THE ROLE OF NMDA RECEPTORS IN THE VENTRAL TEGMENTAL AREA IN THE ACQUISITION OF REWARD-RELATED LEARNING

by

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Abstract

THE ROLE OF NMDA RECEPTORS IN THE VENTRAL TEGMENTAL AREA IN THE ACQUISITION OF REWARD-RELATED LEARNING

by

Margaret R. Zellner

Advisor: Professor Robert Ranaldi

The ability to learn about conditioned stimuli (CS) associated with rewards is a crucial adaptive mechanism. Activity in the mesocorticolimbic dopamine (DA) system, a well-established neural component in reward-related learning and motivational processes, as well as in the ventral tegmental area (VTA), the source of mesocorticolimbic DA, is correlated with responding to and learning about CSs. The mechanism by which VTA neurons become activated by signals associated with environmental stimuli is currently unknown. This dissertation tested a hypothesis arising from a model suggesting that NMDA receptor stimulation in the VTA allows previously weak glutamate signals carrying putative information about environmental stimuli, coincident with strong excitation correlated with receipt of primary rewards, to be strengthened and thereby acquire the ability to activate VTA neurons in themselves, leading to increased approach behavior. Furthermore, once synaptic strengthening has

taken place, the model suggests that NMDA receptor stimulation is not necessary for the expression of reward-related learning. In particular we assessed whether intra-VTA application of AP-5, a selective competitive NMDA receptor antagonist, would impair the acquisition of reward-related learning. Intra-VTA injection of AP-5 impaired the acquisition of lever pressing for food, and the acquisition of conditioned approach. In contrast, AP-5 did not impair the expression of instrumental learning once acquired. Control studies demonstrated that the impairment in learning was not due to reduction in basic food motivation or generalized activity, as separate groups of rats treated with AP-5 did not differ in consumption of rat chow or in ambulatory activity, while showing a mild increase in stereotypy. Analysis of response-reward latencies indicated that any stereotypy that might have occurred during instrumental learning did not cause AP-5 treated rats to experience a reduction in reward contingencies. Finally, rats consuming operant chamber pellets under the influence of intra-VTA AP-5 demonstrated that the reward value of pellets was not impaired by treatment, as AP-5 and control rats emitted similar levels of lever presses for a pellet-associated CS in an extinction session. These findings support the hypothesis that NMDA receptor stimulation in the VTA is necessary for the acquisition, but not expression, of reward-related learning.

Foreword

Portions of this manuscript have been submitted for publication.

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List of Abbreviations

6-OHDA	6-hydroxydopamine
ACC	anterior cingulate cortex
ACh	acetylcholine
AMPA	alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
AP-5	2-amino-5-phosphonovalerate or 2-amino-5-phosphopentanoate
BLA	basolateral nucleus of the amygdala
BNST	bed nucleus of the stria terminalis
CaMK-II	Ca^{2+}/calmodulin-dependent protein kinase II
cAMP	cyclic AMP
CeN	central nucleus of the amygdala
CPP	conditioned place preference
CS	conditioned stimulus
DA	dopamine
EEA	excitatory amino acid
EPSP	excitatory post-synaptic potential
FR1	fixed ratio 1
GABA	gamma-aminobutyric acid
IP	intraperitoneal
ISI	inter-stimulus interval
LDTg	laterodorsal tegmental nucleus

LH	lateral hypothalamus
LTD	long-term depression
LTP	long-term potentiation
mAChR	muscarinic acetylcholine receptor
mOFC	medial orbitofrontal cortex
NAcc	nucleus accumbens
nAChR	nicotinic acetylcholine receptor
NMDA	N-methyl-D-aspartate
PBN	parabrachial nucleus
PFC	prefrontal cortex
PIT	Pavlovian-to-instrumental transfer
PKA	protein kinase A
PPTg	pedunculopontine tegmental nucleus
rNTS	rostral nucleus tractus solitarius
SNc	substantia nigra, pars compacta
TH	tyrosine hydroxylase
US	unconditioned stimulus
VTA	ventral tegmental area

Chapter 1 General Introduction

The ability to learn about stimuli associated with rewards – which may indicate their location, palatability, likelihood of appearing, actions that produce them, or other characteristics – is a crucial adaptive mechanism. Broadly speaking, stimuli associated with rewards are called "conditioned stimuli" (CSs), and an extensive behaviorist literature documents the ways in which conditioned stimuli can affect the acquisition, maintenance, and parameters of behavior (e.g., Morse and Skinner, 1958). The neural circuits underlying the formation of conditioned associations and the behavior influenced by conditioned stimuli has been an active focus of behavioral neuroscience research for over five decades (Stein, 1958). From a basic science perspective, understanding the neural mechanisms underlying the life-sustaining capacity of learning about stimuli associated with reward is important. But it is more immediately significant because disorders of reward-related learning appear to play a significant role in pathologies such as drug addiction (see, for example, Kelley and Berridge, 2002; Kalivas, et al., 2005; Di Chiara and Bassareo, 2007). Drug-associated stimuli induce sympathetic arousal (Ehrman, et al., 1992) and craving in abstinent addicts (Ehrman, et al., 1992; Maas, et al., 1998; Childress, et al., 1999). Contingent presentation of CSs associated with drugs and natural rewards facilitate the acquisition of a novel operant response (e.g., Di Ciano and Everitt,

2004a) and are associated with increased responding during drug self-administration (Di Ciano and Everitt, 2003) and in extinction (Ranaldi and Roberts, 1996; Di Ciano and Everitt, 2003). Non-contingent presentation of drug CSs or contextual cues also increase instrumental responding in extinction (Di Ciano and Everitt, 2003; Bossert, et al., 2004). Similarly, stimuli associated with natural rewards can support or reinstate reward-seeking (e.g., Yun, et al., 2004a). Given that stimuli associated with rewards have such strong effects on behavior, researchers hope that understanding the neural mechanisms underlying these processes will lead to new and more effective treatments for addiction and other reward-related pathologies.

Ever since early studies identified the importance of dopamine (3-hydroxytyramine) (DA) in operant responding for intracranial self-stimulation and "natural" rewards (e.g., Wise, et al., 1978), a large body of evidence has accumulated which implicates the DA system in processes of reward, motivation, and learning. One of the main sources of forebrain DA, which was first discovered in brain tissue in 1958 (Carlsson, et al., 1958), is the group of dopaminergic neurons in the ventral tegmental area (VTA), the A10 cell group. Because these neurons give rise to high-volume projections to olfactory tubercle and nucleus accumbens, which in turn are heavily innervated by hippocampus and amygdala, this system was termed "mesolimbic" by Ungerstedt (1971). VTA neurons also project to cortical regions involved with reward, and therefore the system may also be termed "mesocorticolimbic," with distinct mesolimbic and

mesocortical projections. Overall, the mesocorticolimbic system is considered to be critical for the acquisition and maintenance of reward-related behavior. This system is also involved with fear conditioning (Pezze and Feldon, 2004), although, because this dissertation focused exclusively on positive reinforcement processes, aversive conditioning will not be reviewed further. Activity in the VTA and its terminal regions is robustly linked with behaviors directed towards biologically significant "natural" rewards such as food and sexual partners, "artificial" rewards such as drugs of abuse, and, most importantly from the point of view of the present work, CSs associated with rewards.

From the perspective of learning, which this dissertation is investigating, reward is related to reinforcement, but we will attempt to make a distinction between those terms. Some of the debates about the role of DA in reward and reinforcement (to be discussed below) may be due to the same terms having different meanings for different investigators, or dissociable processes being collapsed under a single term. For purposes of this dissertation, reward is defined as an unconditioned stimulus that elicits approach behavior (Ikemoto and Panksepp, 1999). Reinforcement refers to a behavioral process whereby a particular consequence is associated with an increased likelihood of the emission of a particular behavior (Mackintosh, 1974). As a strictly external, objective measurement, the term "reinforcement" remains agnostic about whether the reinforcer is experienced hedonically or not, although the literature indicates that rewards are hedonically positive, and conversely that substances or experiences

that are positive can function as reinforcers (Panksepp, 1998). In this dissertation, therefore, "reward" will refer to the substance that is unconditionally approached or consumed, and "reinforcement" will refer to the process by which responses are likely to re-occur, given an association between a stimulus or action and a reward.

It should be noted that reinforcement or reward are not necessary for all forms of learning (Bolles, 1972). In latent learning paradigms, for example (Blodgett, 1929; Tolman and Honzik, 1930), animals are exposed to spatial or stimulus cues that are not paired with reward, and when later reinforcement contingencies are introduced, animals demonstrate that they have already learned something about the environment or stimuli. Reward is not necessary for learning stimulus-stimulus associations or spatial mapping of a particular context, and these elements are certainly involved in any situation where animals are learning about cues that are associated with reward. However, in this dissertation we are specifically concerned with reward-related learning.

Conditioned stimuli play a number of roles in reward-related behaviors. For animals and humans, conditioned stimuli may be any of a vast number of stimuli present in situations where rewards are experienced, related to the reward itself, events that occur in proximity to encountering the reward, actions taken to obtain reward, and attributes of the environment in which rewards are obtained. Within experimental paradigms CSs tend to be used in several specifically defined categories. First, stimuli presented contiguously with

rewards can come to elicit responses similar to those elicited by the reward itself. This kind of conditioning is called "Pavlovian" or "classical" conditioning, after the Russian physiologist Ivan Pavlov (Mackintosh, 1974). When stimuli elicit behaviors that are automatically or reflexively directed at the CS itself, rather than the associated unconditioned stimulus (US), such as pecking a key that is spatially distant from a food trough, even when such pecking postpones reward delivery (Williams and Williams, 1969), or pecking a key associated with water delivery in the "drink" position (Jenkins and Moore, 1973), this behavior may be called "auto-shaping" (Brown and Jenkins, 1968) or "sign-tracking" (Hearst and Jenkins, 1974). However, in a broader sense, any stimuli which are passively associated with reward and not contingent on the emission of any specific behavior are considered products of Pavlovian or classical conditioning – for example, when animals approach a food trough after presentation of a CS, or learn to associate a particular flavor with a particular post-ingestive consequence. Such CSs may be said to "predict" reward delivery.

Second, conditioned stimuli are relevant in instrumental or operant responding paradigms, in which presentation of rewards are contingent on an animal emitting a particular action such as pressing a lever (Mackintosh, 1974). In these paradigms, a stimulus (such as a light or tone) is presented simultaneously with a reward. CSs presented in this manner can facilitate the acquisition of an instrumental response. Once the reward association is established, these CSs can maintain responding at higher levels in protocols

which require more responses from the subject, such as second-order schedules in which a certain number of actions elicit a presentation of the CS, and a certain number of CSs must be earned to acquire a reward (Kelleher, 1966).

Third, a stimulus may signal that an action taken will result in reward, in which case it is called a "discriminative stimulus" (DS) (Mackintosh, 1974). The types of CS described so far are typically discrete events, such as tones or lights, although they may be present for prolonged periods or may always inherently be associated with a particular substance, such as flavors. The final category of CSs, contextual cues, are present continuously, such as the smells, textures and visual attributes of an operant chamber or maze. These contextual cues can also control behavior, for example by invigorating exploratory and operant responding when animals are first put in a chamber, or reinstating operant responding by restoring cues that had previously been absent during extinction periods.

All of these categories of conditioned stimuli are involved in orienting, energizing, or maintaining behaviors, both when primary rewards are present and during extinction responding when primary rewards are not delivered. The ability of CSs to attract attention and elicit approach is a well-established phenomenon in behavioral studies (Hearst & Jenkins 1974; Tomie, Brooks & Zito, 1989). There is a long-standing debate in the behavioral literature as to whether Pavlovian and instrumental conditioning represent two absolutely distinct processes, or whether Pavlovian conditioning is always a component of instrumental learning; this is often referred to as "two-process learning theory"

(see Rescorla and Solomon, 1967 for history and critique of the concept). The desire to illuminate whether these processes are indeed separate or overlapping at the neural level has catalyzed a wide variety of research efforts in behavioral neuroscience in recent decades. Although there may be debate about the exact role that CSs play in reward-related behavior, what is certain is that, in all of the paradigms in which CSs do strongly affect behavior, a previously neutral stimulus must become associated with some aspect of the receipt of reward, and thereby become a conditioned stimulus. In recent decades, an active effort has been underway to map out the neural circuits involved with the effects of conditioned stimuli on behavior. As will be reviewed below, a tremendous amount of research has documented the extent to which activity in the mesolimbic DA system is highly correlated with behavior related to conditioned stimuli. However, it is not yet clear how associations between stimuli and reward are formed at the neural level.

Activity in the VTA and its terminal regions is correlated with consumption of primary rewards, as well as with the presentation of novel and aversive stimuli (see Fields, et al., 2007 for review). This activity appears to be linked to pathways that carry information about rewards and unconditioned environmental stimuli, such as excitatory projections from the cholinergic pontine nuclei or the superior colliculus. What remains to be addressed is the neural mechanism by which activity in the VTA and terminal regions also becomes correlated with the presentation of conditioned stimuli. The question is,

in other words, how does the VTA "come to know" about conditioned stimuli? This dissertation seeks to make a contribution to answering this question. In what follows, we will suggest that one substrate of the acquisition process is glutamatergic stimulation of NMDA (N-methyl-D-aspartate) receptors in the VTA.

Overview of Dopamine Physiology

Because the research to be described in this dissertation involves manipulating a source of mesolimbic DA, a brief review of DA's post-synaptic effects is warranted, which will also provide a context for a discussion of the competing theories of DA's role in reward-related learning.

At the neuronal level, some aspects of the action of DA are fairly well understood. Dopamine performs a modulatory role, acting via second-messenger systems to affect slow synaptic transmission (on the order of tens of milliseconds to seconds, compared to fast transmission on the order of milliseconds, mediated by glutamate and GABA [gamma-amino-butyric acid]) (Greengard, 2000). Receptors for DA are grouped primarily according to their interaction with cyclic AMP (cAMP) (see Kebabian and Caine, 1979 for review). When coupled to the D1 family of DA receptors, DA instigates the activation of cAMP via a G_{olf} activation of adenylate cyclase, initiating a cascade of processes which result in phosphorylation of AMPA (alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptors which increase their sensitivity to glutamate,

making the neuron more excitable by several mechanisms, including enhanced surface expression of AMPA and NMDA receptors (see Surmeier, et al., 2007 for review). In contrast, when bound to the D2 family of receptors, DA tends to have inhibitory effects. D2 receptors couple to $G_{i/o}$ proteins, which, when activated, inhibit adenylate cyclase and have inhibitory downstream effects, including decreasing AMPA currents, reducing opening of Na^+ channels, and promoting opening of K^+ channels (Surmeier, et al., 2007). Moreover, D1 and D2 receptors are present in different ratios in different brain regions, and exist in some areas as autoreceptors as well, leading to opposite effects on DA cells than post-synaptic neurons. It is not surprising, therefore, that early findings on DA's effects presented a somewhat confusing picture (Lavin and Grace, 2001), as it may have an inhibitory or an excitatory influence, depending on the receptors involved and other factors. For example, the application of DA in the nucleus accumbens has been found to inhibit cell firing, whereas single pulses of stimulation of VTA cells are correlated with excitation in some nucleus accumbens (NAcc) cells and inhibition in others (Yim and Mogenson, 1982). Furthermore, NAcc DA application or VTA stimulation has the tendency to reduce amygdala-induced excitation in the NAcc (Yim and Mogenson, 1982). As the NAcc maintains inhibitory connections with the ventral pallidum, these different patterns of excitation and inhibition of NAcc neurons in response to environmental cues may release certain appropriate actions and inhibit tangential ones as animals orient towards and work for rewards.

A clearer picture emerges on a micro time scale, as the effects of DA appear to depend on the state of the neuron (Arbuthnott and Wickens, 2007). Dopamine from the VTA released in the prefrontal cortex (PFC) has dissociable effects on neurons depending on whether they are in an "up" or "down" state (Lewis and O'Donnell, 2000; Lavin and Grace, 2001). PFC neurons exhibit a bistable membrane potential that shifts between a hyperpolarized "down" state during which action potentials are unlikely, and a depolarized "up" state during which action potentials occur. While the transition to the "up" state does not appear to depend on DA, stimulation of D1 receptors appears to prolong the "up" state (Lewis and O'Donnell, 2000) and enhances the excitability of the neuron (Lavin and Grace, 2001). Furthermore, DA release in the prefrontal cortex (PFC) following burst stimulation of DA neurons reduces spontaneous firing, while potentiating evoked firing over a scale of tens of minutes (Lavin et al 2005). It has thus been suggested that the combined influences of these effects of DA may be to filter out irrelevant behavior or stimuli, and facilitate associative processes between strong or coincident inputs because of prolonged excitation and recruitment of NMDA receptors (Lewis and O'Donnell, 2000). In other words, as Fields and colleagues (2007) argue, because DA appears not to mediate rapid postsynaptic potentials, "dopamine's action on target neurons is critically dependent on concomitant activity in other afferents to those neurons" (p. 299).

Finally, in addition to affecting how postsynaptic neurons respond to other input, DA also plays a role in synaptic plasticity (see Beninger and Miller,

1998; Jay, 2003 for review). DA binding at the D1 receptors activates adenylate cyclase, increasing the amount of cAMP produced, which activates PKA. PKA phosphorylates AMPA and NMDA receptors, as well as phosphorylates CREB, thereby boosting production of various proteins involved with synaptic activity; and DAARP-32, which increases CaMK-II activity. These events, in conjunction with increased Ca^{2+} currents due to NMDA receptor stimulation, are thought to facilitate synaptic strengthening (Jay 2003).

Theories of Dopamine's Role in Reward-Related Learning

Investigating the VTA's role in conditioned stimuli-related processes takes place within a larger context of research on the DA system and its role in complex behavioral processes such as reward, motivation, addiction, general activity, memory, and executive function. This field of research, while generating a wealth of data addressing a range of constructs, using a variety of techniques and behavioral measures, has yet to yield agreement among researchers about the fundamental role of DA. In what follows, some of the leading perspectives on DA will be very briefly summarized, to set a context for a review of DA's role in relation to conditioned stimuli in particular. Later in the discussion some additional reflections on a role for DA in reward-related learning will be offered, based on that review.

Most researchers would agree that on the broadest level, DA is involved with energizing motor behavior (Beninger, 1983). Treatment with large doses of

DA antagonists, ablations of DA cells and DA knockout models are associated with sluggish or akinetic states (Carli, et al., 1985; Fowler and Liou, 1998; Robinson, et al., 2005), and the DA neuron degeneration found in Parkinsonian patients is correlated with their motor deficits (Marsden, 1984). Conversely, manic episodes and cocaine intoxication, which are both associated with high levels of DA transmission, are marked by psychomotor agitation (American Psychological Association, 2000). However, even from the outset, it is conceptually difficult to separate motor behavior per se from incentive motivation or effort. To put the matter simplistically, are animals or people with DA deficits or blockade sluggish because they *can't* move, or because they are not *motivated* to move? Are cocaine addicts hyperactive because their motor circuits are sensitized, or because they find the world more engaging and salient? However, although there may be a operational and neural overlap between motor behavior and motivation, it appears that aside from massive DA blockade or complete DA knockout, reductions in reward-related behavior result from specific motivational effects and not motoric ones, as it can be demonstrated that animals are still capable of responding although response levels have declined under DA antagonism (see Wise, 2006 for review). Because a review of basic motor processes widens the scope of discussion beyond the specific area of this dissertation, this question will not be resolved here but gives an indication that DA may be seen as affecting processes beyond reward, motivation and learning.

To move to the next most fundamental level beyond general motor activation, DA has been seen as playing a role in basic reward, most familiar in Wise's (1978) "anhedonia" hypothesis that DA blockade reduces the "goodness" of rewards. Supporting evidence, to be discussed in more detail below, for this position includes findings of increases in DA levels in terminal regions (e.g., Taber and Fibiger, 1997) and VTA activity (e.g., Ljungberg, et al., 1992) during consumption of food rewards. However, the weight of evidence currently suggests that DA is not necessary for consummatory processes; blockade of DA does not impair consumption of food (see Kelley, et al., 2005 for review) or positive hedonic reactions to food (see Berridge, 2007 for review).

A more refined version of the basic reward hypothesis is that DA plays a key role in reinforcement (Beninger, 1983; Wise, 2006). In other words, DA release following receipt of reward or perception of conditioned stimuli serves to increase the likelihood of reward-related behavior. Key pieces of evidence for this position include the findings that well-trained animals responding for food under DA blockade respond normally at the outset but progressively reduce responding within and across sessions (Wise, et al., 1978) and animals treated with DA antagonists show a reduction in running speed for reward in a session following one with DA antagonism, not during (McFarland and Ettenberg, 1998). Viewing DA as necessary for reinforcement allows for the possibility that DA transmission is an inherent element in the "goodness" of a reward as part of that process, or that it is dissociable. Therefore, a reinforcement model of DA function

is quite different from an anhedonia one. However, as some theorists who critique the anhedonia hypothesis appear not to recognize (e.g., Salamone, et al., 2007) or only acknowledge after an extensive critique (e.g., Berridge, 2007), Wise has moved beyond that position to one focusing on reinforcement.

The reinforcement perspective on DA encompasses both learning, as behavior need to be reinforced when an animal is naïve in order for reward-related responding to be acquired, and performance, as responses emitted once a behavior has been acquired that are not reinforced will tend to extinguish (Beninger, 1983; Wise, 2004). Some researchers have emphasized one or the other of these two components in their perspectives on DA. While DA is recognized by numerous investigators as playing a role in reward-related learning (e.g., Beninger, 1983; Wickens, 1990), Horvitz and colleagues (Horvitz, et al., 2007) argue that DA transmission plays a greater role in the early stages of learning than in later performance. Evidence for this perspective includes the finding that systemic D1 antagonism reduces head entries both before and during a food-associated cue early in training, but only reduces entries before the cue following extensive training, with responding during the cue preserved (Choi, et al., 2005). This position is further supported by the findings of increased DA immunoreactivity in several terminal regions during the early sessions of Pavlovian and instrumental conditioning, but not later sessions (Hitchcott and Phillips, 1998) and the finding that D1 receptor antagonism in the amygdala, both in the central nucleus (CeN) and basolateral nucleus (BLA)

(Andrzejewski, et al., 2005) impairs the acquisition, but not expression, of instrumental responding.

In contrast, a greater emphasis on performance has been asserted by Salamone (e.g., Salamone, et al., 2007), arguing that DA mediates the expenditure of effort in reward-related behavior. Key pieces of evidence for this perspective include the finding that, when given a choice between a smaller reward that is easy to obtain, and a larger reward that requires more work (such as lever pressing or climbing a barrier), animals under DA antagonism will choose the option requiring less effort, without affecting their consummatory behavior (Salamone, et al., 2007). As reviewed below, the findings that increases in DA levels or VTA firing is often correlated with the presentation of a conditioned stimulus, approach to an operandum, or initiation of a response supports this view. An exclusive role for effort as opposed to reinforcement is made problematic, however, by the evidence referred to above that animals will behave normally during initial periods of DA antagonism and then show reductions in behavior; if DA is only important for effort, antagonism should always result in low levels of reward-related behavior from the outset. Evidence also exists that dopamine antagonists, both systemic and intracerebral, can reduce levels of operant responding before an animal has experienced a primary reward (see, for example, See, et al., 2001; Cervo, et al. 2007), which again demonstrates both the importance of dopamine for incentive motivation, and the

complexity of dopamine's effects and the difficulties in categorizing its function in simple terms.

Another perspective on DA related to performance is the suggestion that a key aspect of DA function is to enable behavioral "switching" (Redgrave, et al., 1999; Nicola, 2007), wherein increases in DA levels following novel or salient events facilitate a change in the animal's orientation or behavioral strategy. However, recent evidence indicates that, at least at the level of the VTA, cells respond more to the outcome-related properties of conditioned stimuli, in the context of both on-going behavior and shifts in responding (Wilson and Bowman, 2006).

Schultz and colleagues (e.g., Fiorillo, et al., 2003) have interpreted the activation of midbrain DA neurons, which occurs in response to unpredicted primary rewards and conditioned stimuli, as will be reviewed below, as a "reward prediction error" signal, that indicates whether or not received rewards match expectancies. As expectancies are matched or not, the DA signal is thought to either maintain or change behavior. This perspective allows for a role in DA in both learning and performance, as "better-than-predicted" rewards induce learning and "worse-than-predicted" rewards induce extinction.

This brief review of the different perspectives does not begin to do justice to the complexity and nuance of current thinking on this topic, but attempts to indicate the differences between various ideas about DA. It is generally recognized that DA is involved with a range of functions, playing a variety of

roles within a complex set of circuitries which organize behavior influenced by DA's global effects at different time courses, energizing and motivating a broad range of learning processes and goal-directed behaviors (e.g., Wise, 2004; Arbuthnott and Wickens, 2007; Berridge, 2007; Schultz, 2007; Alcaro, et al., 2007). While a unified theory of DA function has yet to emerge, most researchers would agree that the mesocorticolimbic DA system plays a central role in motivated behavior, although other circuitries and transmitters are also involved. Therefore this system is obviously a target of research on the underlying neural substrates of the acquisition of conditioned-stimulus associations.

Behaviorally oriented theorists have long proposed that reward-related stimuli gain access to the same motivational neural circuits as primary rewards and thereafter come to activate these circuits and elicit motivational states similar to the primary reward (Bolles, 1972; Bindra, 1974; Beninger and Ranaldi, 1994; Wise, 2004). The exact mechanism by which these processes take place is currently an active area of research, and to date, leading researchers with decades of experience in the field are still in disagreement about some of the unifying principles of what DA does in relation to conditioned stimuli processes. We would argue the role of DA probably plays several different roles in a sequence during the acquisition of reward-related learning. First, DA release during the encounter with primary rewards initially spurs increased investigatory and approach behavior; second, synaptic changes in the VTA allow environmental stimuli to additionally recruit VTA cells, augmenting DA release

and approach behavior, putting the animal in greater proximity to rewards and related cues or operanda; third, DA release at the terminal regions due to primary rewards and CSs facilitate associative processes at the those levels which underlie further stimulus-stimulus, stimulus-response, and stimulus-outcome associations. Once CS associations have been acquired, either primary rewards or CSs can continue to activate VTA cells, providing for DA release that will maintain approach behaviors and motivational processes. It is possible that understanding the role of DA at different levels of the system, and different stages of learning and performance, can set the stage for integrating most of the competing views on DA that have been proposed to date.

In the studies to be described in this dissertation, we examined associative processes in the VTA, which is the source of mesocorticolimbic DA, and did not measure or manipulate activity in terminal regions. Therefore, while we presume DA transmission was affected, we cannot assess whether our effects were due to changes in DA transmission, and are therefore not in a position to make any determinations about reconciling any particular theories of DA function. Indeed, doing such a thing is not even possible within one set of studies; however, a review of the competing ideas is necessary to establish the framework within which the research is conducted. Some speculations about ways to reconcile various disputing ideas about DA will be raised in the discussion in Chapter 9.

Hypothesis Guiding This Research

We and others have suggested that one component of the process by which conditioned stimuli gain access to motivational circuits is synaptic plasticity in the VTA (e.g., Harris, et al., 2004; Sharf, et al., 2006). The VTA receives excitatory input from structures which are likely to convey signals of primary reward, such as the mesopontine cholinergic nuclei, as well as areas that convey information about environmental stimuli, including the superior colliculus and prefrontal cortex, and is therefore a likely site for associative processes arising from convergent stimulation. Long-term potentiation (LTP) is currently thought to be one of the major mechanisms of synaptic changes underlying learning, and is dependent on NMDA receptor stimulation (Citri and Malenka, 2007). NMDA receptors are found in the VTA (Rodriguez, et al., 2000). And in fact, LTP has been demonstrated in VTA DA neurons, where it is NMDA receptor-dependent (Bonci and Malenka, 1999). We propose, therefore, that one component of reward-related learning is synaptic strengthening at glutamate synapses whereby glutamate inputs associated with previously neutral environmental signals come to activate VTA cells, augmenting DA release at terminal regions. This increased DA release triggered by VTA activation through strengthened glutamate synapses might lead to increased approach behaviors, increasing the likelihood of direct contact with rewards or emission of behaviors which lead to reward, or to associative processes upstream in DA terminal regions which will facilitate other aspects of conditioned behavior.

To illuminate the context of this research on associative processes in the VTA that may underlie the acquisition of reward-related behavior, it is necessary first to establish that activity in the mesolimbic DA system, and in the VTA itself, is linked both to food reward and to responses to conditioned stimuli. The putative sources of excitation to the VTA that carry signals of primary reward and environmental stimuli, and the effects of glutamate transmission in the VTA, will be reviewed. Finally, the circuits involved with the acquisition of responses to conditioned stimuli will be discussed. Throughout, the majority of studies cited in this review used rats as the experimental subjects; where other species were used that will be made explicit.

Overview of the Ventral Tegmental Area

The VTA projects to the medial prefrontal cortex; olfactory tubercle and entorhinal cortex; amygdala; ventromedial striatum, particularly the nucleus accumbens; thalamus; posterior, lateral and preoptic areas of hypothalamus; bed nucleus of the stria terminalis; nucleus of the diagonal band; lateral septal nucleus; and brainstem areas including several raphe nuclei, parabrachial nucleus, and locus coeruleus (Beckstead, et al., 1979; Swanson, 1982; Loughlin and Fallon, 1984; Geisler and Zahm, 2005). Projections to the PFC and NAcc – mesoprefrontal and mesoaccumbens, respectively – as well as other structures such as the septum and inferior olive arise from separate, noncollateralized populations (Fallon, 1981; Swanson, 1982; Fallon, et al., 1984; Loughlin and

Fallon, 1984). Afferents to the VTA will be reviewed further on in this discussion.

The VTA is modulated by a host of neurotransmitters and peptides, including glutamate, GABA, serotonin, acetylcholine, norepinephrine, opioids, and peptides including CCK and orexin (see Kalivas, 1993; Meltzer, et al., 1997; Mathon, et al., 2003 for reviews). The VTA contains both DA neurons ("principal" cells), which are distinguished by long duration action potentials and hyperpolarization to DA but not met-enkephalin, and GABA neurons ("secondary" cells), which have short duration spikes and are hyperpolarized by met-enkephalin but not DA (Johnson and North, 1992). The GABAergic neurons project both within the VTA itself (as interneurons) and outside the VTA (Van Bockstaele and Pickel, 1995; Carr and Sesack, 2000b). The VTA also contains a third kind of cell that has yet to be definitively categorized, which responds to neither DA nor opioids (Johnson and North, 1992); recent evidence demonstrates the presence of glutamatergic neurons in the VTA which are non-dopaminergic and non-GABAergic, which may constitute this third type of VTA neuron (Yamaguchi, et al., 2007). It should also be noted that evidence suggests that glutamate is released by DA neurons in the NAcc (Chuhma, et al., 2004) and PFC (Lavin, et al., 2005) which might account for short-latency EPSPs in terminal regions that cannot be initiated by DA, which has a course of action with a longer time frame.

Studies using in vivo recording techniques to investigate VTA activity in response to CSs have used several criteria to determine whether the units recorded are dopaminergic. The classical characteristics of DA neuron firing involves relatively long duration (approximately 2.75 ms) biphasic action potentials with an initial positive wave and a prominent negative phase occurring as spontaneous irregular spikes with interspike intervals of 200-250 ms, and average firing rate of 4.5 spikes/s (Bunney, et al., 1991). Most investigators accept these criteria as identifying DA neurons, but categorical identification of the types of cells being recorded is generally impossible; therefore, any in vivo study must always presume, but cannot prove, that the neurons being recorded are dopaminergic. Indeed, some investigators have found that VTA neurons are more heterogeneous in their firing patterns and types of conducting currents (Kiyatkin and Rebec, 1998; Margolis, et al., 2003; Wilson and Bowman, 2006) and suggest that these criteria need to be substantially modified to reflect the wider categories of VTA neurons (Margolis, et al., 2003; Wilson and Bowman, 2006). For example, a substantial minority (<40%) of tertiary neurons have been found to be dopaminergic (Margolis, et al., 2003). Therefore, although this review will not qualify each citation as pertaining to "presumed DA neurons," any reference to in vivo DA recordings should be read as such.

As we review characteristics of VTA neurons, a similar cautionary statement should be made about the regions included in studies investigating

DA neurons. Many in vivo investigations of responses of VTA cells to reward-related stimuli included measurements of both VTA and substantia nigra, pars compacta (SNc) neurons without reporting a breakdown of data according to placement. However, whenever patterns of activity have been analyzed by region, they have generally been found to be substantially similar (e.g., Ljungberg, et al., 1992; Pan, et al., 2005; also noted by Hyland et al., 2002). In this review, studies which focused on the SNc exclusively are not included, for several reasons. SNc and VTA neurons have divergent, as well as shared, afferents and efferents; the SNc is predominantly dopaminergic and the VTA more heterogeneous, including DA, GABA, and presumed glutamate neurons; and pharmacological differences between the regions have been observed (for review see Korotkova, et al., 2004). In addition, functional differences have been found between the VTA and the substantia nigra in reward-related behavior, such as increased glutamate release in the VTA during the onset of instrumental responding, which is not found in the SNc (You, et al., 2007), and increased DA immunoreactivity in VTA terminal regions (e.g., mPFC, amygdala and NAcc) but not SNc terminal regions (e.g., dorsomedial and dorsolateral striatum) in Pavlovian conditioning and instrumental learning (Phillips, et al., 2003b). Therefore, findings in the substantia nigra should not be taken to apply to the VTA necessarily. References to "midbrain DA neurons" herein will mean that the cited studies investigated both SN and VTA; where investigators have

provided regional categorization, substantial VTA participation was described, or the VTA exclusively was investigated, I will refer to VTA neurons.

DA neurons in the VTA show two types of discharge patterns: single spike mode or bursting mode, with bursts manifesting typically as 3-10 spikes with interspike intervals ranging from 50-100 msec (Bunney, et al., 1991). In anesthetized animals, spontaneous activity is primarily single spike, and in brain slices spiking is exclusively single spiking, in a pacemaker fashion, indicating that excitatory input is required for bursting (Bunney, et al., 1991). After the first determination that bursting in vivo is regulated by excitatory amino acids (EAA), because the broad spectrum excitatory amino acid (EEA) antagonist kynurenate abolishes spontaneous burst firing (Grenhoff, et al., 1988), further results indicated that bursting is triggered by NMDA but not kainate or quisqualate, although high levels of NMDA terminate firing due to a prolonged depolarization block (Wang, et al., 1994). Depolarization induced by NMDA receptors stimulation appears to be due to an inward calcium and sodium current (Mercuri, et al., 1993).

Burst firing leads to higher extracellular concentrations in a manner not linearly related to rate of firing; bursts release twice as much DA as the same number of non-burst spikes (Bunney, et al., 1991; Chergui, et al., 1994). The increase in DA levels found after bursting activity is due to at least two factors: first, significantly more DA is released at the terminal in the second through fourth pulses following the initiation of a train of burst impulses, and second, the

higher amounts of DA overwhelm re-uptake mechanisms in the short time span of bursting release (Chergui, et al., 1994). The functional significance of burst firing, which releases significantly more DA in the synaptic cleft that then can diffuse into the extrasynaptic space, may be that DA signals are available across a longer time span, allowing for the integration of various inputs at terminal regions (Bunney, et al., 1991; Arbuthnott and Wickens, 2007).

Mesocorticolimbic Activity in Relation to Primary Reward

A number of the mesocorticolimbic DA terminal regions demonstrate activity correlated with various phases of reward-related behavior. The region receiving the most investigational attention is the nucleus accumbens, with the medial prefrontal cortex and amygdala also showing numerous indications of activity in relation to reward processes. All of the terminal regions are involved in responding to conditioned stimuli. A review of some of these findings sets the context for studies of the VTA's role in conditioned stimuli processes, as it is the source of DA innervation for those regions. In what follows, therefore, CS-related activity in the mesolimbic terminal regions will be reviewed, to set the context for the smaller number of studies which show similar types of CS-related activity in the VTA. To begin with, however, activity related to food consumption in the nucleus accumbens, which receives the densest innervation of DA fibers from the VTA (Fields, et al., 2007), will be reviewed. Because food is a paradigmatic "natural" reward that must activate DA neurons through sensory

relays, it will be focused on in the review that follows, as opposed to drugs of abuse which globally affect DA transmission exogenously. Increased activity in the NAcc in relation to primary reward provides evidence for an activation in the VTA in relation to primary reward, first because DA in the NAcc comes primarily from release at the terminals due to VTA firing (Phillips, et al., 1992) and DA release mainly depends on action potentials (Gonon, 1988; Phillips, et al., 2003c; Roitman, et al., 2004), although presynaptic glutamatergic facilitation from other NAcc afferents including amygdala, hippocampus, and prefrontal cortex (Fields, et al., 2007) also plays a role, and second, because activity in the NAcc is tightly linked to VTA firing (Yun, et al., 2004b). Therefore, increased DA or activity in the NAcc provides evidence of activation of the VTA in relation to primary reward, which is a necessary element in synaptic strengthening processes which may allow CSs to later activate the same cells.

DA levels in the NAcc increase in food deprived animals during free-feeding and drinking (Taber and Fibiger, 1997; Westerink, et al., 1997; Schilstrom, et al., 1998b), animals consuming a meal signaled by a cue (Phillips, et al., 1993; Wilson, et al., 1995), free-feeding animals consuming a palatable meal (Wilson, et al., 1995; Bassareo and Di Chiara, 1999a), and food-deprived animals engaged in instrumental responding for natural rewards such as food and water (Church, et al., 1987; Hernandez and Hoebel, 1988; Richardson and Gratton, 1996). Clearly consumption of food is linked to increased NAcc DA levels. However, DA release in response to food consumption depends on the context. For example,

DA release in the NAcc shell during unpredicted consumption of a palatable meal is pronounced, whereas DA levels do not change when the meal receipt is signaled by a cue, and DA release in the NAcc core is mild when meal is unpredicted and somewhat higher when it is preceded by a cue (Bassareo and Di Chiara, 1999a).

NAcc neurons have also been shown to fire during consumption of unconditioned stimuli (e.g., Schultz et al., 1992, Wilson and Bowman 2004, Nicola et al. 2004a, b). In addition, NAcc neurons encode palatability based on taste and satiety, as firing patterns tend to increase with palatability, and decrease across a session as satiation occurs (Taha & Fields 2005). Similarly, activity in NAcc and medial orbitofrontal cortex (mOFC) in humans show increased activity during receipt of temporally unpredicted oral rewards (Berns, et al., 2001). However, studies that use techniques measuring DA changes or cell firing on a shorter time scale (in the millisecond to second range, compared to microdialysis techniques which measure DA changes over minutes), have demonstrated that the predominant response of NAcc neurons to consumption of both natural and drug rewards, at least in the context of predicted reward delivery, is a transient inhibition of firing (Carelli, 2002; Wilson and Bowman, 2004; Nicola, et al., 2004a; Nicola, et al., 2004b) and reduced DA currents during food consumption (Kiyatkin and Gratton, 1994; Richardson and Gratton, 1996). Decreases associated with consumption are paralleled by findings in VTA neurons, which also predominantly demonstrate inhibition in relation to

consumption of saccharin (Wilson and Bowman, 2006). Therefore, it is possible that in many of the studies cited above, purported consumption-associated increases are actually accumulated DA release in relation to conditioned cues or operant responses throughout a session. Supporting this presumption is the finding that in a number of terminal regions, DA levels were increased in naïve rats consuming food rewards whose presentation was paired with a novel stimulus compared to rats eating the same number of rewards in the presence of a randomly presented stimulus (Phillips, et al., 2003b).

Mesocorticolimbic Activity in Relation to Conditioned Stimuli

Indeed, a more extensive body of evidence documents a role for DA in the NAcc in relation to conditioned stimuli than in response to consumption. In the context of instrumental behavior, NAcc DA increases with non-contingent presentation of stimuli associated with self-administered cocaine (Gratton and Wise, 1994; Phillips, et al., 2003c) and amphetamine (Di Ciano, et al., 1998). NAcc core DA release also increases in response to the cues (light and insertion of lever) indicating the opportunity to lever press for sucrose; the same cues did not change DA levels in rats who had not experienced sucrose pairing (Roitman, et al., 2004). Non-contingent presentation of a cocaine-associated CS increased Nacc DA in the core, but not the shell (Ito, et al., 2000), although contingent presentations during drug-seeking did not increase DA in either shell or core.

Similarly contingent cocaine CS presentations during extinction did not increase NAcc DA (Neisewander, et al., 1996).

These findings illustrate two important distinctions. First, an anatomical distinction exists in the NAcc between the shell, which is the ventromedial portion, and the core, in the dorsolateral portion (Zahm and Brog, 1992). Many studies on the NAcc, particularly before the late 1990s, have not distinguished between shell and core of the nucleus accumbens, and some investigators argue that this may account for some of the disparate findings on the role of NAcc DA in conditioned stimuli processes (Bassareo, et al., 2007). In this review, where studies made distinctions between regions, the NAcc sub-region will be noted; no such delineation indicates that the study cited did not make such a distinction. Second, DA responses to cues may be influenced by whether the cue is contingent upon responding, or non-contingent, and hence predicted or unpredicted; Nicola (2007) has argued that positive findings on NAcc DA interventions affecting responding to CSs depends on whether cues are presented at short intervals (which would be more predictable) or longer intervals (and hence less predictable), with positive findings congregating in paradigms with long intervals between presentations of cues. These distinctions may account for negative findings such as those from one study in rhesus monkeys, for example, who did not demonstrate increases in ventromedial striatal DA during presentations of a cocaine-associated discriminative stimulus, although DA levels did increase with cocaine self-administration (Bradberry and

Rubino, 2004). In this study, the DS was administered at the same time of day for each animal, which may have increased its predictability and hence reduced its ability to activate DA release.

In the Pavlovian conditioning context, DA levels in the NAcc also increase in relation to the presentation of CSs. A food-associated CS causes increased DA utilization in the NAcc (Blackburn and Phillips, 1989), increased DA current in the NAcc (Phillips, et al., 1993), and an increase in NAcc core DA (Bassareo and Di Chiara, 1999b; Bassareo, et al., 2007). Food deprived animals prevented access to a nearby palatable meal also demonstrate increased NAcc DA levels (Wilson, et al., 1995). A cocaine-associated CS increases NAcc DA (Kiyatkin and Stein, 1996), and CSs associated with either morphine or nicotine also trigger increases in DA in the shell, but not core, of the NAcc (Bassareo, et al., 2007).

Not only does DA release in the NAcc accompany the presentation of CSs, it appears that DA in the NAcc – as well as intact functioning of the NAcc itself – is also involved with responding to CSs. Systemic injections of DA antagonists decrease locomotor activity in response to a CS but do not block consumption of a predicted meal (Blackburn, et al., 1987; Blackburn and Phillips, 1989). Depletions of DA using 6-hydroxydopamine (6-OHDA) lesions in the NAcc core impair Pavlovian approach to a CS (Parkinson, et al., 1999). Similarly, excitotoxic lesions of NAcc core reduce cocaine self-administration maintained by periodic presentations of a CS on a second-order schedule (Ito, et al., 2004). Intra-NAcc injection of the mixed D1-D2 antagonist flupenthixol blocked the acquisition of

operant responding for a conditioned reinforcer (Cador, et al., 1991) and behavioral responding to a DS decreases after injection of the D1 antagonist SCH23390 into NAcc (Wakabayashi, et al., 2004; Yun, et al., 2004a; Yun, et al., 2004b; Nicola, et al., 2005), as well as the D2 antagonist raclopride (Yun, et al., 2004b), although to a lesser extent than D1 blockade. Similarly, flupenthixol reduces conditioned approach to a food-associated stimulus (Di Ciano, et al., 2001).

In contrast to the effects of DA blockade, DA agonism generally increases responding for CSs. Amphetamine injection in the NAcc has been found to potentiate instrumental responding with contingent presentations of Pavlovian CSs (Taylor and Robbins, 1984) and in the NAcc shell to potentiate instrumental responding with non-contingent presentations of Pavlovian CSs (Taylor and Robbins, 1984; Wyvell and Berridge, 2000). Similarly, intra-NAcc DA (Cador, et al., 1991) or amphetamine (Wolterink, et al., 1993) injections dose-dependently potentiate the acquisition of operant responding for a conditioned reinforcer. Systemic administration of amphetamine (Ranaldi, et al., 1995) or D2 agonists (Beninger and Ranaldi, 1992; Ranaldi and Beninger, 1995) increases instrumental responding reinforced by a stimulus previously paired with a reward ("conditioned reward" or CR).

Finally, sub-populations of NAcc neurons change their firing in response to the presentation of conditioned stimuli in a variety of contexts, with predominant changes being excitations, although to a lesser extent inhibition of

activity is also a common response to the presentation of CSs. Although NAcc firing does not necessarily indicate VTA activity (for example, NAcc neurons require glutamate input to fire and the region receives excitatory afferents from a number of structures including amygdala and prefrontal cortex (Mogenson, 1987; Nicola, et al., 2000), it appears that the VTA plays a crucial modulatory role in NAcc firing in relation to CSs, as inactivation of the VTA almost completely abolishes cue-evoked firing in the NAcc while preserving activity correlated with other task-related events such as an operant response and magazine entry (Yun, et al., 2004b). NAcc neurons show phasic changes (short-onset increases, with, in some cases, decreases) in firing to conditioned stimuli presented during instrumental responding: after contingent presentation of alcohol-associated (Janak, et al., 1999) or sucrose-associated (Wilson and Bowman, 2004; Nicola, et al., 2004a) cue and non-contingent presentation of a cocaine-associated CS (Carelli, 2000; Carelli and Ijames, 2001; Carelli, 2002) within sessions of instrumental responding for rewards, and to contingent presentation of a cocaine-associated DS during extinction, with shell neurons increasing more than core (Ghitza, et al., 2003). In primates, a cue signaling the beginning of a food-reward trial also excites ventral striatal neurons (Schultz, et al., 1992). Similarly, in humans, presentations of cues predicting delivery or availability of a monetary reward activates the nucleus accumbens region(Knutson, et al., 2001; Talmi, et al., 2008). These increases in NAcc activity may particularly correlate with linking responses to the cues, as neurons fire to both DSs and related

neutral stimuli before a rat responds to the stimulus, and less or not at all before instances where no response is emitted (Nicola, et al., 2004b), and activity in human NAcc is parametrically associated with the vigor of a manual response to a CS (Talmi, et al., 2008). In Pavlovian paradigms as well, NAcc neurons demonstrate phasic changes in response to sucrose cues (Day, et al., 2006; Wan and Peoples, 2006) with core neurons showing higher levels of excitatory firing (Day, et al., 2006).

Together, the evidence of the role of NAcc activity and NAcc DA in responding to conditioned stimuli is extensive. Other DA terminal regions are also involved in conditioned stimuli processes (see, for example, Baxter and Murray, 2002 on reward processes and the amygdala). Cocaine-related cues have been correlated with increased activity in regions including amygdala, anterior cingulate, and dorsolateral prefrontal cortex in human cocaine users (Maas, et al., 1998). Similarly, in humans, monetary cues are associated with increased amygdala activity (Childress, et al., 1999; Talmi, et al., 2008), and conditioned cues trigger activity in the orbitofrontal cortex in humans, even when subjects are not aware of reward contingencies (Cox, et al., 2005). In rats, presentation of a Pavlovian food-associated cue increases extracellular DA in the amygdala (Harmer and Phillips, 1999) and cues associated with morphine and nicotine increase DA levels in the PFC (Bassareo, et al., 2007). DA immunoreactivity increases in central, anterior basolateral and posterior basolateral amygdala, as well as NAcc shell and mPFC after Pavlovian

conditioning sessions (Phillips, et al., 2003b), and rats lever pressing in a session in which discriminative stimuli were presented showed increased DA levels in the BLA compared to rats lever pressing without a DS (Hori, et al., 1993). Presentation of CSs also increases amygdala activation (Schoenbaum, et al., 1998). Conversely, lesions of the basolateral amygdala suppress cue-induced cocaine reinstatement (Kantak, et al., 2002; Yun and Fields, 2003). A sexual reward CS increases Fos immunoreactivity in a number of reward-related DA terminal regions, including the anterior cingulate and nucleus accumbens (Coria-Avila and Pfaus, 2007).

Conditioned Stimuli and the VTA

Activity throughout the mesolimbic DA system, then, is involved with conditioned stimuli responding. The common element in this system, of course, is the VTA, so it would be reasonable to expect that the VTA itself is also activated in correlation with perceiving and responding to conditioned stimuli. Indeed, numerous studies have documented that VTA neurons demonstrate phasic activity in relation to conditioned stimuli. In primates, midbrain DA neurons respond to cues associated with food delivery or the perception of food rewards, with the majority of responses excitatory and a minority inhibitory (Schultz, 1986; Romo and Schultz, 1990; Schultz, et al., 1993; Mirenowicz and Schultz, 1994; Fiorillo, et al., 2003; Satoh, et al., 2003). Where VTA neurons have been studied specifically in the primate, cells demonstrate the same pattern of

excitation upon perception of food-related stimuli or food itself prior to consumption (Nishino, et al., 1987). Midbrain DA neurons also fire to a stimulus which indicates that a trial is beginning in which a monkey may take an action or make a choice that will lead to reward (Ljungberg, et al., 1992; Satoh, et al., 2003). Moreover, midbrain DA neurons are particularly sensitive to reward-related stimuli, firing in response to touching food but not non-food items such as a bare wire or the walls of an empty food box (Mirenowicz and Schultz, 1994). In addition, cue-evoked firing increases as the probability of reward signified by the cue increases (Fiorillo, et al., 2003).

In rats, VTA neurons are also activated by a range of reward-related stimuli. VTA neurons fire in response to cues associated with heroin (Kiyatkin and Rebec, 2001), food (Miller, et al., 1981), and saccharin (Kosobud, et al., 1994, Wilson & Bowman 2006). Midbrain DA neurons also show burst firing in response to cues predicting food reward (Hyland, et al., 2002; Pan, et al., 2005). Rats show increased Fos immunoreactivity in the VTA when presented with a CS associated with sexual reward (Coria-Avila and Pfaus, 2007), and in both VTA and NAcc when placed in an environment associated with eating (Park and Carr, 1998).

Furthermore, VTA activity seems to be a necessary component of responding to reward-associated cues. Inactivation of the VTA with simultaneous application of $GABA_A$ and $GABA_B$ agonists abolishes Pavlovian-instrumental transfer with a food-associated cue (Murschall and Hauber, 2006)

and dramatically reduces operant responding in rats responding on a second-order schedule reinforced by a cocaine-associated cue before the first injection of cocaine is earned, although responding then reaches normal levels after cocaine injection (Di Ciano and Everitt, 2004b). These effects parallel the abolition of NAcc firing in response to sucrose cues when the VTA is inactivated while activity correlated with other task-related events such as an operant response and magazine entry is preserved (Yun, et al., 2004b).

Acquisition of Mesolimbic Activation in Response to CSs

Until this point the findings summarized have detailed a correlation between activity in the VTA and its mesocorticolimbic terminal regions and the presentation of conditioned stimuli *after* subjects have been trained to associate the stimuli with reward. Is there evidence that activity changes as conditioned associations are formed, or is this activity present even *before* conditioning takes place? In fact, evidence exists for both: the VTA and its terminal regions respond to novel, unconditioned stimuli but tend to habituate to unreinforced stimuli, and also show progressive changes as neutral stimuli are paired with rewards, indicating that associative processes are taking place.

Midbrain DA neurons respond to novel events and aversive events, in addition to rewards and conditioned stimuli (see Horvitz, 2000 for review). Early studies found that various cells in the VTA increase their firing to sound, tail pressing and pricks, and spontaneous movement (Miller, et al., 1981;

Kiyatkin and Rebec, 1998). VTA DA cells in the cat increase both firing rate and bursting activity in response to discrete auditory and visual stimuli not associated with reward, with a shorter latency for auditory stimuli (43±21 ms) than visual stimuli (68±20 ms) (Horvitz, et al., 1997). Midbrain DA neurons receive visual information through a projection from the superior colliculus, synapsing primarily in SNc but also in VTA, with tyrosine hydroxylase (TH) - positive as well as TH-negative cells (Comoli, et al., 2003) and respond with both excitations and inhibitions to the presentation of a light flash at short latency (113 ± 14.2 ms) (Dommett, et al., 2005). Superior colliculus neurons respond 40-60 ms after appearance, disappearance or movement of a stimulus, but not other visual features, making this a suitable structure to transmit relevant information in many experimental paradigms testing conditioned stimuli (Dommett, et al., 2005). Likewise, NAcc DA also increases in response to novel and aversive stimuli (Young, et al., 1998; Young, 2004). Reports in awake primates indicate that DA neurons habituate to non-reinforced stimuli to which they initially respond (Ljungberg, et al., 1992; Schultz, 1998). Consistent with these reports, in the awake rat a transient increase in NAcc DA to a novel stimulus disappears on subsequent sessions when not paired with a reward (Kiyatkin & Stein 1996). In contrast, in the anesthetized rat midbrain DA neurons have been found not to habituate to consistently presented light stimuli (Dommett, et al., 2005), suggesting that part of reward-related learning involves associatively-driven inhibitions when stimuli are not reinforced. Generally, in both primates

(Ljungberg, et al., 1992) and rats (Pan, et al., 2005), midbrain DA neurons fire in response to reward receipt until animals are well trained; thereafter neurons tend to respond only to unexpected reward receipt (Ljungberg, et al., 1992; Schultz, et al., 1993). Similar to findings of NAcc firing in response to consumption, primate VTA neurons have also been found to respond to reward consumption by both activation and inhibition, with inhibitory responses being more prominent (Nishino, et al., 1987).

Activity in the mesolimbic system, then, does correlate to some extent with responses to novel stimuli, but responding to environmental events is not simply automatic – on the contrary, evidence indicates that the activity in connection with conditioned stimuli summarized earlier primarily arises from changes during the course of conditioning. Midbrain DA neurons in the primate respond weakly to a sound preceding a reward early in learning, but strongly activate to the sound over a relatively short number of trials as training progresses (in this study, the amount of training was not specified for the population, but one representative cell demonstrated 41% activation above control levels during trials 1-15, and 107% in trials 16-25) (Mirenowicz and Schultz, 1994). In addition, neurons continued to respond to unexpected reward delivery (even after thousands of trials), but showed no activation to signaled reward receipt late in training (Mirenowicz and Schultz, 1994). Similarly, in the rat, midbrain DA neurons begin to respond to reward-paired tone cues in a classical conditioning paradigm with short-latency responses (excitations at 74 ±

33 ms and inhibitions at 53 ± 9 ms) within the first block of training, with most cells changing firing in parallel with the development of conditioned behavior (Pan, et al., 2005).

An increase in mesolimbic DA activity parallels the changes in VTA activity during the acquisition of conditioned associations. DA immunoreactivity in rats receiving a paired presentation of a stimulus with reward is slightly increased in several terminal regions (central nucleus and posterior basolateral nucleus of the amygdala, NAcc shell, and mPFC) after the first conditioning session, and either robustly increases or increases for the first time after the 4th session (central, anterior and posterior basolateral nuclei, NAcc shell and mPFC) (Phillips, et al., 2003b). NAcc DA efflux parallels this time course, as DA release during consumption of a food reward during instrumental responding increases during the first few trials early in learning, but by the end of the first session and during subsequent sessions, DA does not increase (or actually decreases) during reward consumption, and instead increases after presentation of a cue indicating the start of the session (Richardson and Gratton, 1996). Similarly, VTA neurons (not further characterized) fire in response to a cue signaling food availability, and decrease firing during consumption (Kosobud, et al., 1994).

These changes can happen quite rapidly, either within a single session or across only a few sessions. For instance, within one session NAcc DA increases after presentation of a reward-paired cue, whereas presentation of the same cue

without reward does not increase DA levels (Datla, et al., 2002). Similarly, within one session both a light and a tone increased NAcc DA levels individually after being first presented as a compound stimulus, with the tone thereafter paired with a footshock (Young, et al., 1998). After one pairing, a footshock paired with a tone increased DA levels compared to footshock alone, and a tone paired with footshock increased DA levels when presented alone in a subsequent session (Young, 2004). Furthermore, different patterns of DA response are evident in the NAcc core and shell of rats encountering a palatable food for the first time compared to those who received only one previous exposure to the food (Bassareo and Di Chiara, 1999a). Appetitive behavior emerged in parallel to the changes in DA levels based on exposure, as rats in this study showed no appetitive behavior towards a closed container with novel food, whereas rats who had one previous exposure to the food engaged in vigorous investigation of the container. Similarly, a light stimulus, which triggered a mild transient increase in NAcc DA when first presented, triggered a larger and more sustained increase in NAcc DA when presented at the beginning of a second session, after having been paired with cocaine delivery in the previous session (Kiyatkin and Stein, 1996). Finally, a cue indicating the beginning of a self-administration session did not trigger increased DA signal in the NAcc before the first session, but was associated with increased signal on subsequent sessions of instrumental responding for cocaine (Gratton and Wise, 1994) and food (Richardson and Gratton, 1996).

The Case for Associative Processes in the VTA

The evidence therefore strongly suggests that associative processes are occurring at the neural level which drive the changes in activity associated with conditioned stimuli. Previous authors have suggested that synaptic plasticity in the mesolimbic DA system underlies reward-related learning (for example, Beninger, 1983; Wickens, 1990; Kelley, et al., 2003), and synaptic plasticity processes such as LTP have long been thought to be one of the main mechanisms of learning (Kandel 2001). We have further suggested that synaptic plasticity in the VTA is a component of the acquisition of instrumental responding, as the synapses associated with incoming signals representing environmental stimuli may be strengthened due to coincident excitation from signals representing primary reward, thereby allowing the environmental stimuli to further recruit the motivational circuitry and facilitate increased approach behavior (Sharf and Ranaldi, 2006). The VTA is known to be important for motivation and approach behaviors (Beninger, 1983; Mogenson, 1987). In the context of this review, perhaps one of the most interesting pieces of evidence is that electrical stimulation of the VTA, which causes transient increases in NAcc DA current, is also highly correlated with approach to and initiation of lever-presses for drug self-administration (Phillips, et al., 2003c). In parallel, rats emitting high approach and investigatory behaviors towards a food-related stimulus demonstrate elevated NAcc DA levels, whereas rats not emitting such behaviors

do not (Bassareo, et al., 2007). What follows, therefore, is a review of the attributes of the VTA that make it an appropriate candidate for these associative processes, which require coincident stimulation from both afferents representing primary reward and those representing environmental stimuli, as well as a neural mechanism, namely glutamate transmission at the NMDA receptor, which sets in motion the intracellular processes that lead to synaptic strengthening.

Glutamate May Carry the Environmental Stimuli Signal

First we must review the excitation to the VTA which might be correlated with environmental stimuli. The VTA receives projections from a wide range of brain structures, including prefrontal cortex, dorsal rhinal sulcus, nucleus accumbens, bed nucleus of stria terminalis, olfactory tubercle, amygdala, diagonal band of Broca, substantia inominata, several hypothalamic nuclei including the lateral hypothalamus, lateral habenula, brain stem structures including the superior colliculus, raphe nuclei, parabrachial nuclei, locus coeruleus, and mesopontine and cerebellar nuclei (Phillipson, 1979; Gabbott, et al., 2008). Very recent evidence demonstrates that virtually all afferents to the VTA project some glutamatergic fibers, with the exception of the NAcc and the lateral septum (Geisler, et al., 2007). Recent evidence also suggests that subcortical sources of glutamatergic excitation predominate over cortical influences, as vesicular glutamate transporter type 2, expressed mainly in

thalamic and subcortical axons, is more prevalent in the VTA than vesicular glutamate transporter type 1, which is almost exclusively associated with cortical axons (Omelchenko and Sesack, 2007).

Earlier studies had already demonstrated that several of these afferent regions have either been verified to transmit excitatory amino acids, or give other evidence of being excitatory projections. Medial prefrontal cortex projects to the VTA (Sesack, et al., 1989; Sesack and Pickel, 1992) with cells arising from layer 5, primarily from the infralimbic, prelimbic, and dorsal anterior cingulate sub-regions, as well as sparser projections from dorsal anterior insula, precentral motor area, and orbital cortex (Gabbott, et al., 2008). Evidence suggests that these projections are primarily excitatory. EAAs are the primary neurotransmitters of cortical efferents and excitotoxic lesions of mPFC reduce aspartate in the VTA (Christie, et al., 1985). In addition, monosynaptic connections from medial PFC, which synapse on both DA and non-DA cells in the VTA, make primarily asymmetric (presumed excitatory) synapses on TH-labeled cells (Sesack and Pickel, 1992; Carr and Sesack, 2000b), with prelimbic and infralimbic inputs synapsing selectively on DA but not GABA mesoprefrontal cells, and GABA but not DA mesoaccumbens neurons (Carr and Sesack, 2000b). The clearest evidence of excitatory projections from the mPFC to the VTA is that activation of mPFC by electrical stimulation (Rossetti, et al., 1998) or depolarizing potassium chloride solution (Harte and O'Connor, 2005) causes glutamate release in the VTA. In addition, electrical stimulation of medial

prefrontal (Murase, et al., 1993; Tong, et al., 1996a; Tong, et al., 1996b; Gariano and Groves, 1998; Almodóvar-Fabregas, et al., 2002) and anterior cingulate (Gariano and Groves, 1998) neurons triggers burst firing in VTA DA cells. Finally, electrical stimulation of prefrontal neurons is correlated with increased DA release in the NAcc, which is blocked by a combination of the selective competitive NMDA antagonist AP-5 and the competitive AMPA antagonist CNQX in the VTA (Taber, et al., 1995). Note that these excitatory responses may not predominantly be due to monosynaptic excitatory projections onto DA cells, but either to monosynaptic inhibitory synapses on inhibitory interneurons, or polysynaptic excitation.

The VTA also receives an excitatory input from the ventromedial and ventrolateral bed nucleus of the stria terminalis (vBNST): glutamate stimulation of vBNST increases firing and burst activity of DA neurons, and single-pulse stimulation of vBNST activates presumed DA neurons but not GABA neurons, which is blocked by glutamate antagonists (Georges and Aston-Jones, 2002). A major subcortical glutamate source is the mesopontine tegmentum. The pedunculopontine and laterodorsal tegmental nuclei, which project to the VTA (Oakman, et al., 1995), contain neurons which demonstrate glutamate immunoreactivity and which form synapses on VTA DA neurons (Charara, et al., 1996). These glutamatergic neurons may be separate from cholinergic ones, or glutamate and acetylcholine (ACh) may be colocalized in the same cells (Charara, et al., 1996).

The excitatory inputs described above may carry information about stimuli which can be associated with reward. And indeed, complementing the anatomical studies just cited that demonstrate glutamatergic innervation of the VTA, an extensive body of research demonstrates that the activity of VTA neurons is modulated by glutamate and glutamate agonists and antagonists (see Kalivas, 1993; Meltzer, et al., 1997; Mathon, et al., 2003 for reviews). Glutamate is the focus of this review, as its release in the VTA provides for both the excitation required for synaptic strengthening as well as a route by which conditioned stimuli can continue to recruit VTA cells after associative processes have taken place.

VTA DA cells possess NMDA, AMPA and metabotropic glutamate receptors (Albin, et al., 1992). VTA GABA cells are also modulated by excitatory amino acids, as components of GABA synaptic potentials are abolished by AMPA and NMDA antagonists (Johnson and North, 1992). The NMDA receptor is distinctive because it requires two co-agonists (glutamate and glycine) to be bound at different sites, and because it is both ligand and voltage gated (see Madden, 2002, Kew and Kemp 2005 for review). The receptor is a tetrameric assembly of subunits spanning the cellular membrane with an extracellular amino terminal domain and a pore-forming membrane-residing domain. When two glycine and two glutamate binding sites are occupied, and the local membrane becomes depolarized, dislodging a magnesium ion in the channel pore, the ion channel opens, permitting an influx of calcium and sodium (Mayer,

et al., 1984; Nowak, et al., 1984). The NMDA receptor therefore is able to play a key role in associative neural processes because its activation relies on direct stimulation from a pre-synaptic terminal as well as excitation provided by an additional source (see Citri & Malenka 2007 for review), and therefore has been called a "coincidence detector." NMDA receptor stimulation has both acute effects, as increased ionic currents of calcium facilitate bursting, and over a longer time frame, as the increased influx of calcium through NMDA channels is the substrate for a number of second-messenger processes which lead to LTP (Citri and Malenka, 2007). The NMDA receptor is also involved in long-term depression (LTD), a form of synaptic weakening. However, as this process may be involved with reward-related learning but is not considered to be a necessary component of the model which gives rise to this dissertation's hypothesis, it will not be discussed further.

An activity-dependent increase in AMPA receptors appears to be due at least in part to the activation of protein kinase A (PKA) by inward ionic currents which release unphosphorylated AMPA receptors from a retention interaction which tends to keep them away from the membrane; Ca^{2+}/calmodulin-dependent protein kinase II (CaMK-II) then triggers the cellular machinery which performs the insertion of the receptor into the membrane (Esteban, et al., 2003). This increase in AMPA receptors is thought to represent one aspect of the synaptic strengthening following long-term potentiation.

NMDA receptors are distributed throughout the brain, with highest levels found in the hippocampus, and high density in cortex and various subcortical areas including NAcc, striatum, thalamus, and amygdala, with generally lower densities in midbrain and brainstem regions (Monaghan and Cotman, 1985). NMDA receptors are diffusely distributed throughout the VTA primarily within cell bodies and dendrites, with the paranigral region of VTA showing a higher mean area of density of receptors than the parabrachial region, indicating either more dendrites or more receptors per dendrite (Rodriguez, et al., 2000). This corresponds with findings that mPFC stimulation in the anterior cingulate region correlates with Fos-like immunoreactivity in the paranigral and parabrachial areas, with additional labeling in the nucleus interfascicularis and linearis dorsalis (Rossetti, et al., 1998).

Excitatory amino acids (glutamate and aspartate) are known to stimulate VTA cells and cause release of DA at the terminals. In vitro, ejection of glutamate and the glutamate agonists NMDA, AMPA, kainate or quisqualate increases VTA cell firing (Seutin, et al., 1990; Suaud-Chagny, et al., 1992; Chergui, et al., 1993; Wang, et al., 1994; Tong, et al., 1996a; Gronier and Rasmussen, 1998). In vivo, application of glutamate or its agonists increases DA current in the NAcc (Suaud-Chagny, et al., 1992), and increases levels of DA or DA metabolites in the VTA, NAcc, and mPFC (Wang, et al., 1994; Westerink, et al., 1998). Moreover, glutamate increases VTA DA cell firing (Wang and French, 1993; Wang, et al., 1994; Almodóvar-Fabregas, et al., 2002), which appears to be mediated primarily

by NMDA receptors as both competitive and non-competitive NMDA antagonists, but not AMPA antagonists, block glutamate-induced firing (Wang and French, 1993).

Furthermore, NMDA application in vitro on ventral midbrain DA neurons (Johnson, et al., 1992; Johnson and Wu, 2008) or in vivo in the VTA in anesthetized rats triggers burst or burst-like firing (Chergui, et al., 1993; Wang, et al., 1994). As discussed earlier, burst firing is significant for reward processes mediated by DA because bursting causes larger amounts of extracellular DA. Conversely, AP-5 in the VTA reduces spontaneous burst firing, whereas CNQX does not (Chergui, et al., 1993). This appears to suggest that NMDA receptors are necessary for bursting, although the conclusion that NMDA receptors are primarily responsible for bursting has been questioned (Meltzer, et al., 1997). In addition to the role of glutamate in the induction of bursting, it appears that other influences on the VTA are also involved; for example, inactivation of the laterodorsal tegmental nuclei (LDTg), which projects glutamatergic, cholinergic and GABAergic afferents to the VTA, reduces VTA burst firing (Lodge and Grace 2006). The evidence is clear, though, that glutamate and NMDA application initiate bursting, and antagonists impair bursting. In general, competitive NMDA antagonists tend to reduce firing rate and burst firing in the VTA (Meltzer, et al., 1997), although in certain studies the application of AP-5 in the VTA only reduced baseline firing rates slightly and non-significantly (Georges and Aston-Jones, 2002) and other competitive NMDA antagonists similarly

reduced burst firing but non-significantly (French, et al., 1993). The effects of AP-5 on DA levels and its implications for the findings of this dissertation will be discussed in a later section.

In summary, the VTA receives excitatory inputs from a number of regions which may carry information about stimuli in the environment; glutamate is released in the VTA from those inputs; and glutamate, particularly at the NMDA receptor, stimulates VTA cells, providing both the excitation needed for coincident processes necessary for synaptic strengthening, as well as burst firing which increases DA release at the terminal regions, playing an important role in incentive-motivational processes. These characteristics of glutamate input to the VTA suggest that it is a pathway by which the previously weak signals of neutral stimuli become strengthened through associative processes and thereby become strong signals by which excitation relating to conditioned stimuli activate VTA cells. In fact, glutamate transmission in the VTA has already been implicated in having a role in conveying information about conditioned stimuli. Instrumental responding influenced by cocaine CSs is both correlated with VTA glutamate release and reduced with VTA glutamate blockade (You, et al., 2007). Moreover, inhibition of glutamate release (via metabotropic glutamate receptor stimulation) in the VTA during heroin self-administration training reduces context-induced reinstatement (Bossert, et al., 2004), simultaneous antagonism of AMPA and NMDA receptors blocks the acquisition of cocaine CPP (Harris and Aston-Jones, 2003), and NMDA receptor antagonism alone blocks acquisition of morphine

conditioned place preference (CPP) (Harris, et al., 2004). Therefore, the evidence suggests that a route by which signals relating to CSs excite the VTA, and by which those signals come to be able to excite the VTA, occur via glutamate stimulation of the NMDA receptor. In what follows, the evidence for a excitatory primary reward signal to the VTA mediated by cholinergic afferents from the mesopontine nuclei and related to food reward, which we hypothesize is the other necessary component of associative processes in the VTA underlying the acquisition of responses to food-related conditioned stimuli, will be summarized.

Acetylcholine As the Purported Primary Reward Signal

The VTA contains cholinergic axons (Henderson and Sherriff, 1991) and receives afferents from the cholinergic nuclei in both the pedunculopontine (PPTg) and laterodorsal (LDTg) tegmental nuclei (Henderson and Sherriff, 1991; Oakman, et al., 1995; Garzon, et al., 1999). Some evidence suggests that the primary direct ACh input to VTA is the LDTg, as lesions of LDTg, but not PPTg, abolish intra-VTA anticholinesterase increases in NAcc DA efflux (Blaha, et al., 1996). However, the PPTg also sends significant afferents to the VTA as indicated by an effect of self-stimulation induced ACh release in the VTA which is influenced by ACh antagonists in the PPTg (Chen, et al., 2006). Cholinergic afferents to the VTA synapse on both DA and GABA cells; synapses on DA cells are predominantly asymmetric (presumed excitatory) and are found primarily on mesoaccumbens cells, making four times as many synapses on

mesoaccumbens than mesoprefrontal cells, while GABA synapses are predominantly symmetric (Omelchenko and Sesack, 2006).

DA cells of the VTA possess muscarinic ACh (mACh) and nicotinic (nACh) receptors (Gronier and Rasmussen, 1998). Acetylcholine, acting at both types of receptors, is a powerful modulator of VTA activity. Application of acetylcholine or ACh agonists depolarizes VTA neurons in vitro (Calabresi, et al., 1989; Weed, et al., 1995), increases DA cell firing rate and causes burst firing (Seutin, et al., 1990; Gronier and Rasmussen, 1998), and causes DA release in PFC and NAcc (Westerink, et al., 1998; Schilstrom, et al., 1998b; Miller and Blaha, 2005). Systemic application of nicotine, an ACh agonist, causes increased DA levels in the NAcc, which is blocked by an intra-VTA nicotinic ACh receptor antagonist (Schilstrom, et al., 1998b).

Acetylcholine, with its ability to modulate VTA activity, also appears specifically to play an important role in reward processes. Intra-VTA injection of carbachol, an ACh agonist, produces conditioned place preference (Yeomans, et al., 1985). During lateral hypothalamic (LH) self-stimulation, extracellular ACh increases in the VTA (Rada, et al., 2000; Chen, et al., 2006). This release varies with the intensity of stimulation (Rada, et al., 2000). Furthermore, mACh antagonists in the VTA increase thresholds for self-stimulation of the lateral hypothalamus (Yeomans, et al., 1985) and dorsal tegmentum (Kofman and Yeomans, 1988). Antagonism of nAChRs also increases thresholds for LH stimulation, but to a much smaller extent than mAChR blockade (Yeomans and

Baptista, 1997). Thresholds for self-stimulation are also increased by inhibition of the PPT, the Ch5 cholinergic nucleus (Yeomans, et al., 1993).

Additional evidence also demonstrates a role for ACh transmission for food reward in particular. VTA concentrations of ACh increase during eating and drinking (Garzon, et al., 1999; Rada, et al., 2000). Furthermore, mACh receptor antagonism in the VTA reduces eating in our laboratory and others (Rada, et al., 2000, Sharf and Ranaldi, 2006) and prevents acquisition of a food-rewarded operant task (Sharf, et al., 2006). Nicotinic receptors also play a role in food-related neural processes, as nicotinic antagonism in the VTA reduces, but does not eliminate, NAcc DA increases during free feeding sessions, although the amount of food consumed is not affected (Yeomans, et al., 1993; Schilstrom, et al., 1998b). These findings suggest that VTA ACh receptor stimulation, primarily of the muscarinic subtype, is involved in mediating some of the *unconditional* (i.e., rewarding or incentive motivational) effects of rewards, including food, and provides excitation that could possibly contribute to synaptic strengthening processes.

Synaptic Strengthening in the VTA

In summary, the VTA receives a confluence of signals –- ACh and glutamate –- which could represent some of the unconditioned and conditioned stimulus signals required for reward-related learning. Therefore, it is conceivable that these VTA ACh and glutamate signals are integrated in some

way in the VTA as animals learn about environments and instrumental responses associated with food reward. This kind of neural integration would likely be produced by something like synaptic long-term potentiation (LTP), a phenomenon which has been proposed as a physiological mechanism of learning (see Citri and Malenka, 2007 for review).

LTP has been demonstrated in the VTA, as VTA DA cells demonstrate an increase in synaptic strength following a paired pulse protocol, whereas GABA cells do not; furthermore, this LTP appears to be NMDA receptor-dependent because post-pulse synaptic strengthening is blocked by D-APV (Bonci and Malenka, 1999). This is consistent with the ability of AP-5 to block LTP in the hippocampus (Collingridge, et al., 1983; Davis, et al., 1992). Interestingly, although the induction of LTP appears to depend on NMDA receptor stimulation, its expression does not (Muller, et al., 1988). This distinction suggests that NMDA receptor stimulation may be critical for the acquisition of behavior, but not for the performance of behavior that has already been learned. Other evidence of synaptic changes in the VTA has also emerged, although these studies have investigated the phenomenon of sensitization, in which animals manifest higher degrees of locomotor activity after a period of exposure to certain drugs (see Kauer, 2004 and Jones and Bonci, 2005 for review). VTA cells show increased synaptic strength after one exposure to drugs of abuse (alcohol, amphetamine, cocaine, morphine and nicotine) and acute stress, but not other psychoactive non-addictive drugs like fluoxetine, as measured by an increased

ratio of AMPA to NMDA current (Ungless, et al., 2001; Saal, et al., 2003). However, because these studies on drug exposure investigate a form of non-associative learning—an enhanced unconditioned response to an unconditioned stimulus, their findings, while suggestive that LTP in the VTA is possible, are not evidence of the LTP that would be necessary for associative learning—acquisition by a conditioned stimulus of the ability to produce a conditioned response. Further research is needed to follow up on these initial findings of excitation-induced synaptic changes in the VTA cited above (Bonci and Malenka, 1999) to assess whether they are involved with learning about conditioned stimuli. This dissertation is part of that effort.

Impairments in Acquisition of Reward-Related Learning

To this point, this introduction has focused on activity in the mesolimbic DA system in relation to conditioned stimuli once the associations had been established, and on changes that occur within the system after a process of learning. This has set the context for an exploration of associative processes in the VTA, mediated by the NMDA receptor, which we propose are one part of the acquisition phase of reward-related learning. Because this dissertation involved manipulations in the VTA designed to assess possible effects on the acquisition of both instrumental and Pavlovian reward-related learning, which will be described in greater detail in the next chapter, it is important to know what

findings have already been established on manipulations that yield impairments in reward-related learning.

Broadly speaking, activity at various levels of the DA system has been found to be involved with the acquisition of reward-related behavior (for reviews, see Beninger, 1983; Beninger, 1992; Balleine and Dickinson, 1998; Beninger and Gerdjikov, 2004). Systemic studies first indicated this. For example, rats receiving systemic injections of the non-specific DA antagonist pimozide were dose-dependently impaired in the acquisition of instrumental responding for food (Wise and Schwartz, 1981). Classical conditioning processes are also affected by systemic pimozide, as rats receiving such treatment during sessions in which food delivery is accompanied by a cue show no acquisition of lever pressing in a conditioned reward paradigm, in which lever pressing is reinforced by a reward-associated cue (Beninger and Phillips, 1980), and delayed acquisition of a discriminated lever pressing task, in which lever presses are rewarded only during the presence of a cue (Beninger and Phillips, 1981) when tested subsequent to drug treatment. These findings indicate that DA blockade interfered with some aspect of the acquisition by neutral stimuli of the ability to recruit motivated behavior. However, because these earlier studies used systemic administration of DA antagonists, they were unable to indicate the specific nodes in the mesolimbic system implicated in the impairments shown.

Additional studies have indeed found that particular components of the mesolimbic system are specifically involved with acquisition of reward-related

behavior. First, complete inactivation, DA depletion, or DA blockade in the NAcc impairs the acquisition of reward-related behavior. The acquisition of autoshaping or sign-tracking is blocked or impaired by excitotoxic lesions of the core, but not shell, of the NAcc (Parkinson, et al., 2000), depletion of DA in NAcc using 6-hydroxydopamine (6-OHDA) lesions (Parkinson, et al., 2002), or blockade of NAcc core DA receptors (Di Ciano, et al., 2001). These studies used discrete 10 s presentations of visual stimuli or operanda immediately before reward delivery. In contrast, excitotoxic lesions of NAcc core or shell did not disrupt acquisition of conditioned approach as measured by food cup approaches in a paradigm in which a CS was present for two minutes, during which time reward was delivered, and head entries during CS presentation were measured across sessions (Hall, et al., 2001). In this case, as the authors argue, the lack of impairment in the conditioning phase may have been because US processes were still intact, allowing rats to selectively approach when food pellets were present; however, it appears that learning about the CS was impaired, as indicated by the subsequent impairment in the same animals who received core, but not shell, lesions in acquiring a novel operant response for the CS (Pavlovian-to-instrumental transfer, or PIT). It appears, therefore, that it is incorrect to term their procedure "conditioned approach" if they argue that food-cup responding actually indicates responding to the US, which may help clarify the discrepancy between this finding and others. With such long CS durations, it seems inevitable that food approaches will be confounded with CS approaches.

Consistent with this is the finding that the acquisition of conditioned approach is facilitated when CS duration is shorter, apparently making the delivery of reward more specifically linked to the cue as opposed to translating to the environment in general (Delamater and Oakeshott, 2007). Not surprisingly, therefore, in a similar paradigm to the Hall 2001 study, but with discrete light stimulus presentations before food delivery, approaches to the magazine during the CS or immediately afterwards were impaired across sessions when AP-5 was administered in the NAcc core (Kelley, et al., 1997).

Manipulations in the NAcc also affect the acquisition of instrumental responding. Whereas lesions of NAcc core or shell did not impair the acquisition of instrumental responding (Hall, et al., 2001), more specific dopaminergic manipulations in the NAcc do impair instrumental learning. Antagonism of D1 receptors in the NAcc (Smith-Roe and Kelley, 2000; Hernandez, et al., 2005) impairs the acquisition of instrumental responding for food reward, as does both inhibition and stimulation of cAMP-dependent PKA (Baldwin, et al., 2002a), one of the second-messenger molecules activated by D1 receptor stimulation, although the level of impairment with PKA inhibition is less than D1 blockade. Antagonism of D1 and D2 receptors also impairs acquisition of instrumental responding for a conditioned reinforcer (Wolterink, et al., 1993).

The amygdala also is involved with reward-related acquisition, as DA levels in the amygdala increase during acquisition of Pavlovian conditioning (Harmer and Phillips, 1999; Phillips, et al., 2003b). Conversely, lesions of the

basolateral amygdala impair the acquisition of cocaine conditioned place preference (CPP) (Fuchs, et al., 2002) and of discriminated approach (Burns, et al., 1993). In contrast, lesions in the central and basolateral amygdala in the 2-min CS conditioned approach paradigm mentioned above resulted in no impairment in conditioned approach as well as no impairment in acquiring lever pressing for food reward, but did impair PIT after receiving CeN lesions (Hall, et al., 2001). Finally, antagonism of D1 receptors in both the central nucleus and basolateral amygdala impair the acquisition of lever-pressing for food (Andrzejewski, et al., 2005), and antagonism of NMDA receptors impairs the acquisition of conditioned approach (Burns, et al., 1994).

Lesions of anterior cingulate (ACC) also impair acquisition of autoshaping (Bussey, et al., 1997) (Cardinal, et al., 2003), although interestingly ACC lesions do not disrupt temporally discriminated approach, in which rats approach the food magazine when cued by a single stimulus spatially associated with the magazine (Cardinal, et al., 2003). ACC lesions in these studies resulted in an increase in approach to the CS-, rather than diminished approach to the CS+, suggesting that the ACC is involved with discriminating between stimuli rather than mediating the motivational impact of the CS.

Conversely, acquisition of conditioned approach is facilitated by post-session infusion of a D3 agonist in the central, but not basolateral nucleus of the amygdala (Hitchcott and Phillips, 1998) and the shell, but not the core of the nucleus accumbens (Phillips, et al., 2003a).

In addition to intact mesolimbic DA function being necessary for acquisition of reward-related learning, there is also evidence that NMDA receptor stimulation plays a role. Earlier studies demonstrated a role for NMDA receptor stimulation in others kinds of learning; for example, systemic AP-5 impairs spatial learning at doses that also impaired LTP in the hippocampus in the same animals (Davis, et al., 1992). Further studies similarly demonstrated that systemic NMDA antagonists block acquisition (Tzschentke and Schmidt, 1995) of morphine conditioned place preference.

Because systemic administration affects receptors in a widespread fashion, these results cannot specify a role for NMDA receptors in the mesolimbic system as a whole or the VTA in particular. However, similar results arise from intracerebral administration of NMDA antagonists in reward-related regions. AP-5 in the NAcc core blocks the acquisition of conditioned approach for food reward (Di Ciano, et al., 2001). Similarly, acquisition of instrumental responding for food reward is blocked by antagonism of NMDA receptors in both shell and core (Kelley, et al., 1997). AP-5 alone in the medial PFC does not impair acquisition of instrumental learning, but in conjunction with a D1 antagonist does impair acquisition; however, the results of this study bear further confirmation as vehicle rats also showed low rates of acquisition, and impairments in learning persisted past the treatment phase (Baldwin, et al., 2002b). Finally, in the VTA simultaneous blockade of AMPA and NMDA receptors blocks the acquisition of cocaine CPP (Harris and Aston-Jones, 2003),

and AMPA and NMDA receptor antagonists individually block acquisition of morphine CPP (Harris, et al., 2004).

We have been specifically interested in associative processes at the level of the VTA, the common element to dopaminergic activity at the terminal regions. Although the various structures in the mesocorticolimbic terminal regions may play different roles in reward-related learning, such as stimulus associations being mediated by the amygdala and motoric response to CSs being mediated by NAcc, as already discussed, what is clear is that activity in the VTA has been correlated in different ways with virtually all aspects of reward-related behavior. What this review has aimed to do is to present an overall picture of the activity in the mesolimbic system in relation to conditioned stimuli, both after associations have been learned and during the process of acquisition, and to give an overview of the attributes of the VTA that make it a likely site for associative processes that might underlie the acquisition of reward-related behavior. In particular, we are interested in associative processes that take place in the VTA so that conditioned stimuli come to activate cells in that region, which then presumably have the downstream effects of augmenting approach behaviors and facilitating the acquisition of stimulus-stimulus, stimulus-response, and response-outcome associations in terminal regions. Current evidence indicates that glutamate stimulation in the VTA at the NMDA receptor, coincident with other afferent excitation mediated by acetylcholine, triggers calcium influx and a variety of second-messenger events, leading to long-term potentiation at glutamatergic

synapses. This synaptic strengthening may increase the recruitment of VTA neurons by those environmental stimuli that are in some temporal relationship with reward, thereby increasing the incentive-motivational attributes of those stimuli. This dissertation was designed to test this hypothesis, which will lead to further understanding of both normal and pathological reward-related learning processes.

Chapter 2 Overview of Specific Aims

This dissertation was designed to test a hypothesis arising from a model of reward-related learning which proposes that synaptic plasticity in the VTA underlies the ability of signals relating to conditioned stimuli to activate motivational circuits. This model proposes that glutamate synapses receiving signals associated with previously neutral stimuli become strengthened due to coincident excitation of VTA cells by another afferent, presumably cholinergic, transmitting a signal of primary reward. This synaptic strengthening is mediated by NMDA receptors, whose ion channels are opened in the presence of depolarization, allowing for calcium influx that triggers second-messenger cascades leading to increased excitability at that synapse, presumably because of increased sensitivity of existing AMPA receptors or increased trafficking of AMPA receptors into the post-synaptic membrane. As these glutamate synapses are strengthened, glutamate signals associated with environmental signals begin to acquire the ability to activate VTA cells (presumably dopaminergic) themselves, increasing approach behaviors, and thereby facilitating encounters with stimuli associated with reward. At a behavioral level, this may be expressed in terms of the acquisition of stimulus-approach associations, or the initial attribution of incentive salience to environmental stimuli. Furthermore, the model asserts that NMDA receptor stimulation is necessary for the initiation

of synaptic strengthening, and thereby the *acquisition* of these conditioned associations, but once LTP has occurred, NMDA receptor stimulation is no longer necessary for the activation of VTA cells by glutamate transmission, and therefore not necessary for the *expression* of the reward-related associations. Accordingly, this dissertation tested the hypothesis that blockade of NMDA receptors in the VTA would prevent the acquisition of reward-related learning, but not its expression.

This hypothesis addresses a mechanism by which conditioned stimuli become associated with rewards and drive behavior, both adaptive reward-seeking as well as pathological behavior, which may be manifested in instrumental behavior such as drug-taking. We were therefore interested in beginning the testing of this hypothesis, which relates to reward-related learning in general, in the context of the acquisition of instrumental responding, which is one kind of reward-related learning. An instrumental learning paradigm was particularly useful in assessing the prediction that NMDA receptor blockade would impair the acquisition, but not expression, of reward-related learning, because it would allow us to compare behavior when rats were naïve to the reward-related environment to their behavior after acquisition had occurred. This comparison would allow us to assess whether the formation of conditioned associations per se were being blocked, or whether some more basic motivational processes or motoric abilities were being affected by NMDA receptor antagonism. If performance were reduced after acquisition, that would indicate

that primary reward or motoric ability may have been impaired, making a claim of impaired conditioned associations difficult. Our choice of an instrumental conditioning paradigm was also influenced by the fact that this work was designed to follow previous studies testing the component of our model regarding acetylcholine transmission as the primary reward excitatory component of the associative process (Rada, et al., 2000; Sharf, et al., 2006). In order for us to compare results directly to our previous work on the model we used the same behavioral paradigm.

Accordingly, the first specific aim of this dissertation was to assess the effects of NMDA receptor antagonism during the acquisition and expression of instrumental learning. As mentioned above, instrumental learning is of particular interest because of its relevance to the pathological processes involved with dysregulated reward behaviors which have a strong instrumental component such as drug abuse, eating disorders, gambling, and so on. As a natural reward, food reward was chosen so that we could assess the basic mechanisms of reward associations. For this reason, food reward was used in all of the studies in this dissertation.

For specific aim 1, a paradigm was designed in which rats would receive bilateral microinjections of vehicle or the selective competitive NMDA receptor antagonist AP-5 before each of four instrumental learning sessions, followed by five sessions with no treatment, and a final session with the same treatment as in the first phase of the experiment. Rats would be pre-exposed to food pellets in

their home cages to overcome any neophobia and ensure consumption of pellets earned in the initial sessions. If lever pressing for any AP-5 group did not increase significantly across the first four sessions during treatment, and, as expected, rats receiving vehicle treatment did increase lever pressing, this would indicate an impairment in acquisition of instrumental learning. The subsequent five sessions would allow any rat that had not acquired lever pressing during the first four sessions to do so. Finally, a comparison between the penultimate session (without treatment) and the final session (with treatment) would allow for an assessment of whether AP-5 treatment impaired expression of the learned instrumental behavior. In this first study, a separate group was implanted with cannulae dorsal to the VTA to assess whether any effects might be attributed to diffusion of compounds outside the VTA.

Two additional studies were planned as part of Specific Aim 1 to control for possible causes of any impairment in acquisition that might be found in the initial study. In the first, we assessed whether intra-VTA AP-5 would impair basic food motivation. To test this possibility, rats were given the opportunity to consume rat chow freely during four daily sessions. Rats received intra-VTA injections of saline before the first three sessions in order to assess baseline levels of consumption. Before the fourth and final session, rats received either vehicle or AP-5 injections in the VTA and were again given the opportunity to consume rat chow. Comparisons between sessions 3 and 4 were planned to determine whether AP-5 affected consumption of food. The second study assessed possible

effects of AP-5 treatment on motor activity. Rats received intra-VTA injections of saline before each of three consecutive sessions in activity monitoring chambers to establish baseline levels of ambulatory activity and stereotypy. Before the fourth and final sessions, rats received either saline or AP-5 and again were exposed to the activity chambers, and measures of activity were compared between sessions 3 and 4.

The second specific aim of this dissertation was to further assess possible causes of the impairment of acquisition of instrumental responding in the first study. Having determined that basic food consumption and motor activity was not reduced during instrumental training, it became necessary to ascertain whether AP-5 treatment was interfering with reward processes. Specifically, given that the results of the activity study indicated that AP-5 caused an increase in stereotypy, it was possible that stereotypy impaired the stimulus-reward contingencies experienced by AP-5 treated rats. In other words, although rats were able to press the lever and enter the trough, and appeared motivated to consume food when it was discovered, we needed to assess whether more time elapsed between lever presses and discovery of food pellets under AP-5 treatment due to presumed increased activity in the operant chambers, thus decreasing the stimulus-reward contingency relative to vehicle rats. Therefore we conducted a detailed analysis of latencies between lever presses and head entries (which allowed rats exposure to food pellets) between the vehicle and 0.5 μg AP-5 group, the lowest effective dose in the initial study.

The second component of Specific Aim 2 addressed whether AP-5 impaired the reward value of food pellets, which might have reduced the formation of reward associations due to a weaker reward signal. To do so, we used a reward devaluation paradigm in which rats acquired a lever press response for food pellets in two sessions. In the following session, rats were given the opportunity to consume food pellets in the operant chamber without the presence of levers, after being treated with either vehicle or AP-5 in the VTA. During the fourth and final session that was not preceded by treatment, lever presses which resulted in presentation of the food-associated conditioned stimulus, but not food pellets, were recorded. Comparison of lever presses reinforced by a CS in extinction between groups allowed for a determination of whether consuming food pellets under the influence of intra-VTA AP-5 reduced the reward value of the pellets, as any reduced reward would be reflected in a reduction of a conditioned reinforcement effect in the subsequent session.

Given that a number of factors are involved in instrumental learning (to be discussed in more detail in Chapter 4), Specific Aim 3 was to test the role of NMDA receptors in the VTA in a reward-learning task that did not require the animal to acquire any new behaviors, but simply to form an association between a stimulus and a reward in a classical conditioning paradigm. If NMDA receptor antagonism prevented a CS from influencing behavior in this protocol, this would provide further evidence that the impairment of acquisition in the initial study was due to the blockade of synaptic strengthening in the VTA that allows

environmental signals to specifically recruit approach behavior, rather than disrupting stimulus-response associations. Therefore we designed a conditioned approach paradigm in which rats were exposed to paired presentations of a conditioned stimulus and a food pellet in each of three sessions after intra-VTA injections of either vehicle or AP-5. Head entries before, during and after the presentation of the CS were recorded. After a subsequent extinction session in which neither the CS nor pellets were presented, a final session was conducted in which only the CS was presented, and again head entries before, during and after the CS were measured. If reward-related learning had taken place in the first 3 sessions, animals should emit more head entries during or immediately after the CS as compared to the control period before the onset of the CS. We predicted, in keeping with our hypothesis, that intra-VTA application of AP-5 would impair reward-related learning and thus rats would show no increase in head entries in response to the CS. To provide further support for a possible impairment in associative processes, we also planned to test a third group of rats that would receive vehicle injections and be exposed to random (unpaired) presentations of the CS and food pellets. If AP-5 blocked conditioned stimulus associations, they should behave similarly to vehicle rats receiving random presentations.

In these experiments, the NMDA antagonist AP-5 (2-amino-5-phosphonovalerate or 2-amino-5-phosphopentanoate [Davies et al., 1981]) was chosen because it is highly selective for the NMDA receptor and has been used

extensively in behavioral studies. It has high affinity for the glutamate binding site on the NMDA receptor, and as a competitive antagonist it prevents glutamate from activating the receptor. In addition, it has very little NMDA receptor subtype selectivity, so NMDA receptors in the VTA are most likely affected by it regardless of subunit variability (Kew and Kemp, 2005). Most importantly for the aim of these studies, AP-5 has been shown to block LTP in various brain regions including the hippocampus (Collingridge, et al., 1983; Harris, et al., 1984; Davis, et al., 1992). Rats used in all of the experiments described herein were food-deprived during the period of training and testing.

Chapter 3 General Methodologies

The protocols used in the present experiments were in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals and were approved by the Queens College Institutional Animal Care and Use Committee. This chapter describes procedures that were used for all studies described in this dissertation. Specific details for each experiment will be provided in the following chapters.

Subjects

Subjects consisted of male Long Evans rats, facility-bred from males and females obtained from Charles River Laboratories (Raleigh, NC), with initial free-feeding weights between 275 and 380 grams at the time of surgery. All rats were individually housed and maintained on a 12 h light:12 h dark cycle (lights off 06:00). All experimental sessions were conducted during the dark phase in order to test the rats during their active periods. All animals had unlimited access to food (Purina rat chow) until food deprivation was instituted to prepare animals for experimental sessions, at which time access was restricted to daily rations (approximately 12 grams) until rats reached 85% of their free-feeding weights, and were maintained at that weight for the duration of the experiment. Accordingly, weight gain due to consumption of food pellets during behavioral testing was compensated for by a corresponding reduction in rat chow. Chow rations were delivered pseudo-randomly between 30 minutes and 4 hours after

behavioral testing sessions to minimize association between testing and chow delivery.

Surgical procedure

All animals received an intraperitoneal (IP) injection of atropine sulfate (0.1 ml) and were anesthetized by sodium pentobarbital (65 mg/kg). Stainless steel guide cannulae (0.635 mm outer diameter, 0.3302 mm inner diameter) were bilaterally implanted into the ventral tegmental area (VTA) using the following coordinates: -5.6 mm caudal to bregma, ±2.0 mm from the midline at a 10° angle toward the midline and -8.3 mm below the surface of the skull (Paxinos and Watson, 1986). For the dorsal anatomical control group the coordinates were identical except the guide cannulae were lowered to -7.3 mm. The cannulae were secured by dental acrylic anchored to the skull by four stainless steel screws. Obturators (0.3048 mm diameter), extending 1 mm beyond the tip of the cannulae, were inserted at all times except during microinjections.

Microinjection procedure

While each rat was held gently by an experimenter, a stainless steel injector tube (0.3048 outer diameter, 0.1524 mm inner diameter) delivering either saline or AP-5 was inserted into the guide cannula, the tip of which extended 1 mm past the end of the guide cannula. The test compound was manually injected over a 30-s period and the injector was kept in place for an additional 60 s to allow for the solution to disperse into brain tissue, after which it was removed

and the obturator was replaced. This procedure was repeated on the contralateral side.

Drugs

AP-5 (Sigma-Aldrich, St. Louis, MO) was dissolved in 0.9% saline before the start of the experiments. Each microinjection was delivered in a volume of 0.5 *µl*.

Testing Apparatus

Instrumental conditioning and free-feeding sessions were conducted in operant conditioning chambers measuring 30 x 21 x 18 cm (*l* x *w* x *h*). One wall was equipped with two removable levers, two white stimulus lights and a food trough. Each chamber was housed in a ventilated, sound-attenuating box. Locomotor activity tests were conducted in activity chambers measuring 40.5 x 20.5 x 24.5 cm (*l* x *w* x *h*). Each chamber was equipped with eight photo-emitters positioned along the length of the chamber paired directly opposite a photocell.

Histology

Most VTA microinjection sites were localized in the caudal portion of the VTA (-5.6 to -6.04 mm posterior to bregma) with some injections occurring in the central portion (-5.2 to -5.3 mm posterior to bregma) (see Figures 9 and 10). Dorsal placements were generally 1 mm above the VTA placements, located just ventral to the red nucleus, from -5.6 to -6.04 mm posterior to bregma.

Data Analysis

The specific statistical tests used to analyze the results of each experiment will be detailed in the following chapters. All analyses were conducted using the statistical software package SPSS. Where significant interactions were found following ANOVAs, follow up tests of simple main effects were conducted with ANOVAs using the overall mean error. Criterion for significance was $P < 0.05$ in all cases.

Chapter 4 NMDA Receptor Stimulation is Necessary for Acquisition, but not Expression, of Food-Rewarded Instrumental Learning

Appetitive instrumental learning (also known as instrumental conditioning, operant learning or operant conditioning) refers to an animal learning to emit a particular response in order to produce a particular outcome (Rescorla 1991). Operant learning is found in most animals, even invertebrates (Brembs 2003). Learning to produce rewards through voluntary behavior necessarily involves a number of components, which may possibly be dissociable behaviorally and neurophysiologically. Instrumental learning is thought to involve the acquisition of associations between any of the following: various stimuli associated with the response (such as the visual and tactile qualities of a lever), discrete stimuli which may occur contiguously with the response (such as a light or a tone), the properties of the reward (its hedonic valence, magnitude, and specific sensory qualities), the motivational state elicited by the primary reward, and the motor impulses that compose the particular action, such as pressing a lever. The combinations of associations of these factors may be referred to as stimulus-stimulus, stimulus-reward, stimulus-response, and response-outcome associations. While earlier behaviorist theory held that all instrumental learning could be accounted for by stimulus-response associations, wherein associations "stamped in" by reinforcement would cause a stimulus to

generate a response, current thinking is more resonant with ideas raised by theorists like Bindra (1974) and Bolles (1972) who asserted that through association with reward, stimuli come to acquire incentive properties which energize and orient animals, and make certain responses more likely. In addition, Bindra (1974) highlighted the importance of the motivational state of the animal, which he placed on an equal level with the properties of stimuli with a reinforcement history, such that hunger, for example, will cause an animal to be more sensitive to the impact of stimuli which are related to primary rewards relevant to that state, and increase the likelihood of emitting appetitive and consummatory behaviors corresponding to that state. One recent study, for example, demonstrating that rats emit significantly more lever presses reinforced by a food-associated stimulus when hungry than when satiated (Corbit, et al., 2007), illustrates this model.

Indeed, current thinking on the neural circuitry underlying the acquisition of appetitive instrumental learning confirms earlier theoretical models to a large extent. While the neural circuits underlying reward-related instrumental learning remain to be completely mapped out, investigators are finding that a number of interconnected structures are important in acquisition. These include the midbrain DA neurons, which signal primary and conditioned reward and broadly energize exploration and effortful behavior; the NAcc (both core and shell), which is thought to regulate effort and facilitate the selection of appropriate responses and the inhibition of inappropriate ones; the amygdala

(both CeN and BLA), which forms specific stimulus-reward associations and signals that to the NAcc and other structures; and mPFC, which updates current outcome values and integrates environmental context with specific cues and actions (Mogenson, et al., 1980; Panksepp, 1998; Kelley, et al., 2003; Nicola, 2007; Salamone, et al., 2007). The continuing collection of data which refines the understanding of the role of each of these structures proceeds until the present; for example, the specific roles of the divisions of NAcc and amygdala, respectively, remains an area of active research, with some investigators arguing that the BLA and NAcc shell are more involved with specific sensory associative processes, and CeN and NAcc core more involved with general activational responses to stimuli (Kelley, et al., 2005; Balleine, 2005; Di Chiara and Bassareo, 2007). While the hippocampus may not be necessary for the specific acquisition of instrumental behavior per se (Kelley, et al., 2003), it also appears to play a role in signaling novelty to the VTA (Lisman and Grace, 2005), which may underlie the habituation of VTA cells to non-rewarded stimuli, as well as the spatial and contextual memory which impacts instrumental learning in certain paradigms. Instrumental learning is also mediated by neural integration subcortically in the brainstem and even spinal cord (Grau, et al., 2006).

Even from this brief review, it is clear that some of the various associative processes can be dissociated at the neural level – for example, stimulus-stimulus associations may be primarily mediated by cortex and amygdala, while response-outcome associations may involve mPFC and striatal connections, and

stimulus-approach associations may primarily take place at the level of the midbrain DA neurons – although of course in the intact organism these circuits are highly interconnected and together give rise to the complex behavior.

Bindra's idea of "central motive state" being constituted by the intersection of the animal's current homeostatic condition and the incentive impact of reward-related stimuli is also substantiated by the neural dynamics underlying hunger and eating. The neural circuits which mediate the oral experience of food interact with those that mediate the visceral states of hunger, satiation, and the positive and negative post-ingestive effects of food, and both of these circuits interact with circuits mediating reward and motivation. Because the research in this dissertation addressed food motivation, it would be useful to briefly review those neural circuits (see Simon, et al., 2006 for review). Taste receptor cells on the tongue are stimulated by sugars, salts, fats, acids, and other tastants, and project to the rostral nucleus tractus solitarius (rNTS). Somatosensory neurons surround the taste receptor cells and project information about texture, temperature and other sensory features of foodstuffs via the trigeminal, glossopharyngeal, and vagal nerves. The rNTS receives both taste and somatosensory information, and the caudal NTS receives vagal afferents carrying information about the state of the gastrointestinal system, so the sensory and taste stimuli from the mouth begin to be integrated with visceral information from the gut already at the level of the brainstem. The neural signals relating to foodstuffs in the mouth are then (in the rat) relayed to the parabrachial nucleus

(PBN), which projects both to the ventromedial posterior thalamus which projects to primary gustatory cortex (in the insula), and also to the amygdala and lateral hypothalamus. The primary gustatory cortex projects both to the orbitofrontal cortex, considered secondary taste cortex, and to the CeN, which then projects to the LH and the VTA. Descending afferents from forebrain structures including the CeN, LH and gustatory cortex also modulate the PBN and rNTS.

The VTA is thus in a position to receive information about food reward at a less processed level via the amygdala and LH, which receive projections from the PBN, as well as more elaborated information from primary taste cortex via the amygdala, and additionally elaborated information from the OFC from direct projections.

Because the rats used in our studies, as in many studies addressing food-rewarded behavior, were food deprived, it is also important to have a sense of the effects of food deprivation on the nervous system. Feeding is mediated by a complex network of neurons with the involvement of a range of hormones and neurotransmitters (see Gao and Horvath, 2007 for review). The lateral hypothalamus contains orexin and melanin-concentrating hormone neurons, which are stimulated by circulating hormones that indicate levels of fats and nutrients, including leptin and ghrelin. These neurons project to the VTA as well as other brain areas. LH orexin neurons are activated rapidly by fasting, and promote generalized arousal through projections to various brain stem nuclei

including the locus coeruleus. Of great relevance to our studies is that the excitability of the VTA is sensitized by LH stimulation by orexin. Finally, although the complete story is not yet fully understood, the VTA is also modulated by leptin, insulin, and ghrelin, indicating that the VTA is directly affected by food deprivation states through circulating hormones in addition to signals from the LH and elsewhere (Gao and Horvath, 2007). Ghrelin in the VTA is associated with increased responsivity to glutamatergic stimulation, which in turn is associated with increased DA neuron activity and increased feeding. Moreover, recent evidence indicates that ghrelin promotes changes in synaptic morphology in the VTA (Abizaid, et al., 2006), suggesting that the VTA in food-deprived rats is primed to enable associative processes which may underlie one component of reward learning.

Interestingly, rats can learn about food reward, such as developing flavor preferences, when they are not hungry, indicating the important role of orosensory stimulation in food reward (Sclafani and Ackroff, 2004). Learning about food flavors involves DA transmission (Azzara, et al., 2001; Baker, et al., 2003), although we can assume that this aspect of food learning was not affected in our studies as rats were exposed to operant chamber pellets before any testing and drug treatment.

Certain tastes such as sweets and fats appear to be innately rewarding to rats and many other species, promoting approach behavior and operant learning (Sclafani and Ackroff, 2004). Orosensory features thus play a prominent role in

food reward. Post-ingestive consequences also influence learning about foods, particularly in terms of flavors, as animals can learn to associate tastes with positive (e.g., satiation by carbohydrates) and negative (e.g., malaise induced by lithium chloride injection) consequences of eating (Simon, et al., 2006). However, without oral stimulation, ingestive consequences are not effective in supporting operant learning. Furthermore, as animals become satiated with a given food, neural responses to its taste change, including decreases in LH and OFC neuronal activity.

Thus, the actions of peripheral hormones as well as modulatory influences from the LH on the VTA in food-deprived animals would seem to be the neurophysiological substrate of the "central motive state" of the organism proposed by Bindra (1974). It is reasonable to argue that a hungry animal is oriented towards food and food-related stimuli, and more susceptible to learning about reward cues, because its neural circuits are "tuned," or more sensitive, to the orosensory signals related to food and the other excitatory signals transmitting information about food-related stimuli, thereby selectively enhancing processing of such signals and facilitating associative synaptic changes underlying learning.

Through this very brief review of the interacting neural systems that are thought to underlie instrumental learning and food-related processes, the context is set for the report of the first experiment in this dissertation, which tested the

hypothesis that NMDA receptor antagonism in the VTA would impair the acquisition, but not expression, of food-rewarded instrumental learning.

Subjects and Materials

Please refer to Chapter 3 (General Methodologies) for a full description of the subjects, surgery, apparatus, microinjection procedure, drugs and doses, and histology procedure.

Behavioral procedure

After at least one day of food deprivation and prior to beginning testing, all rats were given 20 food pellets (45 mg, Bio-Serv, Frenchtown, NJ) in their home cages on each of three days. During the instrumental conditioning experiments all rats were exposed to the operant chambers for 10 daily 1-h sessions in which presses on the active lever were reinforced under a fixed ratio 1 (FR1) schedule of reinforcement with one food pellet, accompanied by the illumination of the light above the active lever for 2 s. Presses on the inactive lever produced no consequences.

For sessions 1 to 4 all animals received bilateral microinjections of vehicle (n=11) or AP-5 (0.125, 0.25, 0.5 or 1.0 µg/0.5 µl, ns = 6, 8, 6 and 8, respectively) into the VTA or 1 mm dorsal to the VTA (saline, n=6; 0.5 µg AP-5, n=5) immediately before being placed in the operant chambers. No microinjections were given prior to sessions 5 to 9, which began two days after session 4. For session 10, all rats received the same treatment as the first four sessions. Presses on both levers and head entries into the food trough were recorded, as well as

the time between each active lever press that was followed by a head entry ("food approach latency").

Data Analysis

Active lever presses, inactive lever presses, and food trough head entries during each of the 10 sessions were analyzed using separate mixed-design ANOVAs with dose (between groups) and day (repeated measures) as factors. For each set of data, three ANOVAs were conducted, one for sessions 1-4, another for sessions 5-9, and another for sessions 9-10. The data analysis of food approach latencies will be discussed in Chapter 6. All analyses were conducted using the statistical software package SPSS. Criterion for significance was $P < 0.05$ in all cases.

Results

Active lever

The groups receiving vehicle and 0.125 µg AP-5 showed steep, significant increases in lever pressing across days 1 to 4, the 0.25 µg group showed steep significant increases on days 3 and 4, and the 0.5 and 1.0 µg groups failed to show large increases in responding across days (see Figure 1; a mixed design ANOVA showed a significant session × dose interaction [$F_{(12,102)}$=2.354, p<.010]). Follow-up tests revealed session effects for the vehicle, 0.125 and 0.25 µg groups [$F_{(3,102)}$=13.611, 10.786, and 8.362, respectively, all ps<.05]. During sessions 5 to 9 all groups showed increases in active lever presses (see Figure 1; $F_{(4,136)}$=28.034, p<.001), with AP-5 groups exhibiting fewer presses ($F_{(4,34)}$=48.009, p=.004).

During session 10 all groups showed similar amounts of active lever pressing when compared to session 9 (Figure 1).

Inactive lever

During sessions 1 to 4 all groups generally decreased pressing across days (see Figure 3; $F_{(3,102)}$=3.001, p<.05), with AP-5 groups emitting a higher number of inactive lever presses than the vehicle group ($F_{(4,34)}$=106.801, p<.001). During sessions 5 to 9 all groups continued to reduce inactive lever presses across sessions ($F_{(4,136)}$=2.911, p<=.05). During session 10 all groups showed similar amounts of inactive lever pressing when compared to session 9, except for the 0.5 µg group which showed an increase (significant session × dose interaction [$F_{(4,34)}$=2.778, p<.05]; follow-up tests revealed a significant session effect in the 0.5 µg group [$F_{(1,34)}$=7.722, p<.05]).

Head entries

During sessions 1 to 4 all groups receiving AP-5 emitted more head entries than the vehicle group, although the group differences were not significant (see Figure 4). Head entries showed large within-group variability and generally did not show systematic changes across sessions except for the 1.0 µg group, which showed large daily increases (significant session × dose interaction ($F_{(12,102)}$=2.260, p<.005); follow-up tests revealed a significant session effect in the 1 µg group [$F_{(3,102)}$=10.608, p<.05]). During sessions 5 to 9 the differences among the AP-5 and vehicle groups were not significant (Figure 4).

During session 10 groups receiving AP-5 showed a greater number of head entries than the vehicle group (Figure 4; $F_{(1,34)}$=21.474, p<.001).

Dorsal anatomical controls

To test the regional specificity of the effects of AP-5 on acquisition of instrumental responding two groups were tested with injections of vehicle (n=6) or 0.5 µg AP-5 (n=5), respectively, in a site 1 mm dorsal to the VTA prior to each of four instrumental responding sessions. The number of active lever presses rose steeply across the four sessions in both groups and was not significantly different between groups (see Figure 5; $F_{(3,27}$=16.114, p<.005).

Histology

Most VTA microinjection sites were localized in the caudal portion of the VTA (-5.6 to -6.04 mm posterior to bregma) with some injections occurring in the central portion (-5.2 to -5.3 mm posterior to bregma) (see Figure 6). Dorsal placements were generally 1 mm above the VTA placements, located just ventral to the red nucleus, from -5.6 to -6.04 mm posterior to bregma.

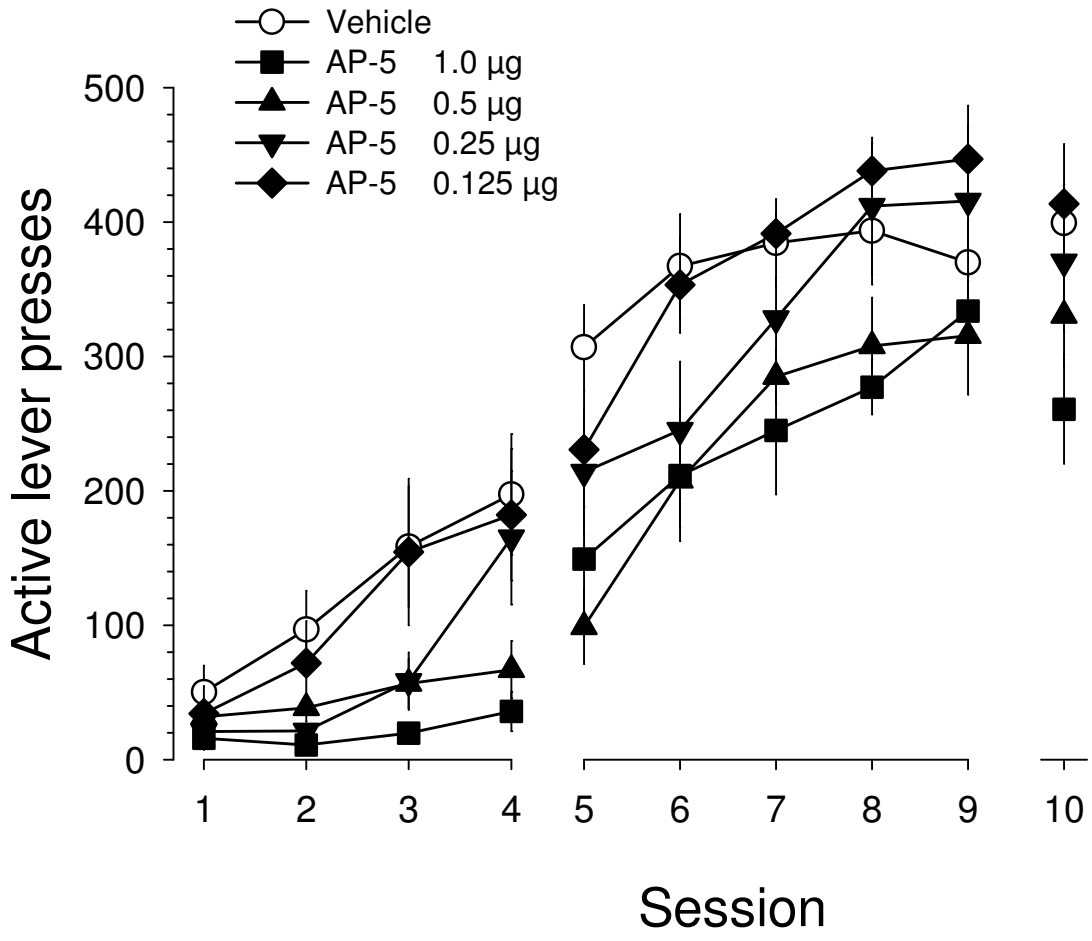


Figure 1. Mean active lever presses for groups tested in ten instrumental conditioning sessions. Rats received bilateral intra-VTA microinjections of 0.125, 0.25, 0.5 or 1.0 µg/0.5 µl of AP-5 (ns = 6, 8, 6 and 8, respectively) or 0.9% saline (vehicle; n=11) immediately prior to sessions 1 to 4 and 10. Vertical bars represent the standard error of the mean (SEM).

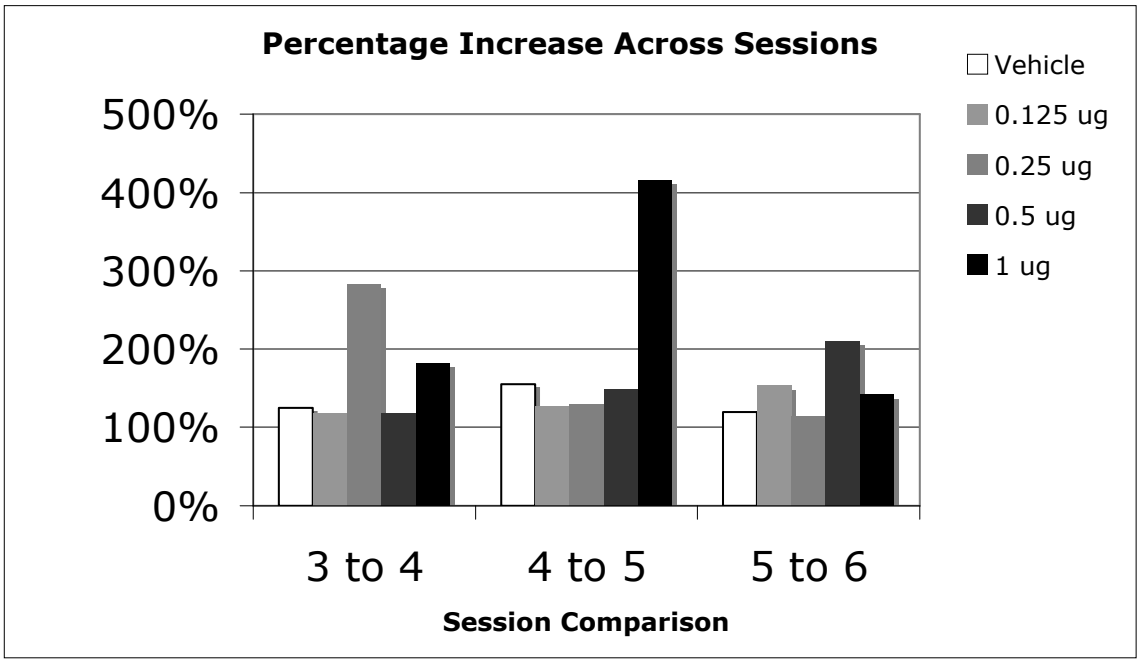


Figure 2. Percentage increase in active lever pressing by group across session. Each average is calculated as a percentage of the average of the previous session.

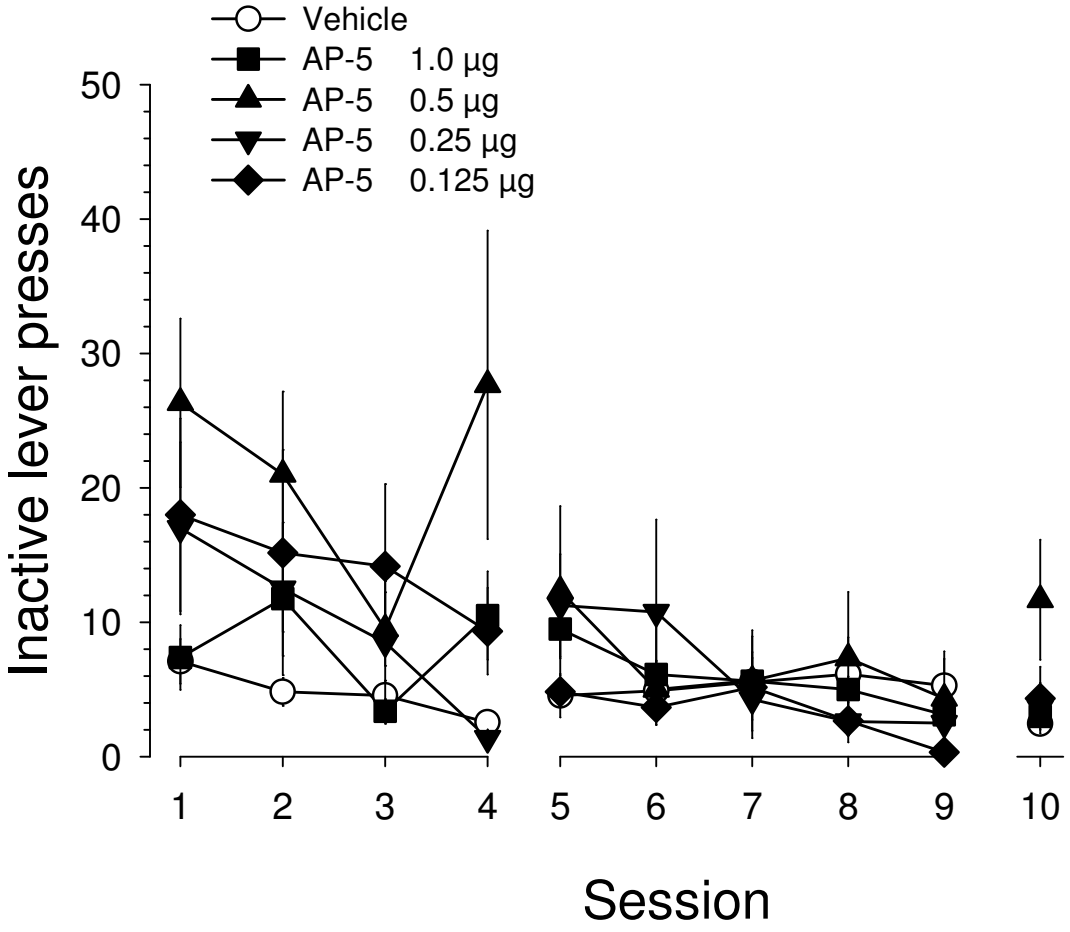


Figure 3. Mean inactive lever presses for the same groups tested in Figure 1. Vertical bars represent the standard error of the mean (SEM).

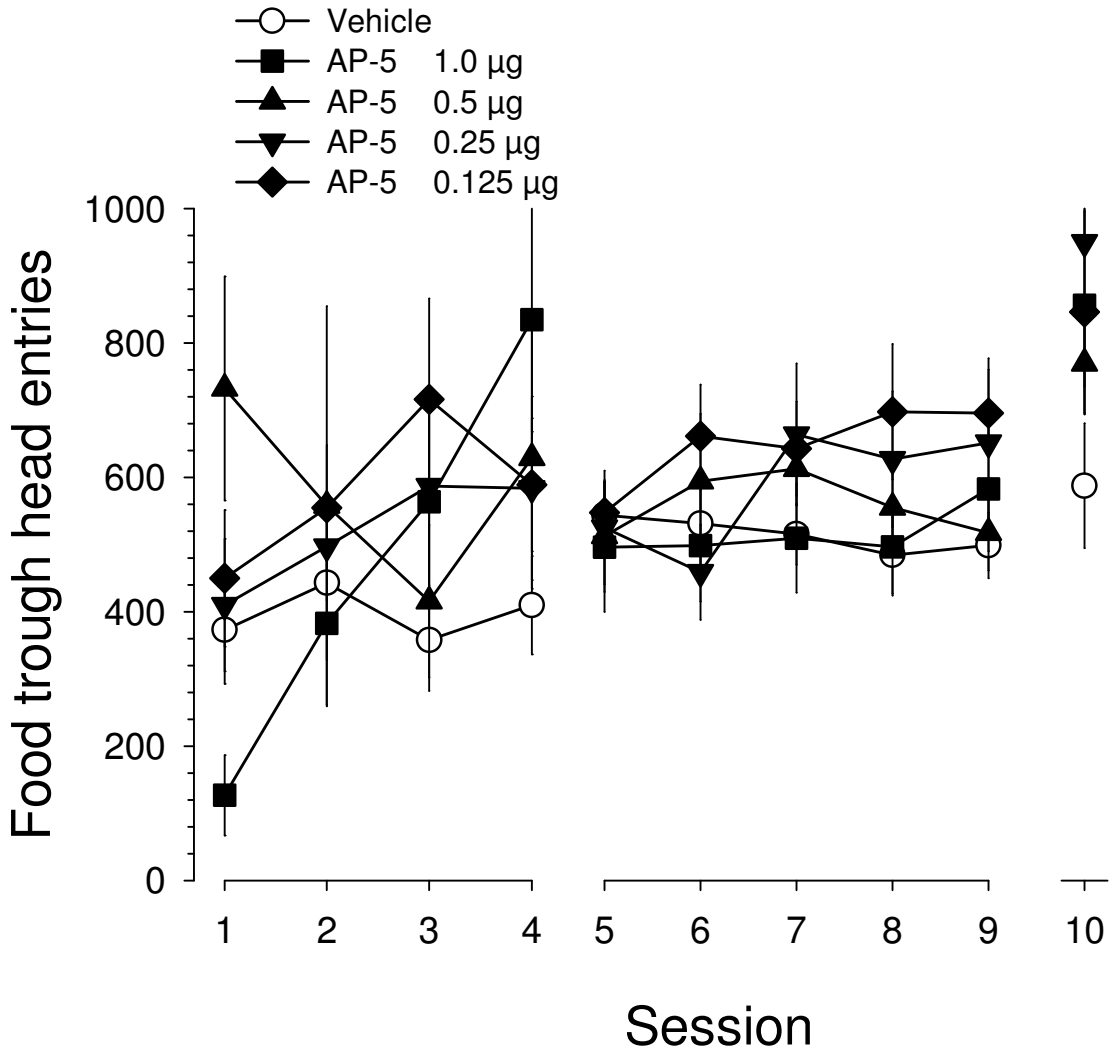


Figure 4. Mean head entries across ten instrumental responding sessions for the same groups tested in Figure 1. Vertical bars represent the standard error of the mean (SEM).

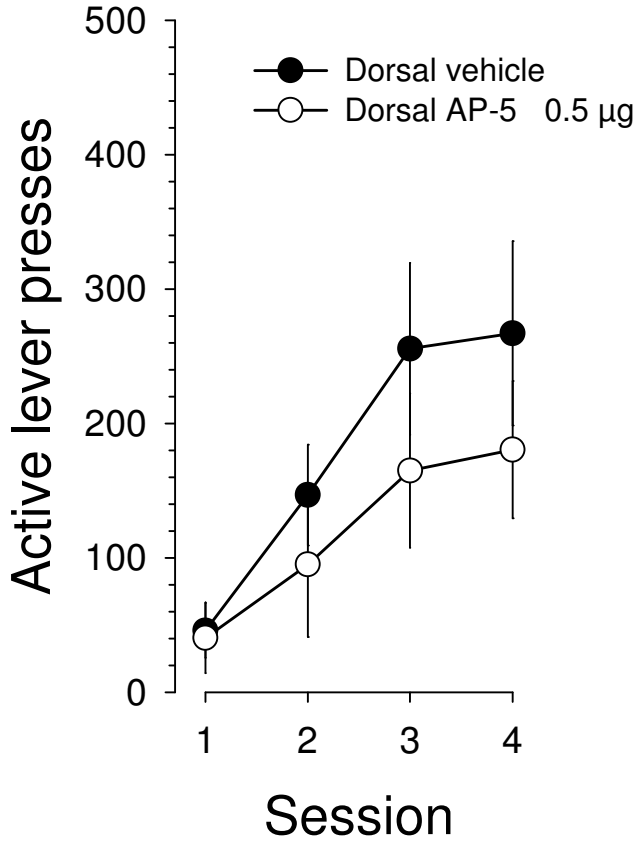
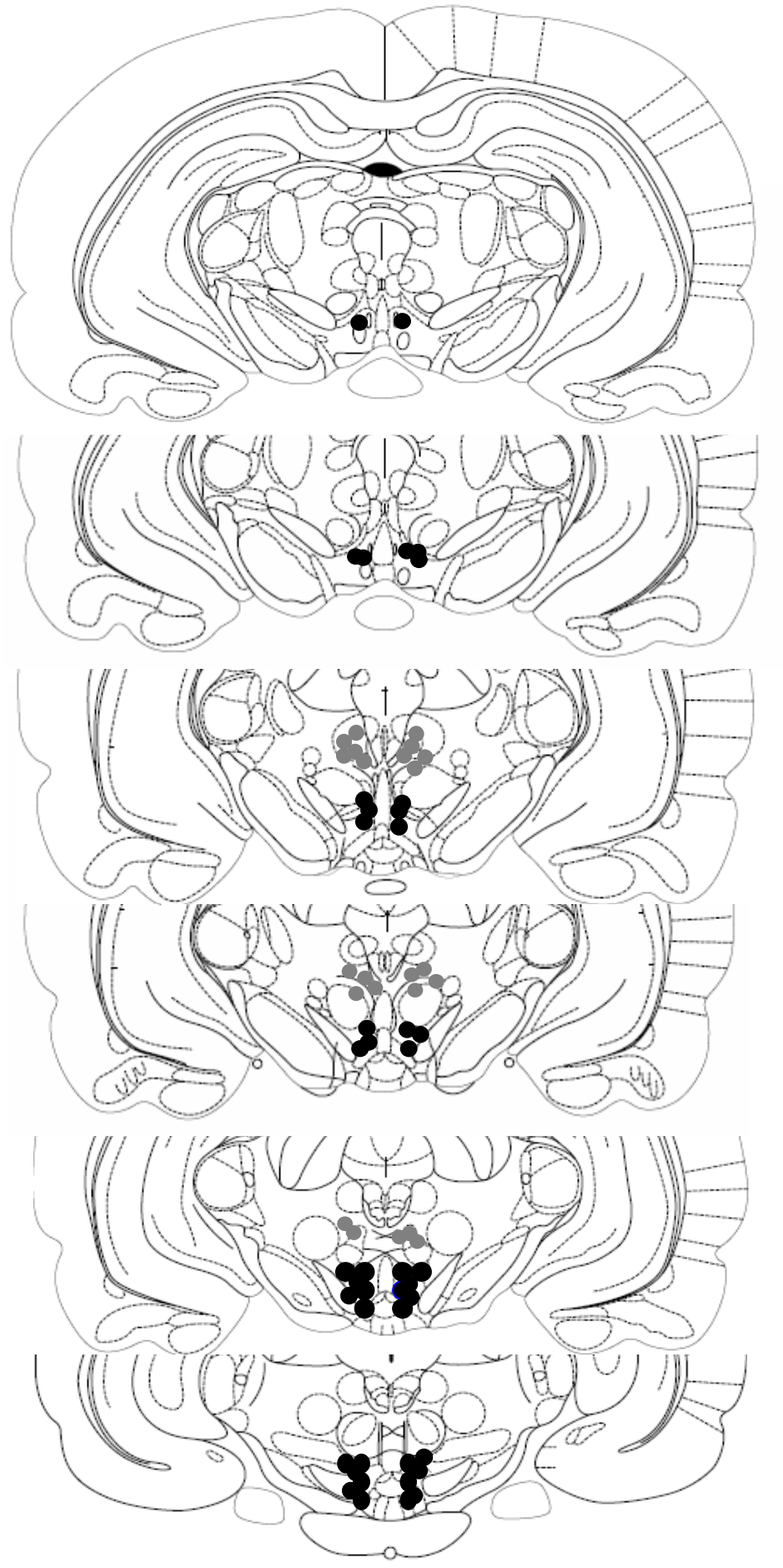


Figure 5. Mean active lever presses for groups receiving injections of vehicle (n=6) or 0.5 µg AP-5 (n=5) in a site 1 mm dorsal to the VTA site. Injections were made immediately prior to each session. Vertical bars represent the standard error of the mean (SEM).

Figure 6. Histological reconstruction of injection sites adapted from Paxinos and Watson (1986). Black circles represent injections in the VTA group; grey circles represent injections in the dorsal control group. The numbers to the right of each section indicates the distance posterior to the bregma.

Figure 6:



Discussion

In the present experiment, the selective competitive NMDA receptor antagonist AP-5 impaired the acquisition of lever pressing in a food-rewarded instrumental learning paradigm. When animals were treated with the two higher doses of intra-VTA AP-5, lever pressing for food was not acquired, but when they were relieved of this treatment, it was. These findings support our hypothesis that NMDA neurotransmission in the VTA is necessary for the acquisition of reward-related instrumental learning. Furthermore, microinjections of AP-5 into the VTA after lever pressing was learned had no effect, indicating that NMDA receptor stimulation in the VTA is not necessary for the expression of learned instrumental responding.

The impairment in acquisition found in this experiment is similar to those found with AP-5 in several mesolimbic terminal regions, including NAcc (see Kelley et al 2003, for review). The relation between these findings and other manipulations which yield impairments in acquisition but not expression will be discussed in Chapter 9.

Because compounds injected into brain tissue may diffuse along the cannulae track, it is always possible that positive effects of treatment we attribute to the VTA may be accounted for by effects dorsal to the injection site (Wise and Hoffman, 1992). The fact that there was no significant difference in lever pressing across sessions between in the rats receiving AP-5 or vehicle 1 mm

dorsal to the VTA argues that the impairments found in the VTA groups can be attributed to effects in the VTA with a high level of certainty. Additionally, there is no evidence that the stress of microinjections or other effects of handling accounted for low levels of lever pressing, as a comparison of group increases from sessions 3-4, 4-5, and 5-6 demonstrates no pattern of augmented behavior between sessions not preceded by injections compared to those that were (see Figure 2).

With any treatment that results in reductions in behavior, one possible explanation for the outcome is the occurrence of global motor deficits or sedation. However, the impairment in acquisition found in this experiment cannot be accounted for by any global reduction in activity, as AP-5 groups emitted more head entries than the vehicle group during the first four sessions, although group differences were not significant. AP-5 groups also pressed the inactive lever more than the vehicle group. This indicates that AP-5 did not impair the abilities to enter the food trough or to press a lever, making it difficult to argue that an inability to perform these responses accounted for the lack of increase across the first four sessions.

The significantly higher inactive lever pressing found in the AP-5 groups raises the possibility AP-5 treated rats received a sparser reinforcement ratio relative to active lever presses, or treated rats tended to generalize between levers, and therefore were impaired in making reward associations with the active lever. The findings described in the conditioned approach study, to be

described in Chapter 8, argue that the primary deficit in treated rats was forming specific associations with reward-related stimuli, not a sparser reinforcement ratio or an inability to distinguish between levers. However, the possible interference caused by increased inactive lever pressing should be addressed in future work, ideally in a paradigm which only presents rats with the opportunity to press one lever.

Regarding the lack of reductions in lever pressing on Day 10, one possible explanation might be that drug delivery was deficient, due to tissue damage or cannulae blockage. The evidence indicates that this was not the case, however, because head entries in the AP-5 groups increased on Day 10, demonstrating that AP-5 was still producing behavioral effects. Further effects of AP-5 will be discussed in Chapter 4. The lack of reduction of lever pressing together with a continuation of behavioral activation on Day 10 supports the interpretation that NMDA receptor stimulation was not necessary for reward-related learning to be expressed.

Although the alternative explanations cited above for the lack of acquisition found in this experiment are not likely, due to the performance of the animals within the experiment, one alternative explanation which was impossible to assess within this paradigm is that AP-5 treatment in the VTA may have interfered with food reward. In addition, although head entries and inactive lever presses indicated that motor activity was not reduced by the treatment, our planned activity test was still necessary as an additional measure.

Therefore we moved on to the control studies planned for Specific Aim 1, assessing effects of AP-5 on food motivation and activity. Given that 0.5 µg AP-5 was the lowest dose to produce a significant effect in this study, this dose was chosen for all subsequent experiments.

Chapter 5 Impairment in Acquisition Is Not Due to Reduction in Activity Levels or Basic Food Motivation

Numerous studies demonstrate that psychostimulants and DA agonists tend to increase motor activity, while neuroleptics and other DA antagonists tend to reduce activity (Beninger, 1983; Wise, 2006). In addition, application of glutamate and NMDA in the VTA, as well as both broad-spectrum glutamate antagonists and NMDA-specific antagonists, affect motor activity, although in contrast to DA manipulations, both agonism and antagonism of glutamate receptors in the VTA tends to increase activity. Since our treatment was aimed at a key source of forebrain DA, and affected glutamate transmission, it was necessary to plan to test any possible effects on activity following a significant effect on the acquisition of instrumental responding.

Similarly, if animals do not acquire a food-rewarded instrumental response, another possible explanation for the impairment could be that basic motivation towards food was impaired. Feeding is regulated by a host of factors, as discussed in the previous chapter. It is possible that NMDA receptor antagonism in the VTA, in the context of the other modulatory hormones and neurotransmitters associated with food deprivation, may have interfered with the responsiveness to primary reward signals. Two separate experiments,

therefore, were conducted to assess the affects of AP-5 on locomotor activity and on free feeding.

Subjects and Materials

Please refer to Chapter 3 (General Methodologies) for a full description of the subjects, surgery, apparatus, microinjection procedure, drugs and doses, and histology procedure.

Activity experiment

Two separate groups of rats were tested for ambulatory activity and stereotypy during four consecutive daily 60-min sessions in the activity chambers. Immediately before the first three sessions, all rats were given microinjections of vehicle. Rats were then ranked according to their average activity counts during the first three sessions, and assigned to either a vehicle (n= 6) or treatment (0.5 µg AP-5; n= 7) group in an ABAB pattern. Each rat received bilateral microinjections of either vehicle or drug according to group before the fourth session.

Free feeding experiment

Two separate groups of rats were tested for the effects of treatment on food consumption. Operant chamber levers were removed, and approximately 25-30 g of rat chow placed on a mesh grid on the chamber floor. A tray beneath the floor collected chow remains. Animals were placed in the food-loaded chambers for four consecutive daily 60-min sessions. After each session, the rat chow remaining on the floor and in the collection tray was weighed and

subtracted from the original weight to determine the amount consumed. Immediately prior to the first three sessions, all rats received bilateral vehicle microinjections. After the third session, rats were assigned to either a vehicle or treatment group based on ranked average chow consumption. Immediately prior to session four one group received saline (n = 7) and the other AP-5 (0.5 µg/0.5 µl, the lowest dose that significantly impaired acquisition of lever pressing; n=9).

Data Analysis

The feeding and activity data were analyzed using a mixed-design ANOVA with dose and day as factors. Interactions were followed up with tests of simple main effects with ANOVAs using the overall mean error.

Results

Free feeding experiment

Groups receiving vehicle or 0.5 µg/0.5 µl AP-5 ate the same amount of rat chow on the test session as they did on the baseline session before the test (see Figure 7). A mixed-design ANOVA yielded no significant effects of day or group, and no significant day x group interaction.

Activity testing

The 0.5 µg dose of AP-5 had little effect on ambulatory activity but caused an increase in stereotypy (see Figure 8; analyses revealed a significant session × group interaction [$F_{(1,11)}$=8.938, p<.05]). Visual observation of rats before and after activity sessions suggested that increased stereotypy mainly consisted of

increased circling behavior. No stereotypical behavior such as gnawing or repetitive grooming was ever observed.

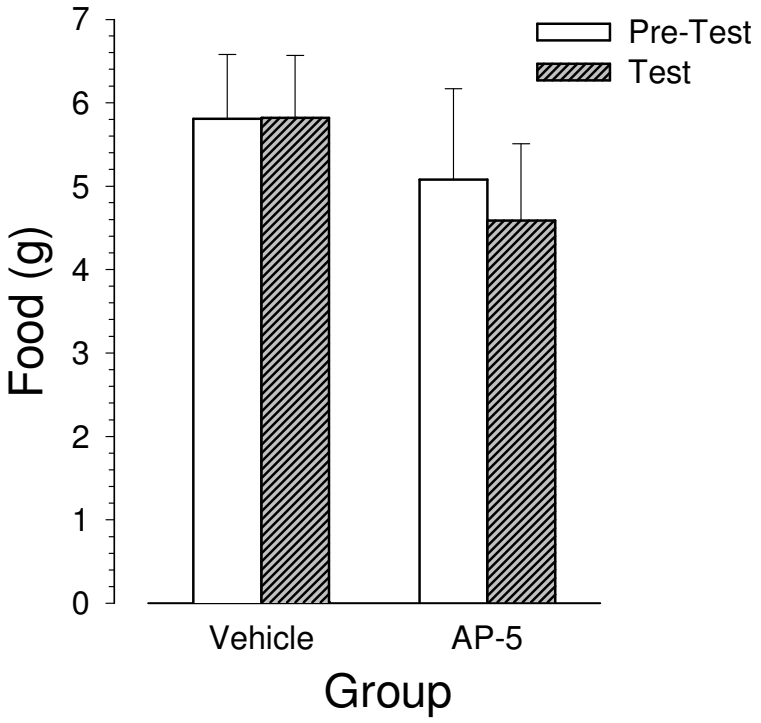


Figure 7. Mean consumption of rat chow on session 3 of baseline training and session 4 for the vehicle and AP-5 groups. Both groups received bilateral VTA microinjections of vehicle before session 3 and either vehicle (n=7) or 0.5 µg/0.5 µl AP-5 (n=9) before session 4. Vertical bars represent the standard error of the mean (SEM).

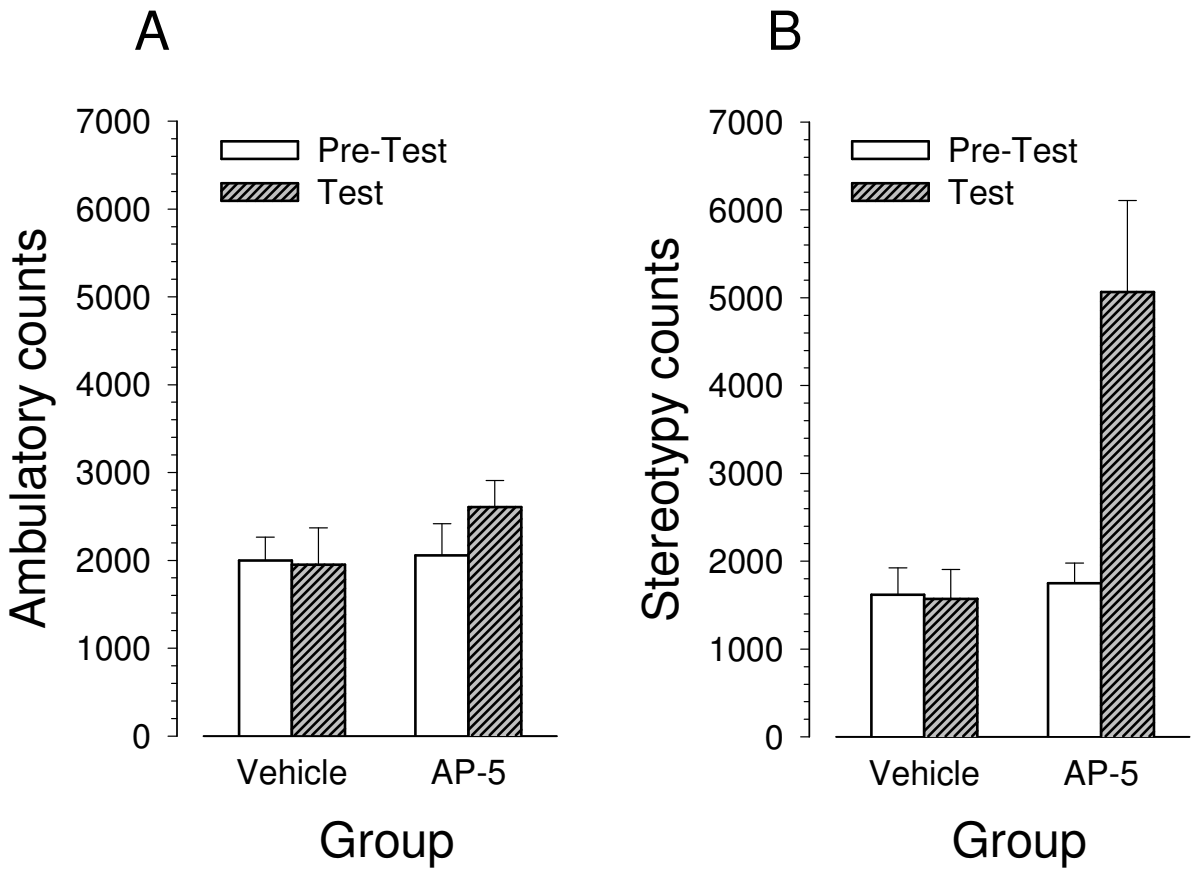


Figure 8. Mean total number of ambulatory (A) and stereotypy (B) counts on sessions 3 and 4 of activity testing. Both groups received bilateral VTA microinjections of vehicle before session 3 and either vehicle (n=6) or 0.5 µg/0.5 µl AP-5 (n=7) before session 4. Vertical bars represent the standard error of the mean (SEM).

Discussion

These control experiments provided further indication that reduced responding in the initial instrumental sessions with AP-5 was not likely due to reduced food motivation or motoric ability. Rats in the free-feeding experiment treated with intra-VTA AP-5 ate as much rat chow as those treated with vehicle, which is consistent with the fact that animals treated with AP-5 in the lever pressing acquisition experiment retrieved and consumed earned food pellets. This indicates that primary food reward was not affected by treatment and that food reward is not dependent on VTA NMDA receptor stimulation.

Effects on motoric activity were assessed in a separate experiment in which AP-5 produced no effects on ambulatory activity but increased stereotypy. This stereotypy may have consisted of increased circling behavior, which was visually observed, but not systemically measured, after injections. No other repetitive movements such as gnawing or grooming were ever observed. It should further be noted that, while stereotypy was measured in the activity chambers, we are unable to determine whether similar stereotypy actually occurred in the operant chambers, where rats were exposed to additional stimuli with the potential to orient behavior. The effect found on behavior in this study is similar to other reports of AP-5 in the VTA. For example, VTA application of AP-5 [APV] increased forward locomotion with no signs of stereotyped sniffing, an effect which lasted approximately 40 minutes (Dawbarn and Pycock, 1981).

The increased locomotor activity induced by intra-VTA AP-5 is not due to alterations in accumbens DA transmission (Cornish, et al., 2001). Although the mechanisms of NMDA receptor antagonist-induced increased locomotor activity are not yet understood, reduced activity of GABA neurons may play a role, as selective lesions of VTA GABA neurons results in increased activity (Shank, et al., 2007).

Taken together with the increased head entry measures found in the initial experiment during the sessions with drug treatment, these data all indicate that AP-5 did not reduce motoric behavior, demonstrating that impairments in acquisition were not attributable to such an effect. A more detailed analysis of the possible effects of stereotypy on response-reward contingencies is found in Chapter 6.

Generally manipulations that affect the VTA or terminal regions which impact acquisition of reward-related behavior do not alter basic consummatory behavior (Kelley et al. 2003): for example, manipulations of the NAcc that impaired conditioned stimulus learning did not affect eating (Kelley, et al., 1997; Baldwin, et al., 2002a; Baldwin, et al., 2002b), nor did manipulations in CeN or BLA (Andrzejewski, et al., 2005), indicating that in these procedures, learning was impaired as opposed to reduction of basic unconditioned processes. The findings of this experiment are in accord with those studies.

Chapter 6 Analysis of Response-Reward Contingencies

One of the factors which influences reward-related learning is the contingency between responses, reward and conditioned stimuli. In general, the tighter the link between responses and rewards, the more acquisition is facilitated. These links might be temporal, such that short inter-stimulus intervals (ISIs), when reward is presented within seconds following a response or conditioned stimulus, facilitate acquisition better than longer ISIs (Mackintosh, 1974). Similarly, the greater the frequency with which responses are reinforced, the faster instrumental responding is acquired.

As discussed in Chapter 5, the results of the activity study indicated that AP-5 resulted in an increase in stereotypy, and no change in ambulatory activity. Because analysis of head entries in the first experiment demonstrated that all groups receiving AP-5 entered the food trough as much or more than the vehicle group (with the exception of the 1 μg group on Day 1), together with the similarity in ambulatory activity, it is reasonable to conclude that AP-5 did not impair activity in general or the ability to enter the food trough. However, it is possible that stereotypy may have caused rats to experience longer delays between instrumental responses, together with the presentation of a light stimulus, on the one hand, and encountering food pellets, on the other hand. Longer delays between lever presses and food pellet exposures could possibly have reduced learning, just as longer ISIs impair acquisition. Alternatively, a

greater number of active lever presses not followed by a food trough entry might have provided rats with only partial reinforcement, similarly impairing learning.

Therefore, to assess whether the stereotypy induced by AP-5 caused drug-treated rats to experience different response-reward contingencies from vehicle-treated rats because they tended to move around the chamber more between lever presses and head entries, thus prolonging the periods between responses and reward, we analyzed the latencies between any active lever press that was followed by a head entry (as opposed to another active lever press or an inactive lever press). This measure was chosen because only those lever presses that were followed by a head entry ("food approach trials") afforded the possibility of experiencing a response-reward contingency. In all groups, it was common, at least early in training, for rats to occasionally emit two or more active lever presses in a row before entering the food trough. Therefore it was necessary to first ascertain the range of active lever presses to be compared. Visual inspection of active lever presses followed by head entries in the vehicle group indicated that food approach latencies declined after the 20th trial, and an analysis of the 0.5 µg AP-5 group revealed that an average of 40 active lever presses occurred before reaching the 20th food approach trial. The first 40 active lever presses was therefore chosen as the range within which the vehicle and 0.5 µg AP-5 groups would be compared.

Method

The following values were calculated and analyzed for the first 40 active lever presses emitted by the vehicle and 0.5 µg AP-5 groups: (1) the number of active lever presses required to reach the criterion where 20 of these presses were followed by a food trough head entry ('response-reward contingencies"), (2) the number of active lever-press clusters (defined as two or more active lever presses occurring within one sec of each other), (3) the time between active lever press and head entry for the 20 response-reward contingencies, (4) a standard food approach latency for the 20 response-reward contingencies (defined as the sum of the average latency in the vehicle group for the response-reward contingencies and the standard deviation), and (5) the percentage of food approach latencies that were equal to or less than this value. One-way ANOVAs were conducted to compare group differences on the number of active lever presses required to reach 20 response-reward contingencies, the number of clusters within the first 40 active lever presses, and the percentages of food approach latencies equal to or less than the standard value.

Results

Of the first 20 food approach trials, the percentage of approach latencies equal to or less than the standard established in the vehicle group was not significantly different between the groups (see Figure 9A). The number of active lever presses required to reach 20 food approach trials (Figure 9B) was slightly higher for the AP-5 group, as was the number of clusters within the first 40 active

lever presses (Figure 9C), but neither was significantly different from the vehicle group.

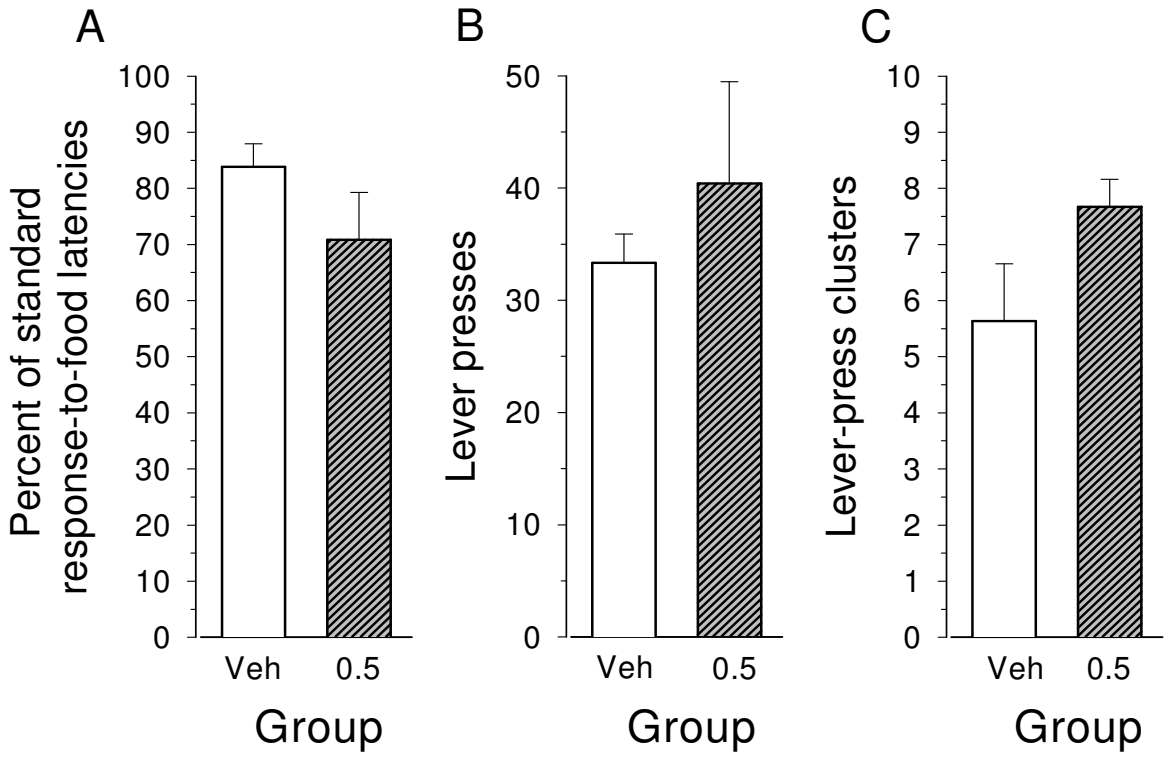


Figure 9. The relationships between active lever presses and food trough head entries emitted by the animals receiving vehicle or 0.5 µg AP-5 in Figure 1. (A) The percentage of food approach latencies (time between an active lever press and food trough head entry) that were equal to or less than the standard food approach latency (determined as the mean latency plus 1 standard deviation for the vehicle group). (B) Mean number of active lever presses accumulated by each group at the time when the criterion of 20 food approach trials (defined as an active lever press followed by a food trough head entry) was reached. (C) Number of clusters of active lever presses by each group within the first 40 active lever presses. Vertical bars represent the standard error of the mean (SEM).

Discussion

The results of our analysis indicate that the impairment of lever-press acquisition in rats receiving AP-5 cannot be attributed to a reduced response-reward contingency. As indicated in Figure 9A, AP-5 rats approached the food trough after emitting an active lever press in the first 20 trials at the same speed as vehicle rats, demonstrating that both groups experienced equivalent levels of reward contingency. In other words, the number of times that AP-5 rats entered the food trough (and presumably discovered a food pellet) within the same time frame as vehicle rats was equivalent. However, because vehicle rats went on to acquire lever-pressing for food, and AP-5 rats did not, something other than a reduced reward contingency must have been operating.

AP-5 rats tended to press the active lever more times in succession than vehicle rats, as shown in Figures 8B and 8C, although this difference was not significant. If there were to be any effect of this increased lever pressing on acquisition, it should have been a facilitatory one as AP-5 rats would then be more likely to find greater numbers of food pellets on approach trials. This further supports our conclusion that AP-5 rats did not experience any reduction in response-reward contingencies.

Thus, it appears that if AP-5 produced any stereotypy in the operant chambers, it did not affect response-reward contingencies. This being the case, it

was appropriate to move on to the next planned experiment to test whether the reward value of food pellets was affected by AP-5 treatment.

Chapter 7 Intra-VTA Application of AP-5 Does Not Reduce Reward Value of Food Pellets

In recent years mounting evidence suggests that two processes control the acquisition and expression of instrumental learning. During acquisition, responding is largely determined by goal-directed actions and susceptible to manipulations that devalue the reward. After acquisition, responding is largely habit-driven and relatively less susceptible to reward devaluation (Adams, 1982; Dickinson, et al., 1995). Thus, it is conceivable that in the initial experiment AP-5 selectively impaired acquisition, but not expression, of lever pressing by devaluing the reward of the food pellets, which would have reduced goal-directed behavior during acquisition but not once animals had received sufficient opportunity to practice the response. Further testing of the impact of AP-5 on reward processing over and above a free-feeding test is necessary because it is possible that although rats consumed food pellets because basic consummatory processes were intact, as the findings described in Chapter 5 demonstrated, the encoding of reward value was affected, which might have impacted subsequent sessions. A devaluation procedure can assess whether the previous reward value representation affects current responding.

Devaluation is sometimes induced after instrumental responding for a reward has been acquired (Holland and Straub, 1979) using techniques such as post-ingestive malaise provoked by lithium chloride injection following

consumption of that food (e.g., Parkinson, et al., 2005) or pre-feeding before re-exposure to the food reward (e.g., Corbit and Balleine, 2003). When the reward is devalued, animals typically will respond less for a reward-associated CS in extinction following the devaluation experience. If such a reduction in responding is found, it can be concluded that the memory of the reward value of the unconditioned stimulus (US), now reduced, associatively reduces the incentive-motivational impact of the CS.

Accordingly, if intra-VTA AP-5 devalues the reward associated with food pellets, it should also reduce responding for a food-associated cue. We therefore designed an experiment in which animals acquired instrumental responding for food pellets while free of treatment, were then given free access to food pellets during a session after microinjections, and finally given the opportunity to lever press with only the food-associated cue as a consequence. The parameters of this experiment allowed for animals to acquire an instrumental response with a minimum of reward history, in order to maximize the possibility that responding on the test day would reflect the representation of value of the reward rather than a highly rehearsed automatic response. Furthermore, we specifically chose only one session of treatment to test whether any reward devaluation accounted for the lack of increase in active lever pressing in the AP-5 groups relative to the vehicle group, which was already evident on Day 2. We hypothesized that AP-5 would not affect the reward value of food pellets and therefore that AP-5 treated

rats would show no decrease in lever pressing reinforced by the conditioned stimulus.

Methods

Subjects and Materials

Please refer to Chapter 3 (General Methodologies) for a full description of the subjects, surgery, apparatus, microinjection procedure, drugs and doses, and histology procedure.

Behavioral Procedure

Two separate groups of rats were tested in operant chambers to assess whether intra-VTA injection of AP-5 reduces the reward value of Bio-Serv food pellets. Rats received two instrumental conditioning sessions in which presses on the active lever resulted in the delivery of a food pellet and a 2 s presentation of the light above that lever. Presses on the inactive lever were recorded but resulted in no consequences. Sessions were terminated after 30 active lever presses or 60 minutes. Any rat that pressed the active lever 30 times during at least one session continued on to the next phase. Rats were then randomly assigned to either a vehicle (n=6) or 0.5 µg AP-5 (n=7) group. In session 3, each rat received a bilateral microinjection of either saline or AP-5, and was then immediately placed in the operant chambers in which levers were absent and 50 food pellets available in the food trough, for 15 minutes. During the fourth and final session, rats were again placed in the operant chambers with access to levers. Pressing the active lever resulted in presentation of the light stimulus

only. Active and inactive lever presses and food trough head entries were recorded.

Data Analysis

The number of active and inactive lever presses in session 4 were compared between groups using a two-way ANOVA with group and lever as factors.

Results

Following a session in which rats ate operant chamber food pellets after receiving intra-VTA injections of vehicle or 0.5 µg AP-5, each group emitted more active than inactive lever presses, as a two-way ANOVA revealed a significant effect of lever ($F_{(1,22)}$=24.532, p<.005). However, there were no significant differences between groups nor a significant group x lever interaction.

During the treatment session, some rats did not eat all pellets available, due to a combination of leaving pellets in the trough or knocking pellets out of the trough and onto the chamber floor where they were inaccessible. Any rat who left more than 50% of pellets in the trough was excluded from the test session (4 rats in the AP-5 group). Of the remaining rats, 1 rat in the vehicle group left 20% of pellets in the trough, so any AP-5 rats who left equivalent pellets was permitted to proceed to the test session. All rats who were tested ate at least 50% of available pellets, with the majority (4 of 6 vehicle rats and 4 of 7 AP-5 rats) eating at least 70% of available pellets.

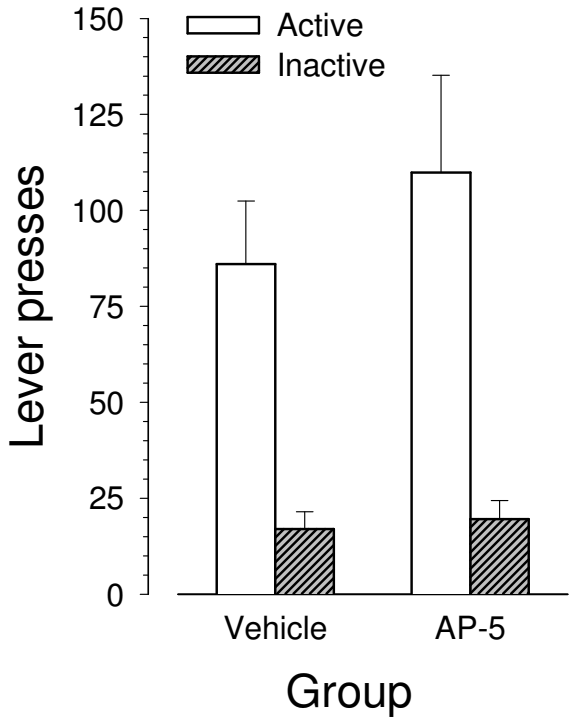


Figure 10. Mean number of active and inactive lever presses during an extinction session for rats that received either vehicle (n=6) or 0.5 µg AP-5 (n=7) immediately prior to a previous session with free access to 50 operant chamber food pellets. Vertical bars represent the standard error of the mean (SEM).

Discussion

In our food pellet devaluation experiment, animals that consumed food pellets under AP-5 or vehicle treatments during acquisition responded at similar levels during a subsequent extinction test. Because extinction responding represents goal-directed behavior and is indicative of remembered reward (goal) value, this test indicates that food pellet devaluation did not occur when food pellets were consumed by AP-5-treated animals. Therefore, the selective impairment of acquisition seen here cannot be adequately explained as differential effects of AP-5 on the representation of reward value which then differentially affects responding that is either goal-directed or habit-driven.

Chapter 8 Intra-VTA Application of AP-5 Impairs Acquisition of Conditioned Approach

The results of the first study demonstrated that acquisition of instrumental responding was impaired by intra-VTA application of AP-5, and subsequent experiments suggested that this impairment was not due to reductions in motoric activity, food consumption or food reward. That initial experiment was designed to test the role of conditioned stimuli in acquisition, with the presumption that part of acquiring instrumental responding involves learning about the conditioned stimuli in the environment – the sound of the pellet dropping, the light presentation accompanying the lever press, the feel of the lever and the sensation of pressing on it – and that these CSs also energize further approach behavior, facilitating acquisition. However, it is possible that intra-VTA treatment impaired some part of the *motoric* aspect of acquisition, so that while animals may have begun to associate the light presentation with food delivery, for example, they were not able to consolidate the motor pattern of lever pressing.

In order to test the hypothesis in a more simplified way, therefore, we planned to test animals in a basic conditioned approach paradigm in which no new motor behavior had to be acquired. In this study, we would assess whether intra-VTA AP-5 treatment would impair the ability of a conditioned stimulus to

control a behavior that rats naturally emit when investigating a novel chamber or retrieving food – namely, inserting their heads into the food trough.

To the extent that our paradigm presented the CS before the US, allowing associations to be formed between a stimulus and an outcome, this paradigm addresses Pavlovian conditioning. Because we did not assess approach to the CS itself, but rather approach to the magazine influenced by presentation of the CS, this is not properly called a Pavlovian approach paradigm but rather a discriminated approach paradigm. However, because we were unable to assess whether rats were indeed approaching the CS itself, we have chosen to call this a conditioned approach paradigm to allow for both possibilities. Whether the rats are reflexively approaching the food trough during the CS because of its association with reward, or approaching the magazine because the CS generates an expectancy of a food pellet presentation, we are unable to say.

As noted in Chapter 1, there are differences in approach paradigms based on the use of extended or discrete presentation of conditioned stimuli, which may account for conflicting results on manipulations assessing involvement of particular circuits or neurochemistries in acquisition. When stimuli are present in an extended time frame, such as a 2-min presentation of a light, during which pellets are presented on a random 30-s schedule, rats may show higher levels of head entries during CS periods, but that may simply be due to responding to food presentations. In contrast, discrete presentations of 3-10 s allow for a more accurate assessment of whether rats are responding to the CS per se, as head

entries during the CS can be compared with a period immediately preceding the CS. This avoids the possible confounds of using an extended CS punctuated by several deliveries of a primary reward, which may conflate approach to the reward with approach conditioned by the CS. In addition, shorter presentations of the CS facilitate acquisition of discriminated approach (Delamater and Oakeshott, 2007). Therefore we tested rats in a paradigm in which discrete light presentations were paired with food deliveries, to assess whether head entries would increase during CS periods relative to an equal control period. In addition, we trained a separate group of rats with saline injections who received random presentations of food and light. We predicted that if 0.5 μg AP-5 impaired the ability of rats to acquire a conditioned association to the light presentation, their performance on a test day of light presentation would be comparable to the rats trained with random light presentations.

Method

Subjects and Materials

Please refer to Chapter 3 (General Methodologies) for a full description of the subjects, surgery, apparatus, microinjection procedure, drugs and doses, and histology procedure.

Behavioral procedure

Just as in the previous food-rewarded experiments described above, after recovery from surgery and at least one day of food deprivation rats were given

20 Bio-Serv food pellets in their home cage before being given their daily rations for each of three days before beginning testing, to allow rats to become acquainted with the attributes of the novel food.

Subjects were given one 20-min magazine training session in the operant chambers in which 20 food pellets were delivered on a random schedule, to allow rats to become acquainted with magazine delivery of food pellets. Rats were then randomly assigned to groups and received bilateral microinjections of either vehicle or 0.5 µg AP-5 immediately before three 60-min conditioning sessions given once daily. During conditioning sessions, 30 food pellets were delivered on a random 2 min 40 sec schedule. For two groups (the "saline paired" and 0.5 µg AP-5 groups), each pellet delivery was preceded by a 3-s presentation of a light on the left side of the trough. For a third group (the "saline random control" group), food pellets and a 3-s light were each presented on a random 2 min 40 sec schedule, such that food delivery and light presentations had no correlation.

After the 3 days of conditioning with intra-VTA treatment, all rats received one 30-minute extinction session with no pre-treatment during which no light or food presentations were programmed. This extinction session was designed to reduce responding to similar levels between groups, thereby maximizing the potential of observing differential CS-related responding on the test day. Finally, a test session was administered in which, following saline microinjection for all groups, rats were presented with light presentations on the

same random schedule as that used during training, with no further consequences. All sessions (habituation, 3 training, extinction, and test) were conducted on consecutive days.

Measures

All head entries into the magazine were recorded as follows: during the 6 s immediately prior to the CS presentation ("Pre-CS"), during the 3-s CS presentation ("CS"), and during the 3 s immediately after the CS presentation ("Post-CS"). The number of head entries during the Pre-CS period provided a measure of baseline responding, as it randomly sampled activity of the same duration as the combined CS and Post-CS periods, which were predicted to increase if reward-related learning occurred. Head entries at all other times were designated as "Other." The sum of these four categories provided the total head entries during the entire session.

Data Analysis

Total head entries and conditioned approach ratios across the three conditioning sessions were compared with a repeated measures one-way ANOVA, with group as the between-subjects factor and session as the repeated measure. Conditioned approach ratios on the test day were compared with a separate one-way ANOVA using group as the between-subjects factor. Follow up tests of significant effects were conducted with tests of simple main effects with one-way ANOVAs using the overall mean for the training phase, and a least significant differences test for the test session.

Results

As shown in Figure 11, across the three conditioning sessions total head entries increased for the AP-5 group, and remained steady for the saline paired and saline random group. A repeated measures ANOVA revealed no significant effect of day, but a significant effect of group ($F_{(2,26)}$=10.451, p=.000) and a significant day × group interaction ($F_{(4,52)}$=4.921, p<.002). Follow up tests indicated that the AP-5 group showed a significant effect of day ($F_{(2,52)}$=6.992, p=<.05), whereas the other groups did not. Total head entries for all three groups decreased markedly during the extinction session, and there was no significant difference between groups on the test day.

Because total head entries were different between groups during the three conditioning sessions, the most representative way to compare any possible effects of the CS on head entries between groups was to express head entries as a ratio of the sum of the CS and Post-CS (in the numerator) to the Pre-CS (in the denominator) (the "conditioned approach ratio"). The conditioned approach ratio during conditioning increased for the saline experiment group, but not the AP-5 or saline random control rats (see Figure 12); a repeated measures ANOVA on the conditioned approach ratios for each group across the three conditioning sessions revealed a significant effect of day ($F_{(2,52)}$=3.089, p=.05); a significant effect of group ($F_{(2,26)}$=6.673, p=.005); and a significant day × group interaction ($F_{(4,52)}$=2.563, p<.05). A one-way ANOVA on the conditioned approach ratios on

the test day revealed a significant effect of group ($F_{(2,26)}$=3.697, p<.05), and a post-hoc LSD test showed that the saline experimental group was significantly different from both the AP-5 and saline random control groups, which in turn did not significantly differ from each other.

In absolute terms, AP-5 rats increased their head entries in both the pre-CS and CS/Post-CS periods, whereas Saline Paired rats only increased head entries in the CS/Post-CS period, and Saline Random rats showed a decline in head entries in both periods across sessions (see Figure 13). On the test day, only the head entries during the CS/Post-CS periods for the Saline Paired groups was increased. These data were not analyzed for statistical significance because they had already been used for the analysis mentioned above, and are only shown to illustrate the actual behavior of the groups. It should be noted that absolute head entries do not directly correspond to approach ratios reported above, because of numerical transformations performed to calculate ratios to account for some zero values in head entries for some subjects.

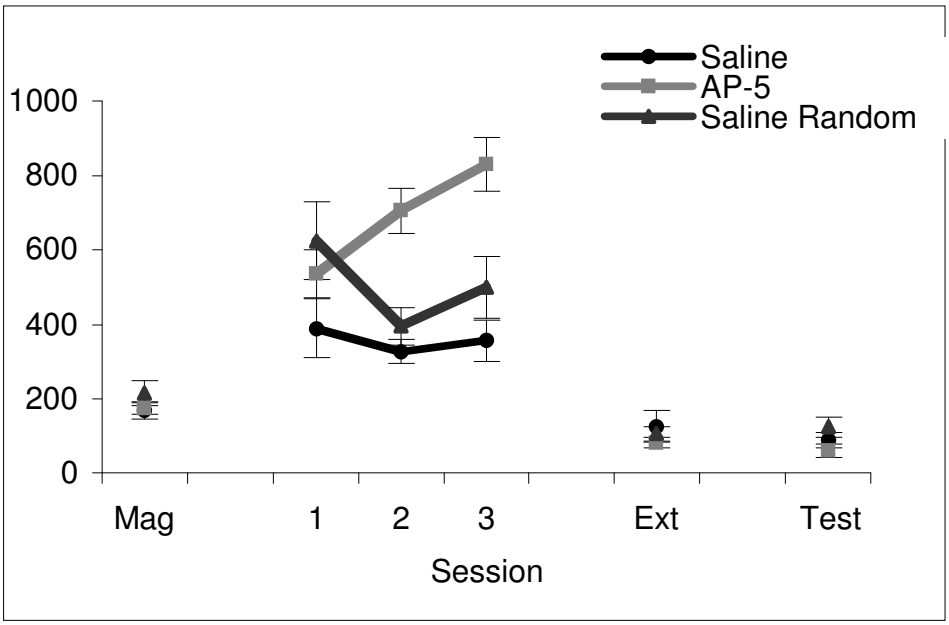


Figure 11. Mean number of total head entries across sessions. After one session of magazine training ("Mag"), rats received 3 conditioning sessions after being treated with intra-VTA AP-5 ("AP-5", n=9) or saline ("Saline", n=11) and trained with paired presentations of light and food pellets; another group treated with saline received presentations of light and food random to each other ("Saline Random"; n=9). All groups then received one extinction session ("Ext") and one test session consisting of random presentations of the light stimulus ("Test"). Vertical bars represent the standard error of the mean (SEM).

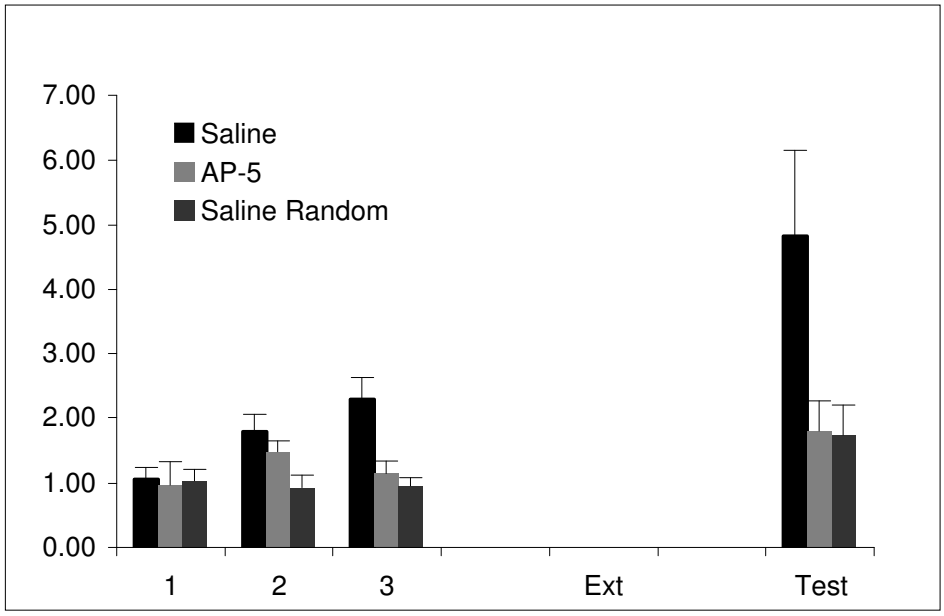


Figure 12. Mean ratios for each group as described in Figure 11 representing the total head entries during CS and Post-CS periods combined, compared to total head entries during the Pre-CS period across the three conditioning sessions and test session. Vertical bars represent the standard error of the mean (SEM).

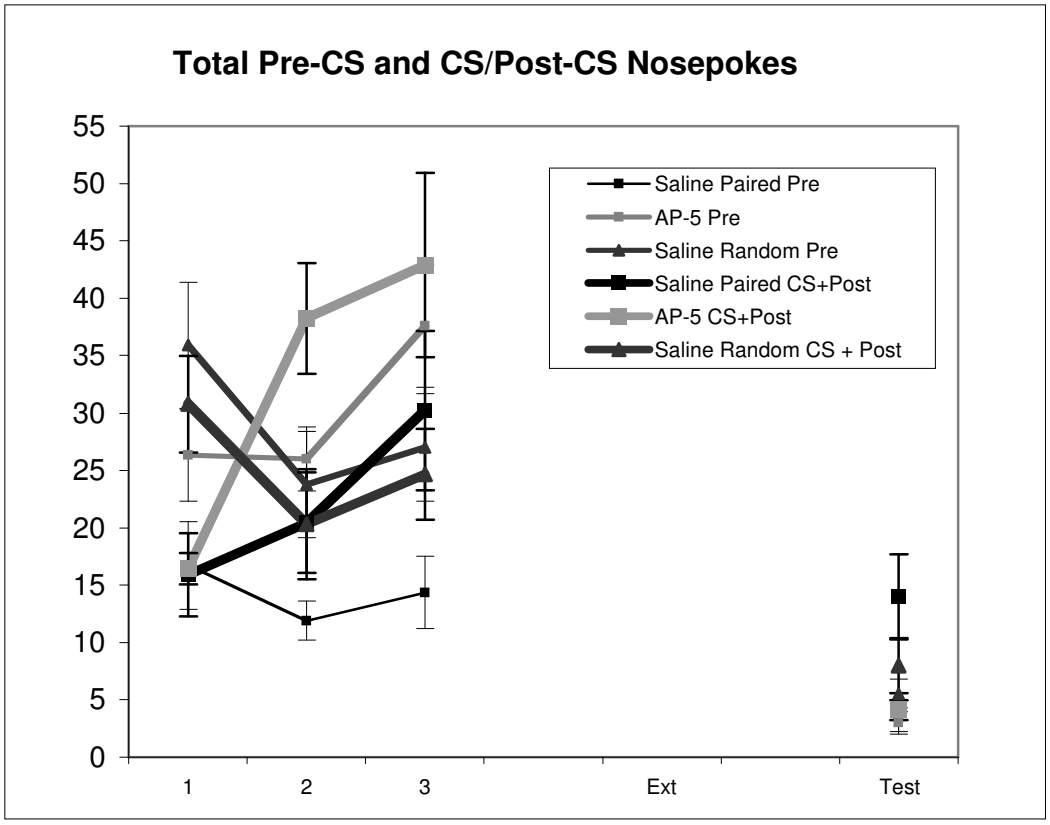


Figure 13. Total head entries per group during the Pre-CS period and the CS+Post-CS period, during the three training sessions and the test session. Vertical bars represent the standard error of the mean (SEM).

Discussion

In this experiment, rats that received intra-VTA injections of AP-5 showed no evidence of acquiring conditioned approach cued by a food-associated light cue. This was indicated first by a lack of increase in the ratio of CS and Post-CS head entries to Pre-CS entries across three conditioning sessions by the AP-5 treated rats, like the saline random group, while the conditioned approach ratio of the saline experimental group did increase across those sessions. More importantly, during the test session, which directly followed saline injection for all groups, the conditioned approach ratio associated with the presentation of the light cue was significantly higher in the saline paired group than the AP-5 group, which in turn was not significantly different from the saline random group. Because the test session was conducted without any acute effects of AP-5 treatment, the lack of conditioned approach provides even stronger evidence that NMDA receptor blockade prevented the acquisition of learning in relation to the CS.

These results strongly support the hypothesis that NMDA receptor stimulation in the VTA is necessary for the acquisition of reward-related learning. In this experiment, NDMA receptor blockade, while leading to an overall increase in activity, as it did previously in both the instrumental learning and activity studies, prevented an increase in head entries relative to the CS, both during training following pre-treatment with drug, and during extinction with

no pre-treatment. AP-5 treated rats behaved as if the CS had acquired no special significance, just as it appeared to remain meaningless to the saline random rats.

The findings of this study, in conjunction with those of the first on instrumental learning, suggest that synaptic plasticity in the VTA is a key step in the process by which Pavlovian stimuli exert their activational and directional influences on instrumental behavior (Estes 1948; Rescorla and Solomon, 1967; Bolles, 1972; Bindra, 1974). The findings of the present study, seen in this context, are consonant with those demonstrating that VTA inactivation reduces Pavlovian-to-instrumental transfer (Murschall and Hauber, 2006), reducing both general and outcome-specific PIT (Corbit, et al., 2007). Together, these data suggest that signals representing CSs activate VTA cells, thereby increasing approach and incentive-motivational processes which facilitate instrumental responding, and furthermore that associative processes in the VTA allow for those signals to become established as activators of VTA cells.

In addition to the stimulus-approach associations at the level of the VTA, NMDA blockade may also have blocked a normal phasic increase in DA in terminal regions including NAcc, amygdala, and PFC, where stimulus-response, stimulus-reward, and response-outcome associations would ordinarily take place. It may therefore be the case that the impairment in reward-related learning found in these studies indicates that under normal conditions, a primary synaptic strengthening of environmental signals in the VTA leads to a secondary facilitation of plasticity in terminal regions.

The findings of the present study are consonant with those finding impairment in the acquisition of conditioned approach with manipulations of mesocorticolimbic terminal regions cited previously, including the NAcc (Kelley, et al., 1997; Parkinson, et al., 2000; Di Ciano, et al., 2001; Parkinson, et al., 2002) amygdala (Burns, et al., 1993; Burns, et al., 1994), and anterior cingulate (Bussey, et al., 1997; Cardinal, et al., 2003). Now our findings add to the understanding of the neural circuitry of the acquisition of Pavlovian conditioning by indicating that NMDA receptor stimulation in the VTA is also necessary.

The increase across the three training sessions in total head entries emitted by the AP-5 group deserves closer examination. This change in behavior suggests the possibility that some kind of learning was taking place in these animals, although we are unable to determine specifically what that might have been. It is possible that AP-5 rats were acquiring some association between reward and the operant chamber, or the food trough, but in the absence of the ability to associate food delivery with the discrete cue, were indiscriminately increasing their investigation of the food trough. It is also possible that the stimulation of food reward, in the context of an inability to link reward to any specific stimuli, resulted in heightened activity. The ability of intra-VTA AP-5 to facilitate context learning or other aspects of reward-learning which are not related to discrete cues therefore merits additional investigation. The scant evidence which exists to date would argue against this being the case, at least in the case of drug administration with VTA NMDA blockade (Harris and Aston-

Jones, 2003; Harris, et al., 2004) where NMDA receptor blockade is associated with impaired context conditioning**;** to our knowledge context-related learning with food reward has not been tested with this manipulation and therefore remains an empirical question. The results of this experiment also argue against context learning; although the total head entry data during days 1 to 3 suggest a possibility of learning, pre-CS responding on the test day was no higher than controls, which ought to have been the case if AP-5 treated rats had learned a reward association with the environment. In any case, given that AP-5 rats on a test day with no drug pre-treatment emitted similarly low levels of head entries to vehicle-treated rats, it appears that even if some kind of learning were taking place across the training sessions, AP-5 rats were specifically impaired in being able to acquire an association with the discrete reward-paired cue.

One final note is that the importance of NMDA receptor stimulation in the VTA is additionally indicated by the fact that animals received sufficient pairings to induce conditioned approach. In similar paradigms, control animals begin to differentially approach a food magazine or CS within approximately 40-60 pairings (Parkinson, et al., 2000; Dalley, et al., 2002; Delamater and Oakeshott, 2007). Our animals received 90 pairings, exceeding that range and giving them substantial opportunity to learn about the CS-US contingency. This suggests that the blockade of NMDA receptors in the VTA robustly impairs learning about a cue associated with reward.

Chapter 9 General Discussion

This dissertation tested the hypothesis that NMDA receptor stimulation in the VTA is necessary for the acquisition, but not expression, of reward-related learning. The findings described in the previous chapters support this hypothesis. Broadly speaking, in both an instrumental learning paradigm and a Pavlovian conditioning paradigm, bilateral microinjections of the selective competitive NMDA receptor antagonist AP-5 into the VTA impaired or abolished reward-related learning. Additional studies demonstrated that these impairments were not due to reductions in motor activity, basic consummatory processes, or the reward value of the food reward. Together, these findings indicate that at least some of the neural changes critical for reward-related learning occur in the VTA, and that these neural changes involve NMDA receptor stimulation.

The findings of this dissertation are summarized as follows. In an instrumental learning procedure, rats receiving intra-VTA microinjections of the selective competitive NMDA receptor antagonist AP-5 at the two higher doses showed no increase in lever pressing across four treatment sessions, indicating impairment in acquisition, while rats receiving vehicle or lower doses of AP-5 did increase their pressing, indicating acquisition of the response. All groups increased lever pressing over five sessions with no pre-treatment. In a final session, intra-VTA AP-5 did not reduce lever pressing, indicating that NMDA

receptor stimulation is not necessary for the expression of reward-related learning. Moreover, the lack of effect on the final treatment session further supports our assertion that the initial impairment in acquisition was not due to an inability to emit a lever press, as rats on Day 10 indicated that they were fully able to do so.

Similarly, in a conditioned approach paradigm intra-VTA AP-5 blocked an increase in CS-related approach across three conditioning sessions in rats receiving paired presentations of a light cue and food pellets. This lack of increase was in contrast to vehicle-treated rats, which did show increased approach to the food magazine during and immediately after the CS, and was comparable to rats that experienced random presentations of light and food. More importantly, conditioned approach was significantly higher on a test day in extinction with no pre-treatment in rats that had not experienced NMDA receptor blockade during conditioning than rats who had experienced NMDA receptor blockade or random presentations. The findings of this final study support the notion that the key element impaired in the instrumental learning experiment was the ability of the CS to control behavior. Specifically, it appears that NMDA receptor stimulation in the VTA is necessary for CSs to elicit incentive-motivational behavior, contributing to the "central motive state" to perform reward-related behavior.

Because the application of a selective NMDA antagonist in the VTA prevented animals from acquiring reward-related responses, it is possible that

the underlying mechanism of this impairment was the inhibition of synaptic plasticity, thereby preventing signals representing environmental stimuli to acquire the ability to activate cells in the VTA. We presume, therefore, that under normal physiological conditions, the synaptic plasticity induced by NMDA receptor stimulation allows environmental signals to come to activate VTA cells. This stimulation of VTA neurons would elicit an appetitive motivational state and energize approach behaviors, which are both necessary for engaging in reward-directed behavior in themselves, thereby increasing the animal's encounters with reward-related stimuli. At the same time, increased DA release at the terminal regions would allow for downstream associative processes to occur, which underlie additional associative processes important in reward-related learning, such as stimulus-response and response-outcome associations.

The attribution of a specific impairment in CS-related learning to NMDA receptor blockade was further supported by the additional control studies conducted. Impairment in acquisition of instrumental learning could not be attributed to a general reduction of motor behavior, as animals receiving AP-5 treatment in the instrumental paradigm emitted equal or greater numbers of head entries and inactive lever presses as vehicle-treated rats, and animals receiving AP-5 in a separate activity study demonstrated equal ambulatory activity and elevated stereotypy, which appears to have been due to increased circling behaviors and not repetitive stationary behaviors such as gnawing which

might have kept them away from the food magazine. This supposition was supported by close analysis of latencies to approach the magazine after a lever press, which was not significantly different in AP-5 treated rats, indicating that an impairment in acquisition was not due to different response-reward contingencies. Furthermore, AP-5 did not reduce basic motivation to eat food, nor did it reduce the reward value of the food pellets, as demonstrated by equal consumption of rat chow between AP-5 and vehicle-treated rats, and by an absence of a reward devaluation effect in rats who consumed pellets under AP-5 and then responded in extinction for a pellet-associated light cue. Additionally, there is little likelihood of delayed or post-session effects of AP-5 treatment on food consumption, as daily chow rations were consumed by all rats in all experiments before any subsequent session.

NMDA receptor stimulation in the VTA proved not to be necessary for expression of reward learning, which is similar to other studies which blocked NMDA receptors in the NAcc (Kelley, et al., 1997; Di Ciano, et al., 2001; Hernandez, et al., 2005). This was indicated by the fact that animals did not significantly reduce their lever pressing on a final session working for food pellets after pre-treatment with AP-5. The finding that NMDA receptor antagonism did not impair expression of reward-related learning is consonant with our hypothesis that once glutamatergic synapses carrying environmental stimuli-related signals have been strengthened, NMDA receptor stimulation is

no longer required for those signals to activate VTA cells by themselves, thus supporting the maintenance of reward-related behavior.

These findings make an important contribution to answering the question posed in the introduction: how does the VTA "come to know" about conditioned stimuli? As discussed earlier, a wealth of evidence from different lines of research demonstrates that the VTA is responsive to conditioned stimuli. Now we are beginning to understand how the VTA comes to be activated by conditioned stimuli.

Our findings that glutamate transmission in the VTA plays a necessary role in the acquisition of reward-related learning add to a growing body of data on VTA glutamate. The extensive evidence covered in Chapter 1 that glutamate and NMDA trigger burst firing in VTA DA neurons, and that burst firing is related to reward-related stimuli as well as primary rewards, already suggested a role for VTA glutamate in CS-related processes. Recent evidence demonstrates that glutamate in the VTA is indeed a critical substrate for behavior associated with CSs: in animals lever-pressing in extinction reinforced by a cocaine-related cue, extracellular glutamate concentrations in the VTA showed a steep increase at the beginning of the session, which was not found either dorsal to the VTA or in the substantia nigra (You, et al., 2007). Interestingly, while glutamate levels peaked to the same extent in self-administration sessions, it appears that glutamate was more related to the cues present at the onset of the session than the cocaine itself, because glutamate levels declined while cocaine self-

administration continued. Furthermore, VTA glutamate levels were correlated with reward-related behavior such as activity towards the lever or the lever slot, and blockade of glutamate in the VTA (but not the dorsal site or the SN) dramatically reduced lever pressing in extinction. That study complemented earlier findings that reduction of glutamate transmission in the VTA reduced contextual-cue reinstatement of heroin seeking (Bossert, et al., 2004).

The evidence strongly suggests, then, that glutamate signals in the VTA are a substrate for the activational or directional influences of conditioned stimuli. This is consonant with other recent findings that inactivation of the VTA reduces the effect of CSs on behavior or neuronal activity: GABA agonist injection into the VTA abolishes NAcc firing in response to conditioned stimuli (Yun, et al., 2004b), markedly reduces instrumental responding reinforced by a CS (Di Ciano and Everitt, 2004b), and dramatically reduces or abolishes Pavlovian-to-instrumental transfer (Murschall and Hauber, 2006; Corbit, et al., 2007). Indeed, the authors of one of these studies assert that "conveying Pavlovian influences on instrumental behavior requires neural activity in the VTA" (Murschall and Hauber, 2006, p. 123).

Of course, VTA glutamate being important for the *effects* of conditioned stimuli does not necessarily mean that it is important for the *acquisition* of those processes. But as reviewed in Chapter 1, previous evidence does suggests that NMDA receptor stimulation in the VTA is necessary for acquisition of reward-related associations. The findings of this dissertation complement and extend

those findings. In particular, these findings make a new contribution to the literature in several ways. Previous studies indicated that VTA glutamate was involved with the acquisition of drug-related associations involving contextual cues (Harris and Aston-Jones, 2003; Harris, et al., 2004). However, our study is distinct from these two and therefore extends the findings on VTA NMDA stimulation in several ways. First, only the 2004 study tested NMDA blockade individually on acquisition; since the 2003 study did not test expression, and the 2004 tested simultaneous NMDA and AMPA blockade on expression, our findings are the first that clearly demonstrate that NMDA receptor antagonism by itself blocks acquisition, but not expression. Second, those studies did not evaluate possible impact on reward value, which our study did, indicating that NMDA receptor antagonism in the VTA does not degrade reward, at least for food. Finally, our study examined reward-related learning in regard to distinct cues, rather than general context, and with food reward instead of drugs of abuse. Our findings are therefore the first evidence that NMDA receptor-mediated associative processes in the VTA are a necessary element in the acquisition of food reward-related associations, that these associative processes in the VTA can mediate discrete CS associations, and that NMDA receptor stimulation is not necessary for expression.

Moreover, the current findings are significant in indicating a fundamental role for synaptic changes in the VTA as one of the first in a sequence of reward-related learning processes, because NMDA receptor blockade in the VTA

impaired the acquisition of both instrumental and Pavlovian conditioning, which some investigators have argued are dissociable at the neural level (Hitchcott and Phillips, 1998; Dickinson, et al., 2000). It would appear that the ability of signals relating to environmental cues to begin to activate VTA cells, thereby augmenting approach behaviors and, presumably, DA release at terminal regions, is a necessary first link in a chain of events leading to reward-related learning. In other words, it is possible that without synaptic strengthening at glutamate synapses carrying information about the presentation of reward-related cues, such as the onset of a light, the sound of a pellet dropping, the sound of a lever being depressed, and so on, the animal remains naïve to the situation and encounters these stimuli each time as if it were the first. It may be that the temporal sequence involved in acquiring reward-related behavior is that the CS begins to stimulate approach behavior as stimulus-approach associations are being formed at the level of the VTA, and after several response-reward iterations, stimulus-response and response-outcome associations begin to be consolidated at higher levels such as amygdala and NAcc. But without the initial recruitment of VTA cells by CSs due to coincident stimulation with the primary reward, the conditions for forming additional reward-related associations may not be present at the terminal regions, and the animal may not be motivated to increase its level of motivated exploration directed towards reward-related stimuli. Of course the findings of this dissertation cannot provide evidence for this supposition and further research is needed to explore it further.

The current findings are also consonant with those from an earlier set of studies in our lab using a similar paradigm, which found an impairment in the acquisition, but not expression, of food reward-related learning following blockade of mACh, but not nACh, receptors in the VTA (Sharf, et al., 2006). Together, these studies support the model set out in Chapter 1, which proposes that synaptic plasticity in the VTA is a mechanism of reward-related learning, in which acetylcholine, as a signal mediating primary reward, activates VTA cells, providing excitation which allows for NMDA receptors, being simultaneously stimulated by glutamate which putatively carries signals about environmental stimuli, to open and trigger a cascade of events leading to synaptic strengthening. Our lab has now demonstrated that mACh receptors and NMDA receptors must be stimulated in the VTA to permit the acquisition of reward-related learning.

The findings of this dissertation also fit into a larger body of literature which is currently mapping the neural structures involved with the acquisition of reward-related behavior, most of which are terminal regions of the mesocorticolimbic DA system. As reviewed in Chapters 1 and 4, the acquisition of reward-related learning engages the VTA, NAcc (both core and shell), amygdala (both CeN and BLA), and mPFC. The acquisition of Pavlovian conditioning is impaired when the following are lesioned: NAcc core (but not shell) (Parkinson, et al., 2000), BLA (Fuchs, et al., 2002), anterior cingulate (Bussey, et al., 1997). However, lesions of amygdala (both CeN and BLA) and

NAcc (core and shell) also have been found to be ineffective in impairing acquisition of instrumental responding (Hall, et al., 2001); the instrumental learning took place after Pavlovian conditioning in a PIT paradigm, so it is unclear whether the lack of impairment was due to some reward learning having already taking place during the Pavlovian conditioning phase. It may also be that lesions, which knock out all the cells of a structure, as opposed to specific pharmacological manipulations, have different effects. Lesions of mediodorsal or anterior thalamus (Corbit, et al., 2003) and hippocampus (Kelley, et al., 2003) also do not impair acquisition of instrumental behavior.

Given that DA levels in all the terminal regions increase during acquisition of both instrumental and Pavlovian conditioning, although sometimes to differing degrees (Phillips, et al., 2003b), it is not surprising that systemic blockade of DA impairs both instrumental learning (Wise and Schwartz, 1981) and Pavlovian conditioning (Beninger and Phillips, 1980; Beninger and Phillips, 1981). However, because DA antagonism also reduces performance of reward-related responses (for example, Wise, et al., 1978), any systemic manipulation of DA may not be blocking learning, per se, but the opportunity to learn due to reduced exploration, or blocking the motivation to respond. This also remains an issue for studies which find that intracerebral DA antagonism impairs the acquisition of instrumental learning – for example, when administered in the NAcc (Wolterink, et al., 1993; Smith-Roe and Kelley, 2000; Hernandez, et al., 2005), amygdala (both CeN and BLA) (Andrzejewski, et al.,

2005), and mPFC (Baldwin, et al., 2002b). Classical conditioning is also impaired by NAcc DA depletion [non-sub-region specific] (Parkinson, et al., 2002; Dalley, et al., 2002) or blockade [core] (Di Ciano, et al., 2001). As some of these studies also demonstrated impaired performance following learning (e.g., Hernandez, et al., 2005) it is difficult to make the argument that learning per se was impaired, as opposed to performance.

The confound of treatments affecting both acquisition and performance appears not to be an issue when it comes to the NMDA receptor. As already discussed in previous chapters, NMDA receptor blockade affects acquisition of learning in the same structures described above, but unlike DA manipulations, generally does not affect performance of behavior. NMDA receptor antagonism in NAcc core (Kelley, et al., 1997; Hernandez, et al., 2005), and shell (Kelley, et al., 1997), impairs instrumental learning, whereas NMDA antagonism in NAcc core does not reduce instrumental responding once acquired (Kelley, et al., 1997; Smith-Roe and Kelley, 2000; Hernandez, et al., 2005). NMDA receptor antagonism in NAcc core also impairs classical conditioning (Di Ciano, et al., 2001). NMDA receptor antagonism also impairs instrumental learning in lateral and basolateral amygdala and in mPFC (Baldwin, et al., 2000). In contrast, NMDA receptor antagonism in the hippocampus does not impair instrumental learning (Baldwin, et al., 2000).

While this review of findings on impairment of reward-related learning is not intended to be comprehensive, it does demonstrate that the intact operation

of a number of interconnected structures is necessary for the acquisition of reward-related behavior, both instrumental and Pavlovian. One thing becomes clear from a cursory review, however – interruptions of normal functioning of structures downstream from the VTA all impair reward-related acquisition to a certain degree, which implies an important role for the VTA.

It is notable that acquisition was impaired in *both* paradigms, because animals had more than sufficient pairings to ensure acquisition. In the instrumental learning experiment, for example, the 0.5 µg AP-5 rats typically experienced an average of 194 food pellets across four sessions, and in the conditioned approach paradigm, 90 each. Given the number of CS-US pairings experienced, the impairment of reward-related learning induced by NMDA receptor blockade in the VTA indicates the critical role for synaptic plasticity in this region.

Alternative explanations

It is possible that the interference with learning was accounted for by preventing VTA cells from burst firing in response to primary reward or environmental stimuli, instead of interfering with LTP. As discussed in the introduction, NMDA receptor stimulation triggers burst firing in VTA cells, which is blocked by competitive NMDA receptor antagonists. If burst firing is necessary to increase phasic DA levels at terminal regions, facilitating both current reward transmission (performance) and long-term synaptic changes

(learning), blocking NMDA receptors may have had its primary effect by abolishing burst firing. Indeed, there is some evidence that NMDA receptor antagonists reduce baseline DA levels, but findings have been contradictory. On the one hand, AP-5 (200 μM) together with CNQX (50 μM) precipitated a decrease of about 20% in NAcc DA levels, and stronger effects have been found with AP-5 alone at a higher dose (500 μM), reducing baseline DA to 75% of basal levels in PFC(Takahata and Moghaddam, 1998; Westerink, et al., 1998) and in the ventral striatum (Karreman, et al., 1996). On the other hand, though, other studies have found no effect of AP-5 (.3 mM or 1.0 mM) in VTA on basal DA levels in the NAcc (Schilstrom, et al., 1998a), and 100 μM did not affect basal levels in one of the previously cited studies (Karreman, et al., 1996). Similarly, CPP in the VTA did not affect baseline levels of DA in NAcc (Westerink, et al., 1996; Westerink, et al., 1997) but did in PFC (Westerink, et al., 1998). Further studies are therefore needed to assess the possibility that the effects of NMDA receptor blockade were more significant in the terminal regions than on VTA cells themselves.

It is also possible that our findings were due more to effects on GABA neurons than DA neurons. Conceivably AP-5 could have blocked synaptic plasticity on GABA cells which affected learning. DA and GABA cells in the VTA express similar AMPA/NMDA current ratios (Bonci and Malenka, 1999)., indicating that GABA cells also possess NMDA receptors. However, while DA cells demonstrate LTP following paired pulse stimulation, GABA cells do not; in

contrast, they demonstrate LTD (Bonci and Malenka, 1999). This is in keeping with work in the hippocampus demonstrating that excitatory synapses on most GABA cells do not express LTP (Maccaferri & McBain 1996). Because NMDA antagonism would most likely not affect LTP on GABA cells, a blockade of GABA cell potentiation cannot account for our findings.

In the immediate phase of treatment, it is possible that NMDA receptor antagonism reduced GABAergic disinhibition of DA cells, as NMDA application in the VTA increases GABA cell firing (Steffensen, et al., 2000) and slightly but significantly increases stimulation-induced discharges in VTA GABA cells (Stobbs, et al., 2004). AP-5 reduces GABA cell spontaneous firing (Steffensen, et al., 1998), blocks the NMDA-triggered increase in GABA cell firing (Steffensen, et al., 2000), and blocks stimulation-induced discharges in VTA GABA cells (Steffensen, et al., 1998). Therefore, any additional activity of GABA cells that may have been triggered by incoming Glu signals during instrumental learning may have been blocked. To the extent that reward learning involves patterns of excitation, inhibition, and disinhibition between the VTA and NAcc or PFC, both of which receive GABAergic projections from the VTA (Carr and Sesack, 2000a; Fields, et al., 2007), it is possible that AP-5 treatment had additional effects that impaired acquisition. However, it is unlikely that effects mediated by NMDA receptor blockade on GABA cells accounted for the impairment in acquisition in these studies, as the net effect of treatment would have been to reduce disinhibition of DA cells and thereby augment reward processes. In support of

this view is a study showing that intra-VTA injections of muscimol increased cocaine reward in a self-administration paradigm (Lee, et al., 2008).

Reflections on Theories of DA Function

As many of the findings cited in the introductory chapter illustrate, developing a coherent theory of the role of DA in reward-related processes is difficult not just because of the complexity of DA's physiological effects, having different effects at different receptors, and depending on the state of the postsynaptic neuron; and it is difficult not just because different cells within particular nuclei demonstrate different patterns of activation to the same behavioral events; but it is difficult also because reward-related processes are composed of numerous components which appear to be mediated by different parts of the highly interconnected series of circuits involved in the mesolimbic system, and because neural processes within that system appear to depend on a host of parameters. The dynamics of neural responses to the presentation of conditioned stimuli appear to differentiate according to a number of dimensions: processes regarding natural rewards are sometimes different from those regarding drug rewards, CSs trigger responses sometimes when they are presented contingently and other times non-contingently, when they appear early in conditioning or late, and so on. Ultimately a theory of the function of DA will need to consider the measures used and the stimuli studied along a number of axes. However, a coherent theory that accounts for these various

categories and how they intersect is not the aim of this review, although it is important to mention as a framework for the specific topic of associative processes in the VTA underlying responding to conditioned stimuli.

As discussed earlier, conditioned stimuli are used in various ways in different paradigms. Generally speaking, in Pavlovian conditioning, CSs appear before and during reward exposure, but in instrumental conditioning, CSs appear after an instrumental response. When CSs are paired with drug infusion, instrumental CSs can be simultaneous with reward, but with food paradigms, because reward consumption must take place some brief time after the instrumental response, CSs may also appear before and during reward exposure. All of this means that the various elements that must become associated in reward-related learning – including stimulus/response, stimulus/stimulus, response/outcome, and stimulus/approach associations – happen in different temporal relations to each other. What is interesting in terms of the findings of this set of experiments is that NMDA receptor blockade in the VTA appears to interfere with processes in which CSs appear before a reward and which appear after a response.

Although investigators have different opinions about the more specific meanings of dopaminergic activity, it is widely accepted that DA is involved with performance, because numerous manipulations that impair DA activity reduce reward-related behavior as well as motoric activity more broadly, and augmenting DA transmission leads to specific facilitation of reward-related

behavior as well as general increases in motor activity. Whether impairing DA transmission in the context of reward-related behavior impacts a primary reward signal, reward prediction error, reinforcement processes, incentive salience, effort or motivation, or sensorimotor integration and selection, remains to be clarified. Behavioral findings can and have been used to support arguments for each of these functions, as summarized earlier.

Because behavioral findings can be interpreted differently, it seems important when attempting to understand the function of DA to integrate an awareness of its physiological effects. One important distinction in DA's effects at the neural level, which may clarify the discussion about its role at the behavioral level, is between DA's effects on neuronal activity during phasic increases (its immediate effects) and DA's facilitation of associative processes (its longer-term effects). When DA acts as a high-pass filter in the PFC, for example, suppressing spontaneous firing while augmenting excitability in response to afferent stimulation and sustaining that excitability over a period of seconds or minutes, it may affect attentional and motivational processes in the moment, driving processes in which the animal orients to, approaches and experiences reward and reward-related stimuli. These immediate effects may be linked either to primary reward processes, incentive salience, or both, for example. Similarly, immediate effects of DA in the NAcc may either energize approach behavior overall, facilitate the selection of particular reward-related actions, or inhibit non-salient actions in the moment, and it may have long-term effects due

to its facilitation of synaptic strengthening at those synapses active during reward-related experience.

In this regard, it is also important when theorizing about the role of DA to synthesize data gathered with techniques that measure changes in DA activity across a wide population of cells, such as microdialysis, with data on activity in single cells or discrete groups of cells. This can clarify, for example, that DA is probably not released indiscriminately for both natural rewards, drug rewards, and conditioned stimuli, but rather that specific cells are activated in particular circumstances, as work by Carelli and colleagues (e.g., Carelli and Ijames, 2001) demonstrates: some NAcc neurons fire in response to natural rewards, while others fire in response to drug rewards, and similarly for reward-related stimuli.

Although this discussion has focused on DA, other neurochemistries in the VTA or its terminal regions may have been affected by our treatment, such as glutamate, GABA, or peptide release from VTA cells, although we have no way of assessing that. Future research will be able to determine what the downstream effects of NMDA receptor blockade in the VTA are. Certainly when VTA cells fire in response to a primary reward or a conditioned stimulus, a variety of post-synaptic influences may result. AP-5 in the VTA may affect one, a few, or all of those. However, it is well substantiated at this point that VTA firing is correlated with DA release according to a number of measures and across a variety of paradigms, and DA blockade in the NAcc has very similar results to inactivation of the VTA in responding to conditioned stimuli.

Therefore it seems appropriate that the experiments conducted for this dissertation be discussed primarily within the framework of DA function, although future findings may clarify the involvement of other transmitters.

As future work is conducted on the role of NMDA transmisision in the VTA during reward-related learning, it is important to keep in mind that differences exist between types of NMDA antagonists. The studies cited in this dissertation have generally been those which used competitive NMDA antagonists, primarily AP-5, because different classes of NMDA antagonists have varying and sometimes opposite effects (Meltzer, et al., 1997). For example, non-competitive NMDA antagonists including MK-801 and phencyclidine (PCP), which operate by a different mechanism – blocking the ion channel pore of the receptor (Kew and Kemp, 2005), and hence only having effects when the channels have been activated – increase firing rate and bursting of VTA DA cells, while competitive antagonists CGS 19755 and (±)CPP either have no effect or non-significantly reduce activity (French, et al., 1993). For future work it may be important to keep the antagonists in separate categories – findings for one class of antagonists do not necessarily predict similar findings with another.

Future work is also required to determine whether NMDA transmission is necessary for expression of reward-related learning in the Pavlovian context. Based on the findings of this dissertation, we would predict that NMDA receptor antagonism would not impair such expression. Indeed, preliminary results from ongoing research being conducted in our lab indicate that in fact AP-5 does not

impair conditioned approach, in a paradigm similar to the one used in the current work. This is not surprising, given that intra-VTA AP-5 did not impair the expression of a more complex reward-related behavior, namely lever pressing, in the current study.

Altogether, the results of the studies described herein, including the instrumental learning study, the conditioned approach study, and the various control experiments, give strong support to the hypothesis with which this dissertation originated. These results suggest that NMDA receptor stimulation in the VTA is necessary for the acquisition of reward-related learning, both instrumental and classical, but it is not necessary for expression of that learning once it has been acquired. We look forward to future work which can extend these studies, thereby adding to a growing literature which we hope will lead us to a complete knowledge of the neural underpinnings of reward-related learning, perhaps supporting the development of new treatments for disorders based on pathologies of these neural circuits, as well as the intellectual satisfaction of understanding the mechanisms of such a basic life-supporting capacity.

Reference List

Abizaid A, Liu ZW, Andrews ZB, Shanabrough M, Borok E, Elsworth JD, Roth RH, Sleeman MW, Picciotto MR, Tschop MH, Gao XB and Horvath TL (2006) Ghrelin modulates the activity and synaptic input organization of midbrain dopamine neurons while promoting appetite. *J Clin Invest* 116:3229-3239.

Adams CD (1982) Variations in the sensitivity of instrumental responding to reinforcer devaluation. *The Quarterly Journal of Experimental Psychology* 34:77-98.

Albin RL, Makowiec RL, Hollingsworth ZR, Dure LS4, Penney JB and Young AB (1992) Excitatory amino acid binding sites in the basal ganglia of the rat: a quantitative autoradiographic study. *Neurosci* 46:35-48.

Alcaro A, Huber R and Panksepp J (2007) Behavioral functions of the mesolimbic dopaminergic system: an affective neuroethological perspective. *Brain Res Rev* 56:283-321.

Almodóvar-Fabregas LJ, Segarra O, Colon N, Dones JG, Mercado M, Meijas-Aponte CA, Vazquez R, Abreu R, Vazquez E, Williams JT and Jimenez-Rivera CA (2002) Effects of cocaine administration on VTA cell activity in response to prefrontal cortex stimulation. *Ann N Y Acad Sci* 965:157-171.

American Psychological Association. Diagnostic and Statistical Manual of Mental Disorders, 4th ed. Test rev. 2000. American Psychological Association. Ref Type: Serial (Book,Monograph)

Andrzejewski ME, Spencer RC and Kelley AE (2005) Instrumental learning, but not performance, requires dopamine D1-receptor activation in the amygdala. *Neurosci* 135:335-345.

Arbuthnott GW and Wickens J (2007) Space, time and dopamine. *Trends Neurosci* 30:62-69.

Azzara AV, Bodnar RJ, Delamater AR and Sclafani A (2001) D1 but not D2 dopamine receptor antagonism blocks the acquisition of a flavor preference conditioned by intragastric carbohydrate infusions. *Pharmacol Biochem Behav* 68:709-720.

Baker RM, Shah MJ, Sclafani A and Bodnar RJ (2003) Dopamine D1 and D2 antagonists reduce the acquisition and expression of flavor-preferences conditioned by fructose in rats. *Pharmacol Biochem Behav* 75:55-65.

Baldwin AE, Holahan MR, Sadeghian K and Kelley AE (2000) N-methyl-d-aspartate receptor-dependent plasticity within a distributed corticostriatal network mediates appetitive instrumental learning. *Behav Neurosci* 114:84-98.

Baldwin AE, Sadeghian K, Holahan MR and Kelley AE (2002a) Appetitive instrumental learning is impaired by inhibition of cAMP-dependent

protein kinase within the nucleus accumbens. *Neurobiology of Learning and Memory* 77:44-62.

Baldwin AE, Sadeghian K and Kelley AE (2002b) Appetitive instrumental learning requires coincident activation of NMDA and dopamine D1 receptors within the medial prefrontal cortex. *J Neurosci* 22:1063-1071.

Balleine BW (2005) Neural bases of food-seeking: affect, arousal and reward in corticostriatolimbic circuits. *Physiol Behav* 86:717-730.

Balleine BW and Dickinson A (1998) Goal-directed instrumental action: contigency and incentive learning and their cortical substrates. *Neuropharmacology* 37:407-419.

Bassareo V, De Luca MA and Di Chiara G (2007) Differential impact of pavlovian drug conditioned stimuli on in vivo dopamine transmission in the rat accumbens shell and core and in the prefrontal cortex. *Psychopharmacology (Berl)* 191:689-703.

Bassareo V and Di Chiara G (1999a) Differential responsiveness of dopamine transmission to food-stimuli in nucleus accumbens shell/core compartments. *Neurosci* 89:637-641.

Bassareo V and Di Chiara G (1999b) Modulation of feeding-induced activation of mesolimbic dopamine transmission by appetitive stimuli and its relation to motivational state. *Eur J Neurosci* 11:4389-4397.

Baxter MG and Murray EA (2002) The amygdala and reward. *Nat Rev Neurosci* 3:563-573.

Beckstead RM, Domesick VB and Nauta WJH (1979) Efferent connections of the substantia nigra and ventral tegmental area in the rat. *Brain Res* 175:191-217.

Beninger RJ (1983) The role of dopamine in locomotor activity and learning. *Brain Res Rev* 6:173-196.

Beninger RJ (1992) D-1 receptor involvement in reward-related learning. *Psychopharm* 6(1):34-42.

Beninger RJ and Gerdjikov T (2004) The role of signaling molecules in reward-related incentive learning. *Neurotoxicity Research* 6:91-104.

Beninger RJ and Miller R (1998) Dopamine D1-like receptors and reward-related incentive learning. *Neurosci Biobehav Rev* 22:335-345.

Beninger RJ and Phillips AG (1980) The effects of pimozide on the establishment of conditioned reinforcement. *Psychopharm* 68:147-153.

Beninger RJ and Phillips AG (1981) The effects of pimozide during pairing on the transfer of classical conditioning to an operant discrimination. *Pharmacol Biochem Behav* 14:101-105.

Beninger RJ and Ranaldi R (1992) The effects of amphetamine, apomorphine, SKF 38393, quinpirole and bromocriptine on responding for conditioned reward in rats. *Behav Pharmacol* 3:155-163.

Beninger RJ and Ranaldi R (1994) Dopaminergic agents with different mechanisms of action differentially affect responding for conditioned reward, in *Strategies for Studying Brain Disorders. Vol. 1: Depressive, Anxiety*

and Drug Abuse Disorders (Palomo T and Archer T eds) Farrand Press, London.

Berns GS, McClure SM, Pagnoni G and Montague PR (2001) Predictability modulates human brain response to reward. *J Neurosci* 21:2793-2798.

Berridge KC (2007) The debate over dopamine's role in reward: the case for incentive salience. *Psychopharmacology (Berl)* 191:391-431.

Bindra D (1974) A motivational view of learning, performance, and behavior modification. *Psychol Rev* 81:199-213.

Blackburn JR and Phillips AG (1989) Dopamine and preparatory behavior: II. a neurochemical analysis. *Behav Neurosci* 103:15-23.

Blackburn JR, Phillips AG and Fibiger H (1987) Dopamine and preparatory behavior: I. Effects of pimozide. *Behav Neurosci* 101:352-360.

Blaha CD, Allen LF, Das S, Inglis WL, Latimer MP, Vincent SR and Winn P (1996) Modulation of dopamine efflux in the nucleus accumbens after cholinergic stimulation of the ventral tegmental area in intact,pedunculopontine tegmental nucleus-lesioned,and laterodorsal tegmental nucleus-lesioned rats. *The Journal of Neuroscience* 16:714-722.

Blodgett HC. The effect of the introduction of reward upon the maze performance of rats. University of California Publications in Psychology 4, 113-134. 1929.

Ref Type: Generic

Bolles RC (1972) Reinforcement, expectancy, and learning. *Psychol Rev* 79:394-409.

Bonci A and Malenka RC (1999) Properties and plasticity of excitatory synapses on dopaminergic and GABAergic cells in the ventral tegmental area. *J Neurosci* 19:3723-3730.

Bossert JM, Liu SY and Shaham Y (2004) A role of ventral tegmental area glutamate in contextual cue-induced relapse to heroin seeking. *J Neurosci* 24:10726-10730.

Bradberry CW and Rubino SR (2004) Phasic alterations in dopamine and serotonin release in striatum and prefrontal cortex in response to cocaine predictive cues in behaving rhesus macaques. *Neuropsychopharmacology* 29:676-685.

Brown PL and Jenkins HM (1968) Auto-shaping of the pigeon's key-peck. *J Exp Anal Behav* 11:1-8.

Bunney BS, Chiodo LA and Grace AA (1991) Midbrain dopamine system electrophysiological functioning: a review and new hypothesis. *s* 9:79-94.

Burns LH, Everitt BJ and Robbins TW (1994) Intra-amygdala infusion of the N-methyl-D-aspartate receptor antagonist AP5 impairs acquisition but not performance of discriminated approach to an appetitive CS. *Behav Neural Biol* 61:242-250.

Burns LH, Robbins TW and Everitt BJ (1993) Differential effects of excitotoxic lesions of the basolateral amygdala, ventral subiculum and medial prefrontal cortex on responding with conditioned reinforcement and

locomotor activity potentiated by intra-accumbens infusions of D-amphetamine. *Behav Brain Res* 55:167-183.

Bussey TJ, Everitt BJ and Robbins TW (1997) Dissociable effects of cingulate and medial frontal cortex lesions on stimulus-reward learning using a novel Pavlovian autoshaping procedure for the rat: implications for the neurobiology of emotion. *Behav Neurosci* 111:908-919.

Cador M, Taylor JR and Robbins TW (1991) Potentiation of the effects of reward-related stimuli by dopaminergic-dependent mechanisms in the nucleus accumbens. *Psychopharm* 104:377-385.

Calabresi P, Lacey MG and North RA (1989) Nicotinic excitation of rat ventral tegmental neurones in vitro studied by intracellular recording. *Br J Pharmacol* 98:135-140.

Cardinal RN, Parkinson JA, Marbini HD, Toner AJ, Bussey TJ, Robbins TW and Everitt BJ (2003) Role of the anterior cingulate cortex in the control over behavior by Pavlovian conditioned stimuli in rats. *Behav Neurosci* 117:566-587.

Carelli RM (2000) Activation of accumbens cell firing by stimuli associated with cocaine delivery during self-administration. *s* 35:238-242.

Carelli RM (2002) Nucleus accumbens cell firing during goal-directed behaviors for cocaine vs. 'natural' reinforcement. *Physiol Behav* 76:379-387.

Carelli RM and Ijames SG (2001) Selective activation of accumbens neurons by cocaine-associated stimuli during a water/cocaine multiple schedule. *Brain Res* 907:156-161.

Carli M, Evenden JL and Robbins TW (1985) Depletion of unilateral striatal dopamine impairs initiation of contralateral actions and not sensory attention. *Nature* 313:679-682.

Carlsson A, Lindqvist M and Waldeck B (1958) On the presence of 3-hydroxytyramine in brain. *Science* 127:471.

Carr DB and Sesack SR (2000a) GABA-containing neurons in the rat ventral tegmental area project to the prefrontal cortex. *s* 38:114-123.

Carr DB and Sesack SR (2000b) Projections from the rat prefrontal cortex to the ventral tegmental area: target specificity in the synaptic associations with mesoaccumbens and mesocortical neurons. *J Neurosci* 20:3964-3873.

Cervo L, Cocco A, Petrella C, Heidbreder CA (2007) Selective antagonism at dopamine D3 receptors attenuates cocaine-seeking behaviour in the rat. *The International Journal of Neuropsychopharmacology* 10(2): 167-181.

Charara A, Smith Y and Parent A (1996) Glutamatergic inputs from the pedunculopontine nucleus to midbrain dopaminergic neurons in primates: Phaseolus vulgaris-leucoagglutinin anterograde labeling

combined with postembedding glutamate and GABA immunohistochemistry. *J Comp Neurol* 364:254-266.

Chen J, Nakamura M, Kawamura T, Takahashi T and Nakahara D (2006) Roles of pedunculopontine tegmental cholinergic receptors in brain stimulation reward in the rat. *Psychopharm* 184:514-522.

Chergui K, Charlety PJ, Akaoka H, Saunier CF, Brunet JL, Buda M, Svensson TH and Chovet G (1993) Tonic activation of NMDA receptors causes spontaneous burst discharge of rat midbrain dopamine neurons in vivo. *Eur J Neurosci* 5:137-144.

Chergui K, Suaud-Chagny MF and Gonon F (1994) Nonlinear relationship between impulse flow, dopamine release and dopamine elimination in the rat brain in vivo. *Neurosci* 62:641-645.

Childress AR, Mozley PD, McElgin W, Fitzgerald J, Reivich M and O'Brien CP (1999) Limbic activation during cue-induced cocaine craving. *Am Jour Psychiat* 156:11-18.

Choi WY, Balsam PD and Horvitz JC (2005) Extended habit training reduces dopamine mediation of appetitive response expression. *J Neurosci* 25:6729-6733.

Christie MJ, Bridge S, James LB and Beart PM (1985) Excitotoxin lesions suggest an aspartatergic projection from rat medial prefrontal cortex to ventral tegmental area. *Brain Res* 333:169-172.

Chuhma N, Zhang H, Masson J, Zhuang X, Sulzer D, Hen R and Rayport S (2004) Dopamine neurons mediate a fast excitatory signal via their glutamatergic synapses. *J Neurosci* 24:972-981.

Church WH, Justice JB, Jr. and Neill DB (1987) Detecting behaviorally relevant changes in extracellular dopamine with microdialysis. *Brain Res* 412:397-399.

Citri A and Malenka RC (2007) Synaptic plasticity: multiple forms, functions, and mechanisms. *Neuropsychopharmacology* 33:18-41.

Collingridge GL, Kehl SJ and McLennan H (1983) Excitatory amino acids in synaptic transmission in the Schaffer collateral-commissural pathway of the rat hippocampus. *J Physiol* 334:33-46.

Comoli E, Coizet V, Boyes J, Bolam JP, Canteras NS, Quirk RH, Overton PG and Redgrave P (2003) A direct projection from superior colliculus to substantia nigra for detecting salient visual events. *Nature Neuroscience* 6:974-980.

Corbit LH and Balleine BW (2003) The role of prelimbic cortex in instrumental conditioning. *Behav Brain Res* 146:145-157.

Corbit LH, Janak PH and Balleine BW. General and outcome-specific forms of Pavlovian-instrumental transfer: the effect of shifts in motivational state and inactivation of the ventral tegmental area. 26(11), 3141-3149. 2007. Ref Type: Generic

Corbit LH, Muir JL and Balleine BW (2003) Lesions of mediodorsal thalamus and anterior thalamic nuclei produce dissociable effects on instrumental conditioning in rats. *Eur J Neurosci* 18:1286-1294.

Coria-Avila GA and Pfaus JG (2007) Neuronal activation by stimuli that predict sexual reward in female rats. *Neurosci* 148:623-632.

Cornish JL, Nakamura M and Kalivas PW (2001) Dopamine-independent locomotion following blockade of N-methyl-D-aspartate receptors in the ventral tegmental area. *J Pharmacol Exp Ther* 298:226-233.

Cox SM, Andrade A and Johnsrude IS (2005) Learning to like: a role for human orbitofrontal cortex in conditioned reward. *J Neurosci* 25:2733-2740.

Dalley JW, Chudasama Y, Theobald DE, Pettifer CL, Fletcher CM and Robbins TW (2002) Nucleus accumbens dopamine and discriminated approach learning: interactive effects of 6-hydroxydopamine lesions and systemic apomorphine administration. *Psychopharmacology (Berl)* 161:425-433.

Datla KP, Ahier RG, Young AM, Gray JA and Joseph MH (2002) Conditioned appetitive stimulus increases extracellular dopamine in the nucleus accumbens of the rat. *Eur J Neurosci* 16:1987-1993.

Davis S, Butcher SP and Morris RG (1992) The NMDA receptor antagonist D-2-amino-5-phosphonopentanoate (D-AP5) impairs spatial learning and LTP in vivo at intracerebral concentrations comparable to those that block LTP in vitro. *J Neurosci* 12:21-34.

Dawbarn D and Pycock CJ (1981) Motor effects following application of putative excitatory amino acid antagonists to the region of the mesencephalic dopamine cell bodies in the rat. *Naunyn Schmiedebergs Arch Pharmacol* 318:100-104.

Day JJ, Wheeler RA, Roitman MF and Carelli RM (2006) Nucleus accumbens neurons encode Pavlovian approach behaviors: evidence from an autoshaping paradigm. *Eur J Neurosci* 23:1341-1351.

Delamater AR and Oakeshott S (2007) Learning about multiple attributes of reward in Pavlovian conditioning. *Ann N Y Acad Sci* 1104:1-20.

Di Chiara G and Bassareo V (2007) Reward system and addiction: what dopamine does and doesn't do. *Current Opinion in Pharmacology* 7:69-76.

Di Ciano P, Blaha CD and Phillips AG (1998) Conditioned changes in dopamine oxidation currents in the nucleus accumbens of rats by stimuli paired with self-administration or yoked-administration of d-amphetamine. *Eur J Neurosci* 10:1121-1127.

Di Ciano P, Cardinal RN, Cowell RA, Little SJ and Everitt BJ (2001) Differential involvement of NMDA, AMPA/kainate, and dopamine receptors in the nucleus accumbens core in the acquisition and performance of pavlovian approach behavior. *J Neurosci* 21:9471-9477.

Di Ciano P and Everitt BJ (2003) Differential control over drug-seeking behavior by drug-associated conditioned reinforcers and discriminative stimuli predictive of drug availability. *Behav Neurosci* 117:952-960.

Di Ciano P and Everitt BJ (2004a) Conditioned reinforcing properties of stimuli paired with self-administered cocaine, heroin or sucrose: implications for the persistence of addictive behaviour. *Neuropharmacology* 47:202-213.

Di Ciano P and Everitt BJ (2004b) Contribution of the ventral tegmental area to cocaine-seeking maintained by a drug-paired conditioned stimulus in rats. *Eur J Neurosci* 19:1661-1667.

Dickinson A, Balleine BW, Watt A, Gonzalez F and Boakes RA (1995) Motivational control after extended instrumental training. *Animal Learning & Behavior* 23:197-206.

Dickinson A, Smith J and Mirenowicz J (2000) Dissociation of Pavlovian and instrumental incentive learning under dopamine antagonists. *Behav Neurosci* 114:468-483.

Dommett E, Coizet V, Blaha CD, Martindale J, Lefebvre V, Walton N, Mayhew JE, Overton P and Redgrave P (2005) How visual stimuli activate dopaminergic neurons at short latency. *Science* 307:1476-1479.

Ehrman RN, Robbins SJ, Childress AR and O'Brien CP (1992) Conditioned responses to cocaine-related stimuli in cocaine abuse patients. *Psychopharm* 107:523-529.

Esteban JA, Shi SH, Wilson C, Nuriva M, Huganir RL and Malinow R (2003) PKA phosphorylation of AMPA receptor subunits controls synaptic trafficking underlying plasticity. *Nature Neuroscience* 6:136-143.

Fallon JH (1981) Collateralization of monoamine neurons: mesotelencephalic dopamine projections to caudate, septum, and frontal cortex. *J Neurosci* 1:1361-1368.

Fallon JH, Schmued LC, Wang C, Miller R and Banales G (1984) Neurons in the ventral tegmentum have separate populations projecting to telencephalon and inferior olive, are histochemically different, and may receive direct visual input. *Brain Res* 321:332-336.

Fields HL, Hjelmstead GO, Margolis EB and Nicola SM (2007) Ventral tegmental area neurons in learned appetitive behavior and positive reinforcement. *Ann Rev Neurosci* 30:289-316.

Fiorillo CD, Tobler PN and Schultz W (2003) Discrete coding of reward probability and uncertainty by dopamine neurons. *Science* 299:1856-1902.

Fowler SC and Liou JR (1998) Haloperidol, raclopride, and eticlopride induce microcatalepsy during operant performance in rats, but clozapine and SCH 23390 do not. *Psychopharmacology (Berl)* 140:81-90.

French ED, Mura A and Wang T (1993) MK-801, phencyclidine (PCP), and PCP-like drugs increase burst firing in rat A10 dopamine neurons: comparison to competitive NMDA antagonists. *s* 13:108-116.

Fuchs RA, Weber SM, Rice HJ and Neisewander JL (2002) Effects of excitotoxic lesions of the basolateral amygdala on cocaine-seeking behavior and cocaine conditioned place preference in rats. *Brain Res* 929:15-25.

Gabbott PL, Warner TA, Jays PR, Salway P and Busby SJ (2008) Prefrontal cortex in the rat: projections to subcortical autonomic, motor, and limbic centers. *J Comp Neurol* 492:145-177.

Gao Q and Horvath TL (2007) Neurobiology of feeding and energy expenditure. *Annu Rev Neurosci* 30:367-398.

Gariano RF and Groves PM (1998) Burst firing induced in midbrain dopamine neurons by stimulation of the medial prefrontal and anterior cingulate cortices. *Brain Res* 462:194-198.

Garzon M, Vaughan RA, Uhl GR, Kuhar MJ and Pickel VM (1999) Cholinergic axon terminals in the ventral tegmental area target a subpopulation of neurons expressing low levels of the dopamine transporter. *J Comp Neurol* 410:197-210.

Geisler S, Derst C, Veh RW and Zahm DS (2007) Glutamatergic afferents of the ventral tegmental area in the rat. *J Neurosci* 27:5730-5743.

Geisler S and Zahm DS (2005) Afferents of the ventral tegmental area in the rat-anatomical substratum for integrative functions. *J Comp Neurol* 490:270-294.

Georges F and Aston-Jones G (2002) Activation of ventral tegmental area cells by the bed nucleus of the stria terminalis: a novel excitatory amino acid input to midbrain dopamine neurons. *J Neurosci* 22:5173-5187.

Ghitza UE, Fabbricatore AT, Prokopenko V, Pawlak AP and West MO (2003) Persistent cue-evoked activity of accumbens neurons after prolonged abstinence from self-administered cocaine. *J Neurosci* 23:7239-7245.

Gonon FG (1988) Nonlinear relationship between impulse flow and dopamine released by rat midbrain dopaminergic neurons as studied by in vivo electrochemistry. *Neurosci* 24:19-28.

Gratton A and Wise RA (1994) Drug- and behavior-associated changes in dopamine-related electrochemical signals during intravenous cocaine self-administration in rats. *J Neurosci* 14:4130-4146.

Grau JW, Crown ED, Ferguson AR, Washburn SN, Hook MA and Miranda RC (2006) Instrumental learning within the spinal cord: underlying mechanisms and implications for recovery after injury. *Behav Cogn Neurosci Rev* 5:191-239.

Grenhoff J, Tung CS and Svensson TH (1988) The excitatory amino acid antagonist kynurenate induces pacemaker-like firing of dopamine neurons in rat ventral tegmental area in vivo. *Acta Physiol Scand 124* 134:567-568.

Gronier B and Rasmussen K (1998) Activation of midbrain presumed dopaminergic neurones by muscarinic chollinergic receptors: An in vivo electrophysiological study in the rat. *Br J Pharmacol* 124:455-465.

Hall J, Parkinson JA, Connor TM, Dickinson A and Everitt BJ (2001) Involvement of the central nucleus of the amygdala and nucleus accumbens core in

mediating Pavlovian influences on instrumental behaviour. *Eur J Neurosci* 13:1984-1992.

Harmer CJ and Phillips GD (1999) Enhanced dopamine efflux in the amygdala by a predictive, but not a non-predictive, stimulus: facilitation by prior repeated d-amphetamine. *Neurosci* 90:119-130.

Harris EW, Ganong AH and Cotman CW (1984) Long-term potentiation in the hippocampus involves activation of N-methyl-D-aspartate receptors. *Brain Res* 323:132-137.

Harris GC and Aston-Jones G (2003) Critical role for ventral tegmental glutamate in preference for a cocaine-conditioned environment. *Neuropsychopharmacology* 28:73-76.

Harris GC, Wimmer M, Byrne R and Aston-Jones G (2004) Glutamate-associated plasticity in the ventral tegmental area is necessary for conditioning environmental stimuli with morphine. *Neurosci* 129:841-847.

Harte M and O'Connor WT (2005) Evidence for a selective prefrontal cortical GABA(B) receptor-mediated inhibition of glutamate release in the ventral tegmental area: a dual probe microdialysis study in the awake rat. *Neurosci* 130:215-222.

Hearst E and Jenkins HM (1974) *Sign-tracking: the stimulus-reinforcer relation and directed action*. The Psychonomic Society: Austin, TX.

Henderson Z and Sherriff FE (1991) Distribution of choline acetyltransferase immunoreactive axons and terminals in the rat and ferret brainstem. *J Comp Neurol* 314:147-163.

Hernandez L and Hoebel BG (1988) Food reward and cocaine increase extracellular dopamine in the nucleus accumbens as measured by microdialysis. *Life Sci* 42:1705-1712.

Hernandez PJ, Andrzejewski ME, Sadeghian K, Panksepp JB and Kelley AE (2005) AMPA/kainate, NMDA, and dopamine D1 receptor function in the nucleus accumbens core: a context-limited role in the encoding and consolidation of instrumental memory. *Learn Mem* 12:285-295.

Hitchcott PK and Phillips GD (1998) Double dissociation of the behavioural effects of r(+) 7-oh-dpat infusions in the central and basolateral amygdala nuclei upon pavlovian and instrumental conditioned appetitive behaviours. *Psychopharm* 140:458-469.

Holland PC and Straub JJ (1979) Differential effects of two ways of devaluing the unconditioned stimulus after Pavlovian appetitive conditioning. *J Exp Psychol Anim Behav Process* 5:65-78.

Hori K, Tanaka J and Nomura M (1993) Effects of discrimination learning on the rat amygdala dopamine release: a microdialysis study. *Brain Res* 621:296-300.

Horvitz JC (2000) Mesolimbocortical and nigrostriatal dopamine responses to salient non-reward events. *Neurosci* 96:651-655.

Horvitz JC, Choi WY, Morvan C, Eyny Y and Balsam PD (2007) A "good parent" function of dopamine: transient modulation of learning and performance during early stages of training. *Ann N Y Acad Sci* 1104:270-288.

Horvitz JC, Stewart J and Jacobs BL (1997) Burst activity of ventral tegmental dopamine neurons is elicited by sensory stimuli in the awake cat. *Brain Res* 759:251-258.

Hyland BI, Reynolds JN, Hay J, Perk CG and Miller R (2002) Firing modes of midbrain dopamine cells in the freely moving rat. *Neurosci* 114:475-492.

Ikemoto S and Panksepp J (1999) The role of nucleus accumbens dopamine in motivated behavior:a unifying interpretations with special reference to reward-seeking. *Brain Res Rev* 31:6-41.

Ito R, Dalley JW, Howes SR, Robbins TW and Everitt BJ (2000) Dissociation in conditioned dopamine release in the nucleus accumbens core and shell in response to cocaine cues and during cocaine-seeking behavior in rats. *J Neurosci* 20:7489-7495.

Ito R, Robbins TW and Everitt BJ (2004) Differential control over cocaine-seeking behavior by nucleus accumbens core and shell. *Nature Neuroscience* 7:389-397.

Janak PH, Chang J-Y and Woodward DJ (1999) Neuronal spike activity in the nucleus accumbens of behaving rats during ethanol self-administration. *Brain Res* 817:172-184.

Jay TM (2003) Dopamine: a potential substrate for synaptic plasticity and memory mechanisms. *Prog Neurobiol* 69:375-390.

Jenkins, HM and Moore, BR (1973) The form of the auto-shaped response with food or water reinforcers. *Journal of Experimental Analysis of Behavior* 20(2):163-181.

Johnson SW and North RA (1992) Two types of neurone in the rat ventral tegmental area and their synaptic inputs. *J Physiol* 450:455-468.

Johnson SW, Seutin V and North R.A. (1992) Burst firing in dopamine neurons induced by N-methyl-D-aspartate: role of electrogenic sodium pump. *Science* 258:665-667.

Johnson SW and Wu YN (2008) Multiple mechanisms underlie burst firing in rat midbrain dopamine neurons in vitro. *Brain Res* 1019:293-296.

Jones S and Bonci A (2005) Synaptic plasticity and drug addiction. *Current Opinion in Neurobiology* 5:20-25.

Kalivas PW (1993) Neurotransmitter regulation of dopamine neurons in the ventral tegmental area. *Brain Res Rev* 18:75-113.

Kalivas PW, Volkow N and Seamans J (2005) Unmanageable motivation in addiction: a pathology in prefrontal-accumbens glutamate transmission. *ne* 45:647-650.

Kantak KM, Black Y, Valencia E, Green-Jordan K and Eichenbaum HB (2002) Dissociable effects of lidocaine inactivation of the rostral and caudal

basolateral amygdala on the maintenance and reinstatement of cocaine-seeking behavior in rats. *J Neurosci* 22:1126-1136.

Karreman M, Westerink BHC and Moghaddam B (1996) Excitatory amino acid receptors in the ventral tegmental area regulate dopamine release in the ventral striatum. *J Neurochem* 67:601-607.

Kauer JA (2004) Learning mechanisms in addiction: synaptic plasticity in the ventral tegmental area as a result of exposure to drugs of abuse. *Annu Rev Physiol* 66:447-475.

Kebabian JW and Caine DB (1979) Multiple receptors for dopamine. *Nature* 277:93-96.

Kelleher, RT (1966) Conditioned reinforcement and second-order schedules. *Journal of Experimental Analysis of Behavior* 9:475-485.

Kelley AE, Andrzejewski ME, Baldwin AE, Hernandez PJ and Pratt WE (2003) Glutamate-mediated plasticity in corticostriatal networks: role in adaptive motor learning. *Annal New York Academy of Science* 1003:159-168.

Kelley AE, Baldo BA, Pratt WE and Will MJ (2005) Corticostriatal-hypothalamic circuitry and food motivation: integration of energy, action and reward. *Physiol Behav* 86:773-795.

Kelley AE and Berridge KC (2002) The neuroscience of natural rewards: relevance to addictive drugs. *Journal of neuroscience : the official journal of the Society for Neuroscience* 22:3306-3311.

Kelley AE, Smith-Roe SL and Holahan MR (1997) Response-reinforcement learning is dependent on N-methyl-D-aspartate receptor activation in the nucleus accumbens core. *Proc Natl Acad Sci USA* 94:12174-12179.

Kew JN and Kemp JA (2005) Ionotropic and metabotropic glutamate receptor structure and pharmacology. *Psychopharmacology (Berl)* 179:4-29.

Kiyatkin EA and Gratton A (1994) Electrochemical monitoring of extracellular dopamine in nucleus accumbens of rats lever-pressing for food. *Brain Res* 652:225-234.

Kiyatkin EA and Rebec GV (1998) Heterogeneity of ventral tegmental area neurons: single-unit recording and iontophoresis in awake, unrestrained rats. *Neurosci* 85:1285-1309.

Kiyatkin EA and Rebec GV (2001) Impulse activity of ventral tegmental area neurons during heroin self-administration in rats. *Neurosci* 102:565-580.

Kiyatkin EA and Stein EA (1996) Conditioned changes in nucleus accumbens dopamine signal established by intravenous cocaine in rats. *Neurosci Lett* 211:73-76.

Knutson B, Fong GW, Adams CM, Varner JL and Hommer D (2001) Dissociation of reward anticipation and outcome with event-related fMRI. *Neuroreport* 12:3683-3687.

Kofman O and Yeomans JS (1988) Cholinergic antagonists in ventral tegmentum elevate thresholds for lateral hypothalamic and brainstem self-stimulation. *Pharmacol Biochem Behav* 31:547-559.

Korotkova TM, Ponomarenko AA, Brown RE and Haas HL (2004) Functional diversity of ventral midbrain dopamine and GABAergic neurons. *Molecular Neurobiology* 29:243-259.

Kosobud AE, Harris GC and Chapin JK (1994) Behavioral associations of neuronal activity in the ventral tegmental area of the rat. *J Neurosci* 14:7117-7129.

Lavin A and Grace AA (2001) Stimulation of D1-type dopamine receptors enhances excitability in prefrontal cortical pyramidal neurons in a state-dependent manner. *Neurosci* 104:335-346.

Lavin A, Noqueira L, Lapish CC, Wightman RM, Phillips PE and Seamans J (2005) Mesocortical dopamine neurons operate in distinct temporal domains using multimodal signaling. *J Neurosci* 25:5013-5023.

Lee DY, Guttilla M, Fung KD, McFeron S, Yan J and Ranaldi R (2008) Rostral-caudal differences in the effects of intra-VTA muscimol on cocaine self-administration. *Pharmacol Biochem Behav* 86:542-549.

Lewis BL and O'Donnell P (2000) Ventral tegmental area afferents to the prefrontal cortex maintain membrane potential 'up' states in pyramidal neurons via D(1) dopamine receptors. *Cerebral Cortex* 10:1168-1175.

Lisman JE and Grace AA (2005) The hippocampal-VTA loop: controlling the entry of information into long-term memory. *ne* 46:703-713.

Ljungberg T, Apicella P and Schultz W (1992) Responses of monkey dopamine neurons during learning of behavioral reactions. *Journal of Neurophysiology* 67:145-163.

Loughlin SE and Fallon JH (1984) Substantia nigra and ventral tegmental area projections to cortex: topography and collateralization. *Neurosci* 11:425-435.

Maas LC, Lukas SE, Kaufman MJ, Weiss RD, Daniels SL, Rogers VW, Kukes TJ and Renshaw PF (1998) Functional magnetic resonance imaging of human brain activation during cue-induced cocaine craving. *Am Jour Psychiat* 155:124-126.

Mackintosh NJ (1974) *The psychology of animal learning*. Academic Press, London.

Madden 2002

Margolis EB, Hjelmstad GO, Bonci A and Fields HL (2003) Kappa-opioid agonists directly inhibit midbrain dopaminergic neurons. *J Neurosci* 23:9981-9986.

Marsden CD (1984) Which motor disorder in Parkinson's disease indicates the true motor function of the basal ganglia? *Ciba Found Symp* 107:225-241.

Mathon DS, Kamal A, Smidt MP and Ramakers GMJ (2003) Modulation of cellular activity and synaptic transmission in the ventral tegmental area. *Eur J Pharmacol* 480:97-115.

Mayer ML, Westbrook GL and Guthrie PB (1984) Voltage-dependent block by Mg2+ of NMDA responses in spinal cord neurones. *Nature* 309:261-263.

McFarland K and Ettenberg A (1998) Haloperidol does not affect motivational processes in an operant runway model of food-seeking behavior. *Behav Neurosci* 112:630-635.

Meltzer LT, Christoffersen CL and Serpa KA (1997) Modulation of dopamine neuronal activity by glutamate receptor subtypes. *Neurosci Biobehav Rev* 21:511-518.

Mercuri NB, Stratta F, Calabresi P, Bonci A and Bernardi G (1993) Activation of metabotropic glutamate receptors induces an inward current in rat dopamine mesencephalic neurons. *Neurosci* 56:399-407.

Miller AD and Blaha CD (2005) Midbrain muscarinic receptor mechanisms underlying regulation of mesoaccumbens and nigrostriatal dopaminergic transmission in the rat. *Eur J Neurosci* 21:1837-1846.

Miller JD, Sanghera MK and German DC (1981) Mesencephalic dopaminergic unit activity in the behaviorally conditioned rat. *Life Science* 29:1255-1263.

Mirenowicz J and Schultz W (1994) Importance of unpredictability for reward responses in primate dopamine neurons. *Journal of Neurophysiology* 72:1024-1027.

Mogenson GJ (1987) Limbic-motor integration. *Progress in Psychobiology and Physiological Psychology* 12:117-170.

Mogenson GJ, Jones DL and Yim CY (1980) From motivation to action: functional interface between the limbic system and the motor system. *Prog Neurobiol* 14:69-97.

Monaghan DT and Cotman CW (1985) Distribution of N-methyl-D-aspartate-sensitive L-[3H]glutamate-binding sites in rat brain. *J Neurosci* 5:2909-2919.

Morse WH and Skinner BF (1958) Some factors involved in the stimulus control of operant behavior. *Journal of the Exerimental Analysis of Behavior* 1:103-107.

Muller D, Joly M and Lynch G (1988) Contributions of quisqualate and NMDA receptors to the induction and expression of LTP. *Science* 242:1694-1697.

Murase S, Grenhoff J, Chouvet G, Gonon F and Svensson TH (1993) Prefrontal cortex regulates burst firing and transmitter release in rat mesolimbic dopamine neurons studied in vivo. *Neurosci Lett* 157:53-56.

Murschall A and Hauber W (2006) Inactivation of the ventral tegmental area abolished the general excitatory influence of Pavlovian cues on instrumental performance. *Learn Mem* 13:123-126.

Neisewander JL, O'Dell LE, Tran-Nguyen LTL, Castaneda E and Fuchs RA (1996) Dopamine overflow in the nucleus accumbens during extinction and reinstatement of cocaine self-administration behavior. *Neuropsychopharmacology* 15:506-514.

Nicola SM (2007) The nucleus accumbens as part of a basal ganglia action selection circuit. *Psychopharm* 191:521-550.

Nicola SM, Surmeier DJ and Malenka RC (2000) Dopaminergic modulation of neuronal excitability in the striatum and nucleus accumbens. *Annu Rev Neurosci* 23:185-215.

Nicola SM, Taha SA, Kim SW and Fields HL (2005) Nucleus accumbens dopamine release is necessary and sufficient to promote the behavioral response to reward-predictive cues. *Neurosci* 135:1025-1033.

Nicola SM, Yun IA, Wakabayashi KT and Fields HL (2004a) Cue-evoked firing of nucleus accumbens neurons encodes motivational significance during a discriminative stimulus task. *Journal of Neurophysiology* 91:1840-1865.

Nicola SM, Yun IA, Wakabayashi KT and Fields HL (2004b) Firing of nucleus accumbens neurons during the consummatory phase of a discriminative stimulus task depends on previous reward predictive cues. *Journal of Neurophysiology* 91:1866-1882.

Nishino H, Ono T, Muramoto K, Fukuda M and Sasaki K (1987) Neuronal activity in the ventral tegmental area (VTA) during motivated bar press feeding in the monkey. *Brain Res* 413:302-313.

Nowak L, Bregestovski P, Ascher P, Herbet A and Prochiantz A (1984) Magnesium gates glutamate-activated channels in mouse central neurones. *Nature* 307:462-465.

Oakman SA, Faris PL, Kerr PE, Cozzari C and Hartman BK (1995) Distribution of pontomesencephalic cholinergic neurons projecting to substantia nigra differs significantly from those projecting to ventral tegmental area. *The Journal of Neuroscience* 15:5859-5869.

Omelchenko N and Sesack SR (2006) Cholinergic axons in the rat ventral tegmental area synapse preferentially onto mesoaccumbens dopamine neurons. *J Comp Neurol* 494:863-875.

Omelchenko N and Sesack SR (2007) Glutamate synaptic inputs to ventral tegmental area neurons in the rat derive primarily from subcortical sources. *Neurosci* 146:1259-1274.

Overaker RF, Dodge BC and Epstein DL A new eyedrop dispensing bottle [In Process Citation]. *Am J Ophthalmol 1999 Sep* 128:368-370.

Pan WX, Schmidt R, Wickens JR and Hyland BI (2005) Dopamine cells respond to predicted events during classical conditioning: evidence for eligibility traces in the reward-learning network. *J Neurosci* 25:6235-6242.

Panksepp J. Affective Neuroscience: The Foundations of Human and Animal Emotions. 1998. Oxford University Press. Series in Affective Science. Ref Type: Serial (Book,Monograph)

Park TH and Carr KD (1998) Neuroanatomical patterns of fos-like immunoreactivity induced by a palatable meal and meal-paired environment in saline- and naltrexone-treated rats. *Brain Res* 805:169-180.

Parkinson JA, Dalley JW, Cardinal RN, Bamford A, Fehnert B, Lachenal G, Rudarakanchana N, Halkerston KM, Robbins TW and Everitt BJ (2002) Nucleus accumbens dopamine depletion impairs both acquisition and performance of appetitive Pavlovian approach behaviour: implications for mesoaccumbens dopamine function. *Behavioral Brain Research* 137:149-163.

Parkinson JA, Olmstead MC, Burns LH, Robbins TW and Everitt BJ (1999) Dissociation in effects of lesions of the nucleus accumbens core and shell on appetitive Pavlovian approach behavior and the potentiation of conditioned reinforcement and locomotor activity by D-amphetamine. *J Neurosci* 19:2401-2411.

Parkinson JA, Roberts AC, Everitt BJ and Di CP (2005) Acquisition of instrumental conditioned reinforcement is resistant to the devaluation of the unconditioned stimulus. *Q J Exp Psychol B* 58:19-30.

Parkinson JA, Willoughby PJ, Robbins TW and Everitt BJ (2000) Disconnection of the anterior cingulate cortex and nucleus accumbens core impairs Pavlovian approach behavior: further evidence for limbic cortical-ventral striatopallidal systems. *Behav Neurosci* 114:42-63.

Paxinos G and Watson C (1986) *The rat brain in stereotaxic coordinates*. Academic, New York.

Pezze MA and Feldon J (2004) Mesolimbic dopaminergic pathways in fear conditioning. *Prog Neurobiol* 74:301-320.

Phillips AG, Atkinson LJ, Blackburn JR and Blaha CD (1993) Increased extracellular dopamine in the nucleus accumbens of the rat elicited by a conditional stimulus for food: an electrochemical study. *Canadian Journal of Physiological Pharmacology* 71:387-393.

Phillips AG, Coury A, Fiorino D, LePiane FG, Brown E and Fibiger HC (1992) Self-stimulation of the ventral tegmental area enhances dopamine release

in the nucleus accumbens: A microdialysis study. *Ann NY Acad Sci*199-206.

Phillips GD, Setzu E and Hitchcott PK (2003a) Facilitation of appetitive pavlovian conditioning by d-amphetamine in the shell, but not the core, of the nucleus accumbens. *Behav Neurosci* 117:675-684.

Phillips GD, Setzu E, Vugler A and Hitchcott PK (2003b) Immunohistochemical assessment of mesotelencephalic dopamine activity during the acquisition and expression of Pavlovian versus instrumental behaviours. *Neurosci* 117:755-767.

Phillips PE, Stuber GD, Heien ML, Wightman RM and Carelli RM (2003c) Subsecond dopamine release promotes cocaine seeking. *Nature* 422:614-618.

Phillipson OT (1979) Afferent projections to the ventral tegmental area of Tsai and interfascicular nucleus: a horseradish peroxidase study in the rat. *J Comp Neurol* 187:117-143.

Rada PV, Mark GP, Yeomans JS and Hoebel BG (2000) Acetylcholine release in ventral tegmental area by hypothalamic self-stimulation, eating, and drinking. *Pharmacol Biochem Behav* 65:375-379.

Ranaldi R and Beninger RJ (1995) Bromocriptine enhancement of responding for conditioned reward depends on intact D_1 receptor function. *Psychopharm* 118:437-443.

Ranaldi R, Pantalony D and Beninger RJ (1995) The D_1agonist SKF 38393 attenuates amphetamine-produced enhancement of responding for conditioned reward in rats. *Pharmacol Biochem Behav* 52:131-137.

Ranaldi R and Roberts DCS (1996) Initiation, maintenance and extinction of cocaine self-administration with and without conditioned reward. *Psychopharm* 128:89-96.

Redgrave P, Prescott TJ and Gurney K (1999) Is the short-latency dopamine response too short to signal reward error? *Trends Neurosci* 22:146-151.

Rescorla RA and Solomon RL (1967) Two-process learning theory: Relationships between Pavlovian conditioning and instrumental learning. *Psychol Rev* 74:151-182.

Richardson NR and Gratton A (1996) Behavior-relevant changes in nucleus accumbens dopamine transmission elicited by food reinforcement: an electrochemical study in rat. *J Neurosci* 16:8160-8169.

Robinson S, Sandstrom SM, Denenberg VH and Palmiter RD (2005) Distinguishing whether dopamine regulates liking, wanting, and/or learning about rewards. *Behav Neurosci* 119:5-15.

Rodriguez JJ, Doherty MD and Pickel VM (2000) N-methyl-D-aspartate (NMDA) receptors in the ventral tegmental area: subcellular distribution and colocalization with 5-hydroxytryptamine(2A) receptors. *Journal of Neuroscience Research* 60:202-211.

Roitman MF, Stuber GD, Phillips PE, Wightman RM and Carelli RM (2004) Dopamine operates as a subsecond modulator of food seeking. *J Neurosci* 24:1265-1271.

Romo R and Schultz W (1990) Dopamine neurons of the monkey midbrain: contingencies of responses to active touch during self-initiated arm movements. *Journal of Neurophysiology* 63:592-606.

Rossetti ZL, Marcangione C and Wise RA (1998) Increase of extracellular glutamate and expression of Fos-like immunoreactivity in the ventral tegmental area in response to electrical stimulation of the prefrontal cortex. *J Neurochem* 70:1503-1512.

Saal D, Dong Y, Bonci A and Malenka RC (2003) Drugs of abuse and stress trigger a common synaptic adaptation in dopamine neurons. *ne* 37:577-582.

Salamone JD, Correa M, Farrar A and Mingote S (2007) Effort-related functions of nucleus accumbens dopamine and associated forebrain circuits. *Psychopharm* 191:461-482.

Satoh T, Nakai S, Sato T and Kimura M (2003) Correlated coding of motivation and outcome of decision by dopamine neurons. *J Neurosci* 23:9913-9923.

Schilstrom B, Nomikos GG, Nisell M, Hertel P and Svensson TH (1998a) N-methyl-D-aspartate receptor antagonism in the ventral tegmental area diminishes the systemic nicotine-induced dopamine release in the nucleus accumbens. *Neurosci* 82:781-789.

Schilstrom B, Svensson HM, Svensson TH and Nomikos GG (1998b) Nicotine and food induced dopamine release in the nucleus accumbens of the rat: putative role of alpha7 nicotinic receptors in the ventral tegmental area. *Neurosci* 85:1005-1009.

Schoenbaum G, Chiba AA and Gallagher M (1998) Orbitofrontal cortex and basolateral amygdala encode expected outcomes during learning. *Nature Neuroscience* 1:155-159.

Schultz W (1986) Responses of midbrain dopamine neurons to behavioral trigger stimuli in the monkey. *Journal of Neurophysiology* 56:1439-1461.

Schultz W (1998) Predictive reward signal of dopamine neurons. *Journal of Neurophysiology* 80:1-27.

Schultz W (2007) Multiple dopamine functions at different time courses. *Ann Rev Neurosci* 30:259-288.

Schultz W, Apicella P and Ljungberg T (1993) Responses of monkey dopamine neurons to reward and conditioned stimuli during successive steps of learning a delayed response task. *J Neurosci* 13:900-913.

Schultz W, Apicella P, Scarnati E and Ljungberg T (1992) Neuronal activity in monkey ventral striatum related to the expectation of reward. *J Neurosci* 12(12):4595-4610.

Sclafani A and Ackroff K (2004) The relationship between food reward and satiation revisited. *Physiol Behav* 82:89-95.

See RE, Kruzich PJ, and Grimm JW (2001) Dopamine, but not glutamate, receptor blockade in the basolateral amygdala attenuates conditioned reward in a rat model of relapse to cocaine-seeking behavior. *Psychopharmacology* 154(3):301–310.

Sesack SR, Deutch AY, Roth RH and Bunney BS (1989) Topographical organization of the efferent projections of the medial prefrontal cortex in the rat: an anterograde tract-tracing study with Phaseolus vulgaris leucoagglutinin. *J Comp Neurol* 290:213-242.

Sesack SR and Pickel VM (1992) Prefrontal cortical efferents in the rat synapse on unlableled neuronal targets of catecholamine terminals in the nucleus accumbens septi and on dopamine neurons in the ventral tegmental area. *J Comp Neurol* 320:145-160.

Seutin V, Verbanck P, Massotte L and Dresse A (1990) Evidence for the presence of N-methyl-D-aspartate receptors in the ventral tegmental area of the rat: an electrophysiological in vitro study. *Brain Res* 514:147-150.

Shank EJ, Seitz PK, Bubar MJ, Stutz SJ and Cunningham KA (2007) Selective ablation of GABA neurons in the ventral tegmental area increases spontaneous locomotor activity. *Behav Neurosci* 121:1224-1233.

Sharf R, McKelvey J and Ranaldi R (2006) Blockade of muscarinic acetylcholine receptors in the ventral tegmental area prevents acquisition of food-rewarded operant responding in rats. *Psychopharm* 186:113-121.

Sharf R and Ranaldi R (2006) Blockade of muscarinic acetylcholine receptors in the ventral tegmental area disrupts food-related learning in rats. *Psychopharm* 184:87-94.

Simon SA, de A, I, Gutierrez R and Nicolelis MA (2006) The neural mechanisms of gustation: a distributed processing code. *Nat Rev Neurosci* 7:890-901.

Smith-Roe SL and Kelley AE (2000) Coincident activation of NMDA and dopamine D1 receptors within the nucleus accumbens core is required for appetitive instrumental learning. *J Neurosci* 20:7737-7742.

Steffensen SC, Nie Z, Criado JR and Siggins GR (2000) Ethanol inhibition of N-methyl-D-aspartate responses involves presynaptic gamma-aminobutyric acid(B) receptors. *J Pharmacol Exp Ther* 294:637-647.

Steffensen SC, Svingos AL, Pickel VM and Henriksen SJ (1998) Electrophysiological characterization of GABAergic neurons in the ventral tegmental area. *J Neurosci* 18:8003-8015.

Stein L (1958) Secondary reinforcement established with subcortical stimulation. *Science* 127:466-467.

Stobbs SH, Ohran AJ, Lassen MB, Allison DW, Brown JE and Steffensen SC (2004) Ethanol suppression of ventral tegmental area GABA neuron electrical transmission involves N-methyl-D-aspartate receptors. *J Pharmacol Exp Ther* 311:282-289.

Suaud-Chagny MF, Chergui K, Chouvet G and Gonon F (1992) Relationship between dopamine release in the rat nucleus accumbens and the discharge

activity of dopaminergic neurons during local in vivo application of amino acids in the ventral tegmental area. *Neurosci* 49:63-72.

Surmeier DJ, Ding J, Day M, Wang Z and Shen W (2007) D1 and D2 dopamine-receptor modulation of striatal glutamatergic signaling in striatal medium spiny neurons. *Trends Neurosci* 30:228-235.

Swanson LW (1982) The projections of the ventral tegmental area and adjacent regions:a combined flourescent retrograde tracer and immunofluorescence study in the rat. *Brain Res Bull* 9:321-353.

Taber MT, Das S and Fibiger HC (1995) Cortical regulation of subcortical dopamine release: mediation via the ventral tegmental area. *J Neurochem* 65:1407-1410.

Taber MT and Fibiger H (1997) Feeding-evoked dopamine release in the nucleus, accumbens: regulation by glutamatergic mechanisms. *Neurosci* 76:1105-1112.

Takahata R and Moghaddam B (1998) Glutamatergic regulation of basal and stimulus-activated dopamine release in the prefrontal cortex. *J Neurochem* 71:1443-1449.

Talmi D, Seymour B, Dayan P and Dolan RJ (2008) Human pavlovian-instrumental transfer. *J Neurosci* 28:360-368.

Taylor JR and Robbins TW (1984) Enhanced behavioural control by conditioned reinforcers following microinjections of d-amphetamine into the nucleus accumbens. *Psychopharm* 84:405-412.

Tolman EC and Honzik CH. Introduction and removal of reward, and maze performance in rats. 4, 257-275. 1930.

Ref Type: Generic

Tong Z-Y, Overton PG and Clark D (1996a) Antagonism of NMDA receptors but not AMPA/kainate receptors blocks bursting in dopaminergic neurons induced by electrical stimulation of the prefrontal cortex. *Journal Neural Transmission* 103:889-905.

Tong Z-Y, Overton PG and Clark D (1996b) Stimulation of the prefrontal cortex in the rat induces patterns of activity in midbrain dopaminergic neurons which resemble natural burst events. *s* 22:195-208.

Tzschentke TM and Schmidt WJ (1995) *N*-Methyl-D-aspartic acid-receptor antagonists block morphine-induced conditioned place preference in rats. *Neurosci Lett* 193:37-40.

Ungerstedt U (1971) Stereotaxic mapping of the monoamine pathways in the rat brain. *Acta Physiol Scand Suppl* 367:1-48.

Ungless MA, Whistler JL, Malenka RC and Bonci A (2001) Single cocaine exposure in vivo induces long-term potentiation in dopamine neurons. *Nature* 411:583-587.

Van Bockstaele EJ and Pickel VM (1995) GABA-containing neurons in the ventral tegmental area project to the nucleus accumbens in rat brain. *Brain Res* 682:215-221.

Wakabayashi KT, Fields HL and Nicola SM (2004) Dissociation of the role of nucleus accumbens dopamine in responding to reward-predictive cues and waiting for reward. *Behavioral Brain Research* 15:19-30.

Wan X and Peoples LL (2006) Firing patterns of accumbal neurons during a pavlovian-conditioned approach task. *Journal of Neurophysiology* 96:652-660.

Wang T and French ED (1993) L-glutamate excitation of A10 dopamine neurons is preferentially mediated by activation of NMDA receptors: extra- and intracellular electrophysiological studies in brain slices. *Brain Res* 627:299-306.

Wang T, O'Connor WT, Ungerstedt U and French ED (1994) N-methyl-D-aspartic acid biphasically regulates the biochemical and electrophysiological response of A10 dopamine neurons in the ventral tegmental area: in vivo microdialysis and in vitro electrophysiological studies. *Brain Res* 666:255-262.

Weed MR, Mackevicius AS, Kebabian JW and Woolverton WL (1995) Reinforcing and discriminative stimulus effects of beta-CIT in rhesus monkeys. *Pharmacol Biochem Behav* 51:953-956.

Westerink BHC, Enrico P, Feimann J and De Vries JB (1998) The pharmacology of mesocortical dopamine neurons: a dual-probe microdialysis study in the ventral tegmental area and prefrontal cortex of the rat brain. *J Pharmacol Ep Ther* 285:143-154.

Westerink BHC, Kwint H-F and De Vries JB (1997) Eating-induced dopamine release from mesolimbic neurons is mediated by NMDA receptors in the ventral tegmental area: a dual-probe microdialysis study. *J Neurochem* 69:662-668.

Westerink BHC, Kwint H-F and deVries JB (1996) The pharmacology of mesolimbic dopamine neurons: a dual-probe microdialysis study in the ventral tegmental area and nucleus accumbens of the rat brain. *J Neurosci* 16:2605-2611.

Wickens J (1990) Striatal dopamine in motor activation and reward-mediated learning: steps towards a unifying model. *J Neural Transm* 80:9-31.

Williams, DR and Williams, H (1969) Auto-maintenance in the pegieon: sustained pecking despite contingent non-reinforcement. *Journal of the Experimental Analysis of Behavior* 21, 125-144.

Wilson C, Nomikos GG, Collu M and Fibiger HC (1995) Dopaminergic correlates of motivated behavior:importance of drive. *The Journal of Neuroscience* 15:5169-5178.

Wilson DI and Bowman EM (2004) Nucleus accumbens neurons in the rat exhibit differential activity to conditioned reinforcers and primary reinforcers within a second-order schedule of saccharin reinforcement. *Eur J Neurosci* 20:2777-2788.

Wilson DI and Bowman EM (2006) Neurons in dopamine-rich areas of the rat medial midbrain predominantly encode the outcome-related rather than

behavioural switching properties of conditioned stimuli. *Eur J Neurosci* 23:205-218.

Wise RA (2004) Dopamine, learning and motivation. *Nat Rev Neurosci* 5:483-494.

Wise RA (2006) Role of brain dopamine in food reward and reinforcement. *Philosophical Transactions of the Royal Society B: Biological Sciences* 361:1149-1158.

Wise RA and Hoffman DC (1992) Localization of drug reward mechanisms by intracranial injections. *s* 10:247-263.

Wise RA and Schwartz HV (1981) Pimozide attenuates acquisition of lever-pressing for food in rats. *Pharmacol Biochem Behav* 15:655-656.

Wise RA, Spindler J, deWit H and Gerber GJ (1978) Neuroleptic-induced "anhedonia" in rats: pimozide blocks reward quality of food. *Science* 201:262-264.

Wolterink G, Phillips G, Cador M, Donselaar-Wolterink I, Robbins TW and Everitt BJ (1993) Relative roles of ventral striatal D_1 and D_2 dopamine receptors in responding with conditioned reinforcement. *Psychopharm* 110:355-364.

Wyvell CL and Berridge KC (2000) Intra-accumbens amphetamine increases the conditioned incentive salience of sucrose reward: enhancement of reward "wanting" without enhanced "liking" or response reinforcement. *J Neurosci* 20:8122-8130.

Yamaguchi T, Sheen W and Morales M (2007) Glutamatergic neurons are present in the rat ventral tegmental area. *Eur J Neurosci* 25:106-118.

Yeomans J and Baptista M (1997) Both nicotinic and muscarinic receptors in ventral tegmental area contribute to brain-stimulation reward. *Pharmacol Biochem Behav* 57:915-921.

Yeomans JS, Kofman O and McFarlane V (1985) Cholinergic involvement in lateral hypothalamic rewarding brain stimulation. *Brain Res* 329:19-26.

Yeomans JS, Mathur A and Tampakeras M (1993) Rewarding brain stimulation: role of tegmental cholinergic neurons that activate dopamine neurons. *Behav Neurosci* 107:1077.

Yim CY and Mogenson GJ (1982) Response of nucleus accumbens neurons to amygdala stimulation and its modification by dopamine. *Brain Res* 239:401-415.

You ZB, Wang B, Zitzman D, Azari S and Wise RA (2007) A role for conditioned ventral tegmental glutamate release in cocaine seeking. *J Neurosci* 27:10546-10555.

Young AM (2004) Increased extracellular dopamine in nucleus accumbens in response to unconditioned and conditioned aversive stimuli: studies using 1 min microdialysis in rats. *J Neurosci Methods* 138:57-63.

Young AM, Ahier RG, Upton RL, Joseph MH and Gray JA (1998) Increased extracellular dopamine in the nucleus accumbens of the rat during associative learning of neutral stimuli. *Neurosci* 83:1175-1183.

Yun IA and Fields HL (2003) Basolateral amygdala lesions impair both cue- and cocaine-induced reinstatement in animals trained on a discriminative stimulus task. *Neurosci* 121:747-757.

Yun IA, Nicola SM and Fields HL (2004a) Contrasting effects of dopamine and glutamate receptor antagonist injection in the nucleus accumbens suggest a neural mechanism underlying cue-evoked goal-directed behavior. *Eur J Neurosci* 20:249-263.

Yun IA, Wakabayashi KT, Fields HL and Nicola SM (2004b) The ventral tegmental area is required for the behavioral and nucleus accumbens neuronal firing responses to incentive cues. *J Neurosci* 24:2923-2933.

Zahm DS and Brog JS (1992) On the significance of subterritories in the "accumbens" part of the rat ventral striatum. *Neurosci* 50:751-767.