. Dickinson (2000a). "Neuronal coding of prediction errors." Annu Rev Neurosci **23**: 473-500.

Schultz, W. and A. Dickinson (2000b). "Neuronal coding of prediction errors." Annu Rev Neurosci **23**: 473-500.

Simon, N. W., I. A. Mendez, et al. (2009). "Effects of prior amphetamine exposure on approach strategy in appetitive pavlovian conditioning in rats." Psychopharmacology (Berl) **202**(4): 699-709.

Smith, K. S. and K. C. Berridge (2005). "The ventral pallidum and hedonic reward: Neurochemical maps of sucrose "liking" and food intake." J Neurosci **25**(38): 8637-8649.

Smith, K. S., K. C. Berridge, et al. (2011). "Disentangling pleasure from incentive salience and learning signals in brain reward circuitry." Proc Natl Acad Sci U S A **108**(27): E255-264.

Stice, E., S. Spoor, et al. (2008). "Relation of reward from food intake and anticipated food intake to obesity: A functional magnetic resonance imaging study." J Abnorm Psychol **117**(4): 924-935.

Suto, N., R. A. Wise, et al. (2011). "Dorsal as well as ventral striatal lesions affect levels of intravenous cocaine and morphine self-administration in rats." Neurosci Lett **493**(1-2): 29-32.

Sutton, R. S. and A. G. Barto (1981). "Toward a modern theory of adaptive networks: Expectation and prediction." Psychol Rev **88**(2): 135-170.

Swanson, L. W. (2005). "Anatomy of the soul as reflected in the cerebral hemispheres: Neural circuits underlying voluntary control of basic motivated behaviors." J Comp Neurol **493**(1): 122-131.

Swanson, L. W. and G. D. Petrovich (1998). "What is the amygdala?" Trends Neurosci **21**(8): 323-331.

Taylor, J. R. and T. W. Robbins (1984). "Enhanced behavioural control by conditioned reinforcers following microinjections of d-amphetamine into the nucleus accumbens." Psychopharmacology (Berl) **84**(3): 405-412.

Timberlake, W., G. Wahl, et al. (1982). "Stimulus and response contingencies in the misbehavior of rats." J Exp Psychol Anim Behav Process **8**(1): 62-85.

Tindell, A. J., K. C. Berridge, et al. (2005). "Ventral pallidal neurons code incentive motivation: Amplification by mesolimbic sensitization and amphetamine." Eur J Neurosci **22**(10): 2617-2634.

Tindell, A. J., K. S. Smith, et al. (2009). "Dynamic computation of incentive salience: "Wanting" what was never "liked"." J Neurosci **29**(39): 12220-12228.

Tindell, A. J., K. S. Smith, et al. (2006). "Ventral pallidum firing codes hedonic reward: When a bad taste turns good." J Neurophysiol **96**(5): 2399-2409.

Tomie, A., M. Lincks, et al. (2012). "Pairings of lever and food induce pavlovian conditioned approach of sign-tracking and goal-tracking in c57bl/6 mice." Behav Brain Res **226**(2): 571-578.

Vanderschuren, L., P. Di Ciano, et al. (2005). "Involvement of the dorsal striatum in cue-controlled cocaine seeking." J Neurosci **25**(38): 8665-8670.

Vanderschuren, L. and B. J. Everitt (2005). "Behavioral and neural mechanisms of compulsive drug seeking." European Journal of Pharmacology **526**(1-3): 77-88.

Volkow, N. D., G. J. Wang, et al. (2002). ""Nonhedonic" food motivation in humans involves dopamine in the dorsal striatum and methylphenidate amplifies this effect." Synapse **44**(3): 175-180.

Volkow, N. D., G. J. Wang, et al. (2002). "Evidence that "non-hedonic" food motivation in humans involves dopamine in the dorsal striatum." Journal of Nuclear Medicine **43**(5): 108p-108p.

Volkow, N. D., G. J. Wang, et al. (2006). "Cocaine cues and dopamine in dorsal striatum: Mechanism of craving in cocaine addiction." J Neurosci **26**(24): 6583-6588.

Wagner, A. R. and R. A. Rescorla, Eds. (1972). Inhibition in pavlovian conditioning: Application of a theory. Inhibition and learning. New York, Acadmic Press.

Wang, H. and V. M. Pickel (1998). "Dendritic spines containing mu-opioid receptors in rat striatal patches receive asymmetric synapses from prefrontal corticostriatal afferents." J Comp Neurol **396**(2): 223-237.

Wassum, K. M., I. C. Cely, et al. (2011). "Mu-opioid receptor activation in the basolateral amygdala mediates the learning of increases but not decreases in the incentive value of a food reward." J Neurosci **31**(5): 1591-1599.

Wassum, K. M., I. C. Cely, et al. (2009). "Disruption of endogenous opioid activity during instrumental learning enhances habit acquisition." Neuroscience **163**(3): 770-780.

Wassum, K. M., V. M. Tolosa, et al. (2012). "Transient extracellular glutamate events in the basolateral amygdala track reward-seeking actions." J Neurosci **32**(8): 2734-2746.

White, N. M. and N. Hiroi (1998). "Preferential localization of self-stimulation sites in striosomes/patches in the rat striatum." Proc Natl Acad Sci U S A **95**(11): 6486-6491.

Will, M. J., W. M. Vanderheyden, et al. (2007). "Striatal opioid peptide gene expression differentially tracks short-term satiety but does not vary with negative energy balance in a manner opposite to hypothalamic npy." Am J Physiol Regul Integr Comp Physiol **292**(1): R217-226.

Willuhn, I., L. M. Burgeno, et al. (2012). "Hierarchical recruitment of phasic dopamine signaling in the striatum during the progression of cocaine use." Proc Natl Acad Sci U S A **109**(50): 20703-20708.

Wilson, C. J. (1987). "Morphology and synaptic connections of crossed corticostriatal neurons in the rat." J Comp Neurol **263**(4): 567-580.

Wise, R. A. (2009). "Roles for nigrostriatal-not just mesocorticolimbic-dopamine in reward and addiction." Trends in Neurosciences **32**(10): 517-524.

Wise, R. A., M. Fotuhi, et al. (1989). "Facilitation of feeding by nucleus accumbens amphetamine injections- latency and speed measures." Pharmacology Biochemistry and Behavior **32**(3): 769-772.

Woolley, J. D., B. S. Lee, et al. (2007). "Nucleus accumbens opioid signaling conditions short-term flavor preferences." Neuroscience **146**(1): 19-30.

Wyvell, C. L. and K. C. Berridge (2000). "Intra-accumbens amphetamine increases the conditioned incentive salience of sucrose reward: Enhancement of reward "wanting" without enhanced "liking" or response reinforcement." J Neurosci **20**(21): 8122-8130.

Wyvell, C. L. and K. C. Berridge (2001). "Incentive sensitization by previous amphetamine exposure: Increased cue-triggered "wanting" for sucrose reward." J Neurosci **21**(19): 7831-7840.

Yager, L. M. and T. E. Robinson (2013). "A classically conditioned cocaine cue acquires greater control over motivated behavior in rats prone to attribute incentive salience to a food cue." Psychopharmacology (Berl) **226**(2): 217-228.

Yin, H. H. (2010). "The sensorimotor striatum is necessary for serial order learning." J Neurosci **30**(44): 1419-14723.

Yin, H. H., B. J. Knowlton, et al. (2004). "Lesions of dorsolateral striatum preserve outcome expectancy but disrupt habit formation in instrumental learning." Eur J Neurosci **19**(1): 181-189.

Yin, H. H., B. J. Knowlton, et al. (2005). "Blockade of nmda receptors in the dorsomedial striatum prevents action-outcome learning in instrumental conditioning." Eur J Neurosci **22**(2): 505-512.

Yin, H. H., B. J. Knowlton, et al. (2006). "Inactivation of dorsolateral striatum enhances sensitivity to changes in the action-outcome contingency in instrumental conditioning." Behav Brain Res **166**(2): 189-196.

Yin, H. H., S. B. Ostlund, et al. (2005). "The role of the dorsomedial striatum in instrumental conditioning." Eur J Neurosci **22**(2): 513-523.

Zahm, D. S. (1999). "Functional-anatomical implications of the nucleus accumbens core and shell subterritories." Ann N Y Acad Sci **877**: 113-128.

Zahm, D. S. (2006). "The evolving theory of basal forebrain functional-anatomical 'macrosystems'." Neurosci Biobehav Rev **30**(2): 148-172.

Zener, K. (1937). "The significance of behavior accompanying conditioned salivary secretion for theories of the conditioned response." The American Journal of Psychology **50**(1/4): 384-403.

Zhang, J., K. C. Berridge, et al. (2009). "A neural computational model of incentive salience." PLoS Comput Biol **5**(7): e1000437.

Zhang, M., C. Balmadrid, et al. (2003). "Nucleus accumbens opioid, gabaergic, and dopaminergic modulation of palatable food motivation: Contrasting effects revealed by a progressive ratio study in the rat." Behav Neurosci **117**(2): 202-211.

Zhang, M. and A. E. Kelley (2000). "Enhanced intake of high-fat food following striatal mu-opioid stimulation: Microinjection mapping and fos expression." Neuroscience **99**(2): 267-277.

Zhu, W. and Z. Z. Pan (2004). "Synaptic properties and postsynaptic opioid effects in rat central amygdala neurons." Neuroscience **127**(4): 871-879.