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Review

How conditioned stimuli acquire the ability to activate VTA dopamine cells: A proposed neurobiological component of reward-related learning

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ABSTRACT

The ability to learn about conditioned stimuli (CS) associated with rewards is a crucial adaptive mechanism. Activity in the mesocorticolimbic dopamine (DA) system, as well as in the ventral tegmental area (VTA), is correlated with responding to and learning about CSs. The mechanism by which VTA neurons become activated by signals associated with conditioned stimuli is not fully understood. Our model suggests that NMDA receptor stimulation in the VTA allows originally weak glutamate signals carrying information about environmental stimuli, coincident with strong excitation correlated with primary rewards, to be strengthened and thereby acquire the ability to activate VTA neurons in themselves, producing approach. Furthermore, once synaptic strengthening occurs, the model suggests that NMDA receptor stimulation in VTA is not necessary for the expression of reward-related learning. In this review we survey evidence that VTA cells respond to cues associated with primary rewards, that this responding is acquired, and that the VTA possesses the attributes to function as a site of integration of signals of primary and conditioned stimuli.

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The ability to learn about rewards – when and where to expect them, for example, or what to do to get them – is a crucial adaptive capacity that higher animals possess. In addition, reward-related learning is implicated in pathologies of motivational processes such as drug addiction and gambling. One key component of reward-related learning is the acquisition of conditioned associations between rewards and stimuli in the environment which predict or accompany those rewards. Such stimuli are called "conditioned stimuli" (CSs), and they influence a wide range of reward-related behavior. (Of course, CSs also play an important role in aversive processes [see (Le Doux, 1998)], but we are focused here on reward-related conditioned stimuli.) CSs signal the availability of rewards and increase motivation to work for them. They are ubiquitous in daily life—for example, the ATM sign that signals the availability of cash, or the sight of a leash that gets a dog excited for a walk. CSs are also thought to play an important role in maintaining pathological reward-related behavior, as the perception of cues associated with drugs can trigger cravings (Childress et al., 1999; Ehrman et al., 1992) which may play a major role in relapse (Childress et al., 1988; Ehrman et al., 1992; Stewart et al., 1984; Wallace, 1989). Given that learning about reward-related stimuli is a key adaptive mechanism, and that CSs play a role in

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pathologies of reward, it becomes important to understand the underlying neurobiology of processes related to conditioned stimuli.

Ever since early studies identified the importance of dopamine (DA) in operant responding reinforced by lateral hypothalamic stimulation or "natural" rewards (e.g., Wise et al., 1978), a large body of evidence has accumulated implicating DA systems in reward, motivation, and learning. It is well established that mesocorticolimbic DA plays an important role in mediating the behavioral effects of primary rewards (for detailed reviews see Berridge and Robinson, 1998; Wise, 2004; Wise and Rompré, 1989). In addition, mesocorticolimbic DA is implicated in mediating the motivational effects of conditioned stimuli. The presentation of CSs is associated with neural activity or DA release in terminal regions of this system, including the nucleus accumbens (NAcc), amygdala, and prefrontal cortex (PFC) (see, for example, Bassareo et al., 2007; Blackburn and Phillips, 1989; Carelli, 2000, 2002; Carelli and Ijames, 2001; Childress et al., 1999; Gratton and Wise, 1994; Janak et al., 1999; Maas et al., 1998; Phillips et al., 2003b; Talmi et al., 2008). As we will review below, activity in the VTA, the source of mesolimbic DA, is also correlated with the presentation of CSs, and indeed may be a necessary condition for responding to conditioned stimuli (Di Ciano and Everitt, 2004; Murschall and Hauber, 2006). This activity seems to be acquired with exposure to stimulus-reward contingencies. However, the neurobiological process by which VTA DA cells "come to know" about previously neutral reward-related stimuli remains unclear. We have previously suggested that neural plasticity in the VTA allows for signals associated with environmental stimuli, through coincident stimulation with primary reward signals, to come to activate DA cells in themselves (Sharf et al., 2006; Zellner et al., 2009). In particular, we suggest that coincident stimulation from afferents carrying a primary reward signal and afferents carrying a signal about environmental stimuli trigger synaptic strengthening, mediated by NMDA receptors, which allows the previously weak signal of the stimulus to come to activate the DA cells in itself. This paper reviews evidence related to this hypothesis.

A note about terminology: in this review, we will attempt to maintain a distinction between "reward" and "reinforcement." We define reward as an unconditioned stimulus that elicits approach behavior (Ikemoto and Panksepp, 1999). Reinforcement refers to a behavioral (and neural) process characterized by an increased likelihood of emitting a particular behavior when it is followed by a particular consequence (Mackintosh, 1974). As a strictly objective measurement, the term "reinforcement" does not indicate 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 pleasurable tend to function as reinforcers (Panksepp, 1998). In this review, 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 a contiguous relation between a stimulus or action and a reward. 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. Of course, reinforcement or reward are not necessary for all forms of learning (Bolles, 1972). In latent learning paradigms (Blodgett, 1929; Tolman and Honzik, 1930), for example, 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 specific 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 related to a reward. However, in this review we will address specifically reward-related learning.

1. Behavioral perspectives on conditioned stimuli

Within experimental paradigms CSs tend to be used in several categories. First, in Pavlovian or classical conditioning, stimuli presented contiguously with rewards can come to elicit responses similar to those elicited by the reward itself (Mackintosh, 1974). These stimuli are passively experienced with reward and their presentation is not contingent on the emission of any specific behavior—for example, when animals are exposed to a tone before food is consumed, or learn to associate a 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 reward is contingent on a particular action such as pressing a lever (Mackintosh, 1974). In these paradigms, a stimulus (such as a light or tone) may be presented in close temporal proximity with a reward. Stimuli presented in this manner come to influence reward-related behavior in a number of ways. Pavlovian CSs can facilitate the acquisition of a novel instrumental response (see Grindley, 1929; Skinner, 1938). Once the reward association is established, 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). The presentation of a CS can also reinstate instrumental responding after it has extinguished (de Wit and Stewart, 1981; Markou et al., 1993). Finally, a stimulus may signal that an action taken will result in reward, in which case it is called a "discriminative stimulus" (DS) (Mackintosh, 1974). As mentioned earlier, these effects of conditioned stimuli on instrumental behavior are considered to be of particular importance in drug addiction, as the "people, places or things" (which recovery programs warn addicts about) that are associated with drug use have been implicated in maintaining active drug use, or triggering craving in abstinent users who then return to active drug use.

The types of CSs 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 a flavor. The final category of CSs, contextual cues, is 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 types of CSs are involved in orienting, energizing, or maintaining behaviors, both when primary rewards are present and during extinction responding when primary rewards are absent. 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 (see, for example, Rescorla and Solomon, 1967). 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. However, 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* affect behavior, a previously neutral stimulus must

become associated with some aspect of the receipt of reward, and thereby become a CS. 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 (Beninger and Ranaldi, 1994; Bindra, 1974; Bolles, 1972; Wise, 2004). This review focuses on one proposed mechanism for this process.

2. Role of dopamine in approach (incentive-motivation)

As this review concerns the associative processes in the VTA which may underlie the ability of CS signals to activate DA cells, a brief mention of the complex physiology and function of dopamine is warranted. Dopamine has both acute and long-term effects on cells. Acutely, DA performs a modulatory role (Greengard, 2001), and tends to increase post-synaptic excitability via coupling to the D_1 family of DA receptors, which results in a cascade of processes leading to increased expression of AMPA and NMDA receptors, and decreases excitability via the D_2 family of receptors, which leads to decreased AMPA currents, reduced opening of Na^+ channels, and facilitated opening of K^+ channels (see Surmeier et al., 2007 for review). Furthermore, DA affects cells depending on their current state, prolonging the "up" state (Lewis and O'Donnell, 2000) and enhancing excitability (Lavin and Grace, 2001), while reducing spontaneous firing (Lavin et al., 2005), perhaps allowing cells to filter out irrelevant behavior or stimuli (Lewis and O'Donnell, 2000). Within a longer time frame, DA plays a role in synaptic plasticity (see Beninger and Miller, 1998; Jay, 2003 for review).

This heterogeneity of effects, depending on the brain region in question, relative concentration of receptor subtypes, current state of affected neurons, and short- versus long-term consequences, may account for the fact that a number of competing views on the function of dopamine exist. These perspectives include those that emphasize DA's role in basic reward (the "anhedonia" hypothesis, Wise et al., 1978), reinforcement and learning (Beninger, 1983; Wise, 2006), effort (Salamone et al., 2007), behavioral "switching" (Nicola, 2007; Redgrave et al., 1999), reward prediction error (Ljungberg et al., 1992; Schultz, 1998), and "wanting" (Berridge, 2007). Each of these various perspectives encompasses a particular aspect of DA function, and a comprehensive account of DA's function is likely to involve a combination of most or all of these perspectives.

In relation to the role of conditioned stimuli in reward-related learning, we are particularly interested in the activation of approach behaviors that is associated with mesolimbic DA activity, particularly in the more medial aspects of the VTA and the medial ventral striatum (see Ikemoto, 2007 for review). Converging evidence demonstrates that exogenous stimulation of mesolimbic areas (either electrically or pharmacologically) results in increased forward locomotion, seeking, and appetitive behaviors, and that approach behavior is linked with mesolimbic cell activity (Beninger, 1983; Berridge, 2007; Berridge and Robinson, 1998; Mogenson, 1987; Panksepp, 1998; Phillipson, 1979; Wise and Bozarth, 1987). Therefore, it appears that one key role that mesolimbic DA plays is to instantiate the incentive-motivational effects of primary rewards, increasing approach and seeking, and thereby maximizing the animal's proximity to rewards.

Given the heterogeneous physiological effects of DA, we argue that activation of the mesocorticolimbic system has at least two domains of action: first, energizing current motivated behavior and sensitivity to reward-related stimuli, and second, facilitating associative processes underlying learning, which allows stimuli and representations of reward to guide future behavior. In this regard, we believe that 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 DA cells, augmenting DA release and approach behavior, putting the animal in greater proximity to rewards and related cues or operanda; and third, DA release at the terminal regions due to primary rewards and CSs facilitate associative processes in those regions which underlie further stimulus–stimulus, stimulus–response, and stimulus–outcome associations. (For associative processes in terminal regions see, for example, Kelley, 1999; Burns et al., 1994; Di Ciano et al., 2001; Parkinson et al., 2000; Kelley et al., 1997.) 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, although CS-related responding declines after some period of time without primary reward.

3. A Hebbian model of reward-related learning in VTA

As indicated already, 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 (Beninger and Ranaldi, 1994; Bindra, 1974; Bolles, 1972; Wise, 2004). 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., Bonci and Malenka, 1999; Harris et al., 2004; Sharf et al., 2006; Stuber et al., 2008; Zellner et al., 2009). Our proposed Hebbian model of synaptic plasticity posits that the acquisition of reward-related learning involves activity-dependent changes in the strength of synaptic connections among CS-relevant and reward (US)-relevant sets of neurons, brought about by the conjoint activity of these neurons on DA neurons of the VTA. The VTA receives excitatory input from structures such as the mesopontine cholinergic nuclei which are likely to convey signals of primary reward, as well as from structures such as the superior colliculus, PFC, and amygdala conveying information about environmental stimuli, and is therefore a likely site for associative processes arising from convergent stimulation. This convergent stimulation creates the conditions for long-term potentiation (LTP), thought to be one of the major mechanisms of synaptic changes underlying learning, which is in many cases dependent on NMDA receptor stimulation (Citri and Malenka, 2007). NMDA receptors are found in the VTA (Rodriguez et al., 2008). And in fact, LTP has been demonstrated in VTA DA neurons, where it is NMDA receptor-dependent (Bonci and Malenka, 1999; Stuber et al., 2008). We propose, therefore, that one component of reward-related learning is strengthening of CS-related synapses whereby inputs associated with previously neutral environmental stimuli come to activate VTA cells, augmenting DA release at terminal regions. Initially, this "neutral environmental stimulus" glutamate signal may be too weak to activate DA cells sufficiently to result in approach behavior. If the reward neurons are firing, as is the case when mACh receptor stimulation associated with reward consumption (i.e., US) is occurring, NMDA receptor stimulation can result in initiation of second messenger cascades implicated in LTP and other intracellular events that lead to neurochemical and structural (i.e., synaptic growth) changes associated with learning. Such synaptic changes could result in glutamate now being able to robustly activate DA cells, causing robust DA release. This increased DA release, triggered by strengthened CS-related synapses in VTA, 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 downstream in DA terminal regions which will facilitate other aspects of conditioned behavior. Thus, the reward-produced mACh receptor stimulation can be considered the physiological US (with the primary reward the environmental US) and the DA activation the physiological unconditioned response (UR; with approach the behavioral UR).

The strengthening of the CS signal could be the result of one or more of several NMDA-dependent structural or functional synaptic changes including proliferation of AMPA receptors (Kessels and Malinow, 2009; Nicoll, 2003), growth of new synapses (Carlisle and Kennedy, 2005), increased presynaptic glutamate release (Lisman and Raghavachari, 2006) or other processes. [Note: This model is not aimed at explaining the acquisition of stimulus–response (S-R), stimulus–stimulus or response–outcome associations, which may involve neurochemical events and neural connections in forebrain regions such as the NAcc, caudate-putamen, amygdala and medial prefrontal cortex (mPFC).]

What follows is evidence that supports this model. This will include evidence that (1) the acquisition of reward-related learning is associated with CS-related changes in VTA DA cellular activity, (2) the VTA receives signals representing unconditioned stimuli (primary rewards) and signals representing conditioned stimuli, and (3) the acquisition of reward-related learning is dependent on VTA NMDA receptor and mACh receptor stimulation.

4. Conditioned stimuli and the VTA

Before we review VTA activity in relation to reward, two caveats need to be made. First, the VTA contains both DA neurons (“principal” cells), and GABA neurons (“secondary” cells) (Johnson and North, 1992) which project both within the VTA itself and outside the VTA (Carr and Sesack, 2000; Van Bockstaele and Pickel, 1995). The VTA also contains a third kind of cell that has yet to be definitively categorized (Johnson and North, 1992); recent evidence demonstrates the presence of glutamatergic neurons in the VTA which are non-dopaminergic and non-GABAergic, and may therefore constitute this third type of VTA neuron (Yamaguchi et al., 2007).

Studies using in vivo recording techniques to investigate VTA activity in response to CSs have therefore had to rely on physiological criteria to determine whether the units recorded are dopaminergic. These criteria include biphasic action potentials of relatively long duration (approximately 2.75 ms), and an average firing rate of 4.5 spikes/s (Bunney et al., 1991). Until recently, most investigators have accepted these criteria as identifying DA neurons, but categorical identification of the types of cells being recorded is currently impossible; therefore, any in vivo study must always presume, but cannot prove, that the neurons being recorded are dopaminergic. Indeed, a number of investigators have found that VTA neurons are heterogeneous in their firing patterns, types of conducting currents, and responsiveness to DA and opioids (e.g., Cameron et al., 1997; Kiyatkin and Rebec, 1998; Margolis et al., 2003; Wilson and Bowman, 2006). Moreover, Margolis and colleagues (Margolis et al., 2006, 2008) provided strong evidence that a number of previously accepted criteria do not reliably distinguish DA and non-DA neurons in the VTA, including an I_h current, action potential duration, and postsynaptic D_2 agonist inhibition. Many of these investigators therefore suggest that the criteria for DA neurons need to be substantially modified to reflect the wider categories of VTA neurons. 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.

Secondly, a similar cautionary statement should be made about the regions included in studies investigating DA neurons. Many in vivo investigations of responses of midbrain DA 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 glutamate neurons; and pharmacological differences between the regions have been observed (for review see Korotkova et al., 2004). In addition, differences have been found between the VTA and the substantia nigra in reward-related behavior (Phillips et al., 2003a; You et al., 2007). Therefore, findings in the substantia nigra should not be taken to apply to the VTA necessarily. In this review, therefore, references to “midbrain DA neurons” mean that the cited studies investigated both SN and VTA, and “VTA neurons” is used when investigators have provided regional categorization, indicated substantial VTA participation in the findings, or exclusively investigated the VTA.

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 (Fiorillo et al., 2003; Mirenowicz and Schultz, 1994; Romo and Schultz, 1990; Satoh et al., 2003; Schultz, 1986; Schultz et al., 1993). Where VTA neurons have been studied specifically, 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 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 and 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-to-instrumental transfer with a food-associated cue (Murschall and Hauber, 2006). In another study, VTA inactivation greatly reduced operant responding in rats working under a second-order schedule reinforced by a cocaine-associated cue before the first injection of cocaine is earned, although responding then reached normal levels after cocaine injection (Di Ciano and Everitt, 2004). These effects parallel the abolition of NAcc firing in response to sucrose cues when the VTA is inactivated while preserving neural activity correlated with the motoric actions of reward tasks such as lever pressing and magazine entry (Yun et al., 2004b).

In addition to activity of VTA cells, the presentation of CSs is also associated with increased DA release in the NAcc. DA in the NAcc

comes primarily from release at the terminals due to VTA firing (Gonon, 1988; Phillips et al., 1992, 2003b; Roitman et al., 2004; Sombers et al., 2009), although presynaptic glutamatergic facilitation from other NAcc afferents including amygdala, hippocampus, and PFC (Fields et al., 2007) also plays a role. In the context of instrumental behavior, NAcc DA increases with presentation of stimuli associated with psychostimulant drugs (Di Ciano et al., 1998; Gratton and Wise, 1994; Ito et al., 2000; Phillips et al., 2003b) and sucrose (Roitman et al., 2004). In the Pavlovian conditioning context, DA levels or DA currents in the NAcc also increase in relation to the presentation of CSs associated with food (Bassareo et al., 2007; Bassareo and Di Chiara, 1999b; Blackburn and Phillips, 1989; Phillips et al., 1993) and drugs of abuse (Bassareo et al., 2007; Kiyatkin and Stein, 1996). Cues associated with food increase extracellular DA levels in the amygdala (Harmer and Phillips, 1999) and cues associated with morphine and nicotine increase DA levels in the PFC (Bassareo et al., 2007). Cues associated with drugs of abuse are also associated with increases in DA immunoreactivity in central, anterior basolateral and posterior basolateral amygdala, as well as NAcc shell and mPFC after Pavlovian conditioning sessions (Phillips et al., 2003a). Finally, rats lever pressing in a session in which food-associated discriminative stimuli were presented showed increased DA levels in the BLA compared to rats lever pressing without a DS (Hori et al., 1993).

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 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 with injections of 6-hydroxydopamine (6-OHDA) 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 D_1–D_2 antagonist flupenthixol blocked the acquisition of operant responding with conditioned reinforcement (Cador et al., 1991) and behavioral responding to a DS decreases after injection of the D_1 antagonist SCH23390 (Nicola et al., 2005; Wakabayashi et al., 2004; Yun et al., 2004a,b) or D2 antagonist raclopride (Yun et al., 2004b) in the NAcc. Similarly, flupenthixol in the NAcc core reduces conditioned approach to a food-associated stimulus (Di Ciano et al., 2001).

In contrast to the effects of DA blockade, enhanced DA neurotransmission generally increases the incentive-motivational effects of CSs. 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 food reward. Similarly, amphetamine injections directly into the NAcc potentiate instrumental responding with conditioned reinforcement (Cador et al., 1991; Taylor and Robbins, 1984; Wolterink et al., 1993) and in the NAcc shell potentiate instrumental responding with non-contingent presentations of Pavlovian CSs (Taylor and Robbins, 1984; Wyvell and Berridge, 2000). Interestingly, the ability of a CS to motivate sucrose reward-seeking (in the absence of sucrose reward itself) is potentiated by intra-NAcc injections of amphetamine (Wyvell and Berridge, 2000). Thus, it appears that NAcc DA plays a role in the incentive-motivational effects of CSs.

Furthermore, 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 (Carelli, 2000, 2002; Carelli and Ijames, 2001; Ghitza et al., 2003; Janak et al., 1999; Nicola et al., 2004; Wilson and Bowman, 2004). Although NAcc firing does not necessarily indicate VTA activity [for example, NAcc requires glutamate input to fire and it receives excitatory afferents from a number of structures including amygdala and PFC (Mogenson, 1987; Nicola et al., 2000), it appears that the VTA plays a crucial modulatory role in NAcc firing in relation to CSs. Inactivation of the VTA almost completely abolishes cue-evoked firing in the NAcc while preserving neural activity correlated with motor actions such as operant responding and magazine entry (Yun et al., 2004b).

Finally, changes in VTA firing in relation to CSs may correlate with behavioral changes during extinction. To our knowledge, no studies have examined VTA cell firing in response to the continued presentation of CSs in the absence of primary reward. However, NAcc shell DA transients (Owesson-White et al., 2008) and DA efflux (Ahn and Phillips, 2007) in response to CSs have been shown to decline across trials of instrumental responding in which primary reward is withheld. This suggests that VTA DA firing decreases over time when no primary reward signal is received. It is beyond the scope of this paper to explore the causes of this decrease (whether additional upstream inhibitory influences encode the absence of primary reward, or new synaptic changes weaken the CS-associated signal, for example). However, the fact that DA transients do not immediately decline, and in fact increase when primary reward is withheld initially (Ahn and Phillips, 2007; Owesson-White et al., 2008) suggests that within some time frame, CS signals in and of themselves maintain the ability to activate VTA DA cells.

5. 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. However, activity in relation to cues does not necessarily imply learning, if that activity is there from the start. Therefore, is there evidence that patterns of VTA activity corresponding with environmental stimuli change as conditioned associations are being 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 show progressive changes as neutral stimuli are paired with rewards, indicating that associative processes are taking place. In what follows we review briefly the evidence for both of these patterns of activity.

Midbrain DA neurons respond to novel events and aversive events, in addition to rewards and conditioned stimuli (see Horvitz, 2000 for review). Animals orient towards novel objects and events, and will work for novel items, indicating that they find novelty rewarding (Bevins et al., 2002; Hughes, 1965); this is thought to be adaptive as animals can expand their repertoire of rewards and reproductive opportunities by exploring new things. Early studies found that various cells in the VTA increase their firing to sound, tail pressing and pricks, and spontaneous movement (Kiyatkin and Rebec, 1998; Miller et al., 1981). 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 (Horvitz et al., 1997). Likewise, NAcc DA also increases in response to novel and aversive stimuli (Young et al., 1998; Young, 2004).

In the absence of reinforcement, however, responses to novel stimuli tend to decline. 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 and 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 and then only to unexpected rewards (Ljungberg et al., 1992; Schultz et al., 1993). The neural mechanisms of this habituation to non-rewarded stimuli are not fully understood, and may involve synaptic plasticity in the VTA in the form of LTP or long-term depression (LTD), the recruitment of new inhibitory influences upstream from the VTA, or other mechanisms.

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 activity in connection with conditioned stimuli 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 (Mirenowicz and Schultz, 1994). In addition, in this same study 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 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 (Phillips et al., 2003a). NAcc DA efflux parallels this time course: 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 but instead increases after presentation of a cue indicating the start of the session (Richardson and Gratton, 1996). Similarly, VTA neurons 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 extracellular DA increases in the NAcc after presentation of a reward-paired cue (Datla et al., 2002), and phasic DA release in the NAcc becomes time-locked to the presentation of a CS across four conditioning sessions (Stuber et al., 2008). Significantly, an increase in the ratio of CS-related DA to reward-related DA was correlated with the number of approaches emitted. Extracellular DA in the NAcc core increased in rats encountering the smell of a palatable food to which they had only been exposed once (Bassareo and Di Chiara, 1999a). Similar to the above-cited study, this increase in NAcc core DA was temporally correlated with vigorous investigation of the food container. In another study 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 does not trigger increased DA signal in the NAcc before the first session, but is associated with increased signal on subsequent sessions of instrumental responding for cocaine (Gratton and Wise, 1994) and food (Richardson and Gratton, 1996).

6. LTP in the VTA as a candidate mechanism for the acquisition of CS-related responding

It is possible that synaptic plasticity in the VTA underlies, at least in part, the changes in neural activity that develops as animals acquire associations between rewards and environmental stimuli. Synaptic plasticity processes such as LTP have long been thought to be one of the main mechanisms of learning (Kandel, 2001). And indeed, LTP has been demonstrated in the VTA. DA cells show 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 the NMDA receptor antagonist D-APV (Bonci and Malenka, 1999). More recent studies have confirmed that VTA DA cells demonstrate LTP (Chen et al., 2008; Luu and Malenka, 2008; Nugent et al., 2008), and that this LTP can be blocked by NMDA receptor antagonism (Stuber et al., 2008; Nugent et al., 2008). 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 VTA NMDA receptor stimulation may be critical for the acquisition of behavior, but not for the performance of behavior that has already been learned.

VTA cells also show increased synaptic strength after 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 (Saal et al., 2003; Ungless et al., 2001). A number of recent studies have confirmed that cocaine induces LTP in the VTA (Argilli et al., 2008; Liu et al., 2005; Mameli et al., 2009; Schumann et al., 2009). [But see Chen et al., 2008, in which only self-administration of cocaine, not yoked administration, was associated with enhanced synaptic function in the VTA.]

It is not surprising that LTP has been demonstrated in the VTA, because a number of attributes of the VTA make it an appropriate candidate for these associative processes. As we will review, the VTA receives substantial afferents able to provide coincident stimulation representing both USs and CSs, and contains a key neural mechanism, namely stimulation of the NMDA receptor, which sets in motion the intracellular processes that lead to neural plasticity following such coincident stimulation.

The VTA receives projections from a wide range of brain structures, including PFC, dorsal rhinal sulcus, NAcc, bed nucleus of stria terminalis, olfactory tubercle, amygdala, diagonal band of Broca, substantia innominata, several hypothalamic nuclei including the lateral hypothalamus, lateral habenula, and brain stem structures including the superior colliculus, raphe nuclei, parabrachial nuclei, locus coeruleus, and mesopontine nuclei (Gabbott et al., 2008; Geisler and Zahm, 2005; Phillipson, 1979). Because it receives projections from such a rich assortment of structures, it is reasonable to assume that associative processes may occur in the VTA to modulate strength at some of those afferent synapses.

Activity in the VTA is modulated by a host of neurotransmitters including glutamate, GABA, serotonin, acetylcholine, norepinephrine, opioids, DA and peptides including CCK and orexin (see Kalivas, 1993; Mathon et al., 2003; Meltzer et al., 1997 for reviews). We focus here on glutamate and acetylcholine, because together they may provide the necessary and sufficient components for mediating associative processes leading to synaptic

strengthening. Glutamate likely carries signals about environmental stimuli, provides for the stimulation of the NMDA receptor which mediates LTP, and is the signal by which conditioned stimuli are likely to continue to recruit VTA cells after associative processes have taken place. Acetylcholine may mediate the primary reward signal of various unconditioned stimuli, as we will detail below.

7. Glutamate in 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; Mathon et al., 2003 and Meltzer et al., 1997 for reviews). 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), which corroborates earlier anatomical and physiological studies (e.g., Pickens and Thompson, 1968; Sesack et al., 1989; Sesack and Pickel, 1992).

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 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 and Malenka, 2007 for review), and therefore has been called a "coincidence detector." As described earlier, NMDA receptor stimulation leads to an increased influx of calcium followed by a number of second-messenger processes which lead to LTP. NMDA receptors are diffusely distributed throughout the VTA primarily within cell bodies and dendrites (Rodriguez et al., 2008).

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 NMDA increases VTA cell firing (Seutin et al., 1990; Tong et al., 1996; Wang and French, 1993; Wang et al., 1994). In vivo, application of glutamate or its agonists increases DA current in the NAcc (Suaud-Chagny et al., 1992), and increases DA concentrations or metabolites in the VTA, NAcc, and mPFC (Wang et al., 1994; Westerink et al., 1998). Moreover, glutamate increases VTA DA cell firing (Almodóvar-Fabregas et al., 2002; Wang et al., 1994), as does NMDA (Chergui et al., 1993). This firing appears to be mediated primarily by NMDA receptors as both competitive and non-competitive NMDA antagonists, but not AMPA antagonists, block glutamate- or agonist-induced firing (Wang and French, 1993; Chergui et al., 1993).

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 the excitation needed for coincident processes necessary for synaptic strengthening. 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 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 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 conditioned place preference (CPP) (Harris and Aston-Jones, 2003) and NMDA receptor antagonism alone blocks acquisition of morphine 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 in and of themselves, thereby becoming conditioend reward signals, is via glutamate stimulation of the NMDA receptor.

With that said, however, it is also possible that VTA glutamate plays a role in primary reward, although this is currently an open question. Manipulations in this region have contradictory effects on reward-related behavior, depending on the receptors, ligands, and rewards in question. On the one hand, reduced glutamate transmission, via administration of glutamate antagonists or stimulation of metabotropic glutamate receptors (mGluRs), has been found to reduce a number of rewards. Stimulation of mGluRs decreases nicotine self-administration (Liechti and Markou, 2007), as does NMDA antagonism (Kenny et al., 2009). AMPA receptor blockade reduces heroin conditioned place preference (in the anterior but not posterior VTA) (Shabat-Simon et al., 2008) and heroin reward (Xi and Stein, 2002; Shabat-Simon et al., 2008). Similarly, NMDA antagonists decrease heroin reward (Xi and Stein, 2002) and the broad-spectrum glutamate receptor antagonist kynurenic acid reduces cocaine reward (You et al., 2007). On the other hand, glutamate antagonism in the VTA has also been found to increase reward. For example, the AMPA receptor antagonist NBQX induced partner preference in monogamous prairie voles (Curtis and Wang, 2005). Similarly, both AMPA and NMDA receptor antagonists have been found to induce mild CPP (Harris et al., 2004), and are self-administered into the VTA (David et al., 1998). Finally, in the case of food reward, recent evidence suggests that reduced glutamate transmission has no effect. Instrumental responding for food is not affected either by NMDA antagonism (Kenny et al., 2009; Zellner et al., 2009) or stimulation of mGluRs (Liechti and Markou, 2007), nor does NMDA blockade reduce free feeding (Zellner et al., 2009). It appears, therefore, that there is a complex interaction between glutamate and other local neurotransmitters that are involved with reward processes, and further research is required to elucidate the role of VTA glutamate in primary reward.

8. Acetylcholine as a purported primary reward signal

In contrast, stronger evidence exists for an 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 conditioned stimuli.

The VTA contains cholinergic axons (Henderson and Sherriff, 1991) originating in the cholinergic nuclei in both the pedunculopontine tegmentum (PPTg) and laterodorsal tegmentum (LDTg) (Garzon et al., 1999; Henderson and Sherriff, 1991; Oakman et al., 1995). 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 synapses on GABA cells are predominantly symmetric (Omelchenko and Sesack, 2006). DA cells of the VTA possess mACh and nicotinic acetylcholine (nACh) receptors (Gronier and Rasmussen, 1998).

Acetylcholine, acting at both types of receptors, is a powerful modulator of VTA activity. Application of ACh or ACh agonists depolarizes VTA neurons in vitro (Calabresi et al., 1989), increases DA cell firing rate and causes burst firing (Gronier and Rasmussen, 1998; Seutin et al., 1990), and causes DA release in PFC and NAcc (Miller and Blaha, 2005; Schilstrom et al., 1998; Westerink et al., 1998). Systemic application of nicotine, an ACh agonist, causes

increased DA levels in the NAcc, which is blocked by an intra-VTA nACh receptor antagonist (Schilstrom et al., 1998).

Acetylcholine, with its ability to modulate VTA activity, appears specifically to play an important role in primary reward processes. Intra-VTA injection of carbachol, a mACh receptor agonist, produces CPP (Yeomans et al., 1985), as does nACh receptor agonism (Laviolette and van der Kooy, 2003; Museo and Wise, 1994). Moreover, carbachol (Ikemoto and Wise, 2002) and nicotine (Maskos et al., 2005; Ikemoto et al., 2006) are self-administered into the posterior (but not anterior) VTA. Lateral hypothalamic (LH) self-stimulation is associated with elevations in extracellular ACh in the VTA (Chen et al., 2006; Rada et al., 2000), the magnitude of which varies with the intensity of stimulation (Rada et al., 2000). Furthermore, mACh receptor 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 nACh receptors also increases thresholds for LH self-stimulation, but to a much smaller extent than mACh receptor blockade (Yeomans and Baptista, 1997). The activity of VTA ACh in relation to cocaine is similar to that of brain stimulation. Recently, You et al. (2007) demonstrated elevations above baseline in VTA ACh concentrations in rats self-administering cocaine. In this study, injections of a mACh receptor antagonist in the VTA resulted in increased rates of cocaine self-administration, indicating a reduction in the rewarding effect of the drug, and implicating VTA mACh receptor stimulation in the primary rewarding effect of cocaine.

Additional evidence also demonstrates a role for ACh transmission in food reward. Extracellular concentrations of ACh in the VTA 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 (Sharf and Ranaldi, 2006) and others (Rada et al., 2000). 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 (Schilstrom et al., 1998; Yeomans et al., 1993). 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 plasticity processes.

ACh may also play other roles in the VTA in reward-related learning or responding. For example, the PPTg and LDTg, which are key sources of ACh to the VTA, appear to control certain aspects of VTA activity which are involved with responding to rewards and reward-related cues; inactivation of PPTg reduces VTA activity in response to CSs (Pan and Hyland, 2005) while activation of the PPTg increases burst firing (Floresco et al., 2003); similarly, the LDTg regulates tonic activity and glutamate-induced burst firing (Lodge and Grace, 2006). However, it should be noted that while the PPTg and LDTg appear to play important modulatory roles in reward-related VTA activity, it is not clear what neurotransmitters are mediating these effects, as both nuclei project cholinergic and glutamatergic afferents (Clements and Grant, 1990; Lavoie and Parent, 1994). The evidence for ACh in mediating a primary reward signal, on the other hand, is substantial, as will be detailed below.

It should be noted that the PPTg-VTA pathway has been implicated in mediating effects of sensory stimuli and CSs. For instance, the PPTg-VTA pathway can convey information about sensory stimuli in general as well as conditioned stimuli (Pan and Hyland, 2005) and neurons in the PPTg show activity correlated either with fixation to the reward-predictor stimuli or with reward delivery (Kobayashi et al., 2002). Thus, the PPTg-VTA pathway contains information about both the US and the CS. However, it is still unclear what neurotransmitters mediate these signals, and whether they are necessary for behavioral responses to CSs.

In summary, the VTA receives a confluence of signals – ACh and glutamate – which could represent some of the US and CS signals required for reward-related learning. In what follows, we review the evidence that blocking one or the other signal in the VTA impairs the acquisition of reward-related learning, further suggesting that coincident stimulation provides for associative processes that allow CSs to come to activate VTA DA cells on their own.

9. Impairments in acquisition of reward-related learning

Here we review the experimental evidence that supports our model of reward-related associative processes in the VTA. First, we have tested the hypothesis that VTA ACh provides a US signal to the VTA by investigating its role in the acquisition of reward-related learning. In one study we found that injections of scopolamine, a mACh receptor antagonist, in the VTA prior to the initial sessions of an instrumental conditioning protocol (lever pressing for food) prevented the animals from acquiring the response, whereas injections made after acquisition of the lever press response had no effect on its performance (Sharf et al., 2006). Thus, blockade of mACh receptor stimulation in the VTA impaired the acquisition, but not the expression, of instrumental learning. This effect was specific to the mACh receptor as injections of mecamylamine, a nACh receptor antagonist, had no effect. In another study we found that intra-VTA injections of scopolamine made prior to the initial sessions where animals had to learn to eat a novel food in a novel environment prevented this learning but injections made after acquisition of this behavior had no effect (Sharf and Ranaldi, 2006). Again, VTA mACh receptor stimulation appeared to be necessary for the acquisition of this reward-related learning but not for its expression. That mACh receptor stimulation appears necessary during acquisition, but not after acquisition, of these reward-related behaviors would be expected if indeed this signal functions as an *unconditioned* stimulus, as a US signal is not needed to produce a *conditioned response* to a CS. Interestingly, recent evidence indicates that VTA ACh can also be involved in mediating the effects of CSs, in addition to USs. You et al. (2008) found that in animals trained to self-administer cocaine but tested in extinction (without cocaine reinforcement) VTA ACh levels rose significantly above baseline during the first hour but returned to baseline for the remainder of the extinction session, suggesting that VTA ACh may be associated with the effect of CSs. This is consistent with the findings cited earlier that the PPN and PPTg pathways are involved with some aspect of a sensory signal. However, this finding is not necessarily inconsistent with our hypothesis that VTA ACh conveys a US signal. Our model does not preclude that a conditioned VTA ACh signal is formed or even that such a signal can facilitate additional approach behavior or act as a secondary reward signal for additional learning and plasticity in the VTA. Our model does suggest, however, that after VTA plasticity has occurred the ACh signal is not necessary for expressing the conditioned approach behavior. However, this remains to be assessed empirically, as the You et al. study did not test whether the VTA mACh signal was necessary for CS-initiated and -maintained conditioned responding. Of course, to the extent that ACh provides a US signal, if it is blocked or absent over a certain period of time, reward-related behavior should extinguish.

Second, we have also tested the component of our model which asserts that coincident NMDA receptor stimulation is necessary for the synaptic strengthening allowing environmental stimulus-related signals to recruit DA cells. Injections of AP-5 into the VTA prior to food-reinforced instrumental conditioning blocked the acquisition of lever pressing, although treatment with AP-5 once lever pressing was acquired had no effect (Zellner et al., 2009). In a separate study, rats treated with intra-VTA AP-5 did not acquire

conditioned approach to a food-related stimulus (Zellner, 2008; manuscript in preparation). These impairments cannot be accounted for by disruptions in basic food motivation or motoric behavior, as AP-5 treated animals did not show a reduction in food consumption or locomotor activity in control experiments. Other studies have indicated that LTP in terminal regions of the mesolimbic DA system is also involved with the acquisition of reward-related behavior, as antagonism of NMDA receptors in amygdala (CeN and BLA) (Burns et al., 1994) and the NAcc core (Di Ciano et al., 2001) impairs the acquisition of conditioned approach for food reward, and in NAcc shell and core and mPFC blocks acquisition of instrumental responding for food reward (Baldwin et al., 2002; Kelley et al., 1997).

Our findings are in line with earlier findings that simultaneous blockade of AMPA and NMDA receptors in the VTA 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). Moreover, these findings are consistent with another recent study which also showed impairment of conditioned approach with intra-VTA injection of AP-5, using a very similar protocol (Stuber et al., 2008). Taken together, these findings provide strong support for the idea that NMDA receptor stimulation in the VTA is necessary for the acquisition by reward-related stimuli of the ability to function as CSs.

As indicated earlier, numerous reports demonstrate that the presentation of CSs is associated with activation of VTA DA cells or DA release from terminals, suggesting that CSs may themselves activate VTA DA cells. And there is considerable evidence that blockade of DA neurotransmission, specifically in mesocorticolimbic terminal regions, impairs the ability of stimuli to function as CSs or conditioned rewards, suggesting a necessary role for DA in the performance of learned reward-related behavior. Altogether, these developments suggest that CSs function as such *because* they activate DA cells. Although this seems plausible, and likely, it must be emphasized that such a conclusion is premature. A causal link between a CS presentation and activation of VTA DA cells, and between CS-activation of DA cells and CS-controlled approach behavior, has not been established. This link would be demonstrated when a single manipulation eliminates the capacities of a CS to both (1) activate VTA DA cells and (2) function as a CS behaviorally. We suggest here, based on the evidence described above, that associative processes in the VTA, namely concurrent stimulation of NMDA and mACh receptors, are necessary for the acquisition by reward-related CSs of the capacities to activate VTA DA cells and to thereby elicit approach responses (i.e.: function as CSs). We are currently testing this hypothesis.

Given the significance of the ability of conditioned stimuli to direct and influence behavior, both adaptive and pathological, it has been the subject of considerable interest in behavioral neuroscience, as the present review has indicated. As the activation of VTA DA cells correlated with CS presentation has been observed for some time now, in a number of paradigms and across a number of species, we are gratified that it appears we are now in the process of understanding the particular mechanisms by which those cells "come to know" when to activate in response to reward-related stimuli. We hope that as work in this area progresses, we will soon be able to contribute to addressing the pathologies of reward-related behavior that involve abnormal strengthening of reward-related cues, as well as contribute to the basic knowledge about this important psychobiological adaptive mechanism.

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