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Dopamine and reward seeking: the role of ventral tegmental area

Abstract: Reward seeking is controlled by conditioned stimuli (CSs). There is a positive relation between mesocorticolimbic dopamine (DA) and the performance of learned reward-directed behavior. The mechanisms by which reward-, including drug-, associated stimuli come to acquire the capacity to activate the DA systems are not fully understood. In this review, we discuss the possible neurochemical mechanisms within the ventral tegmental area that may be involved in how CSs acquire the capacity to activate ventral tegmental area (VTA) DA neurons based on principles of long-term potentiation in the VTA and the role of mesocorticolimbic DA in reward-related learning. We propose that CSs function as such because they acquire the capacity to activate VTA DA neurons. Furthermore, CSs come to acquire this control of VTA DA cells when there is coincident *N*-methyl-D-aspartate receptor stimulation on VTA DA cells and strong depolarization of VTA DA cells, possibly by muscarinic acetylcholine receptor stimulation on these cells. This coincident activity leads to the strengthening of CS-associated glutamatergic synapses and the control by CSs of mesocorticolimbic DA systems and reward-directed behavior.

Keywords: acetylcholine; dopamine; glutamate; learning; motivation; neural plasticity; reward.

DOI 10.1515/revneuro-2014-0019
Received March 3, 2014; accepted April 21, 2014; previously published online May 28, 2014

Introduction

Reward seeking, including drug seeking, is controlled by reward-related conditioned stimuli (CSs). Therefore, if we understand the neural mechanisms through which reward-related CSs acquire control over motivational (i.e., approach) systems then we (1) gain a significantly better understanding of the behavioral significance of these stimuli and (2) identify the neural systems that can be manipulated to eliminate the control of CSs over compulsive drug-, or other maladaptive reward-, seeking using neuropharmacological and/or behavioral strategies.

There is evidence of (1) a positive relation between mesocorticolimbic DA and the performance of learned reward-related behavior (Richardson and Gratton, 1996; Ranaldi et al., 1999; Wightman and Robinson, 2002) and (2) just recently, from our laboratory and others, of a necessary role for ventral tegmental area (VTA) acetylcholine (ACh) (Sharf and Ranaldi, 2006; Sharf et al., 2006) and *N*-methyl-D-aspartate (NMDA) (Stuber et al., 2008; Zellner et al., 2009) receptor stimulation in the acquisition of reward-related learning. However, the crucial studies that link conditioned DA activity to conditioned behavior (reward-related learning) and each of these to the neural mechanisms (e.g., VTA ACh and NMDA receptor stimulation) necessary for both were, until recently, missing. This article will review evidence supporting our model of the neurobiology of reward-related learning based on VTA neural plasticity.

Reward-related stimuli become CSs because they gain access to the same motivational neural circuits that are activated by primary rewards, producing conditioned activation of these neural circuits and eliciting motivational states (i.e., approach) similar to those elicited by primary rewards (Bindra, 1974; Beninger and Ranaldi, 1994; Wise, 2004). Although this idea has existed for at least 40 years (Bindra, 1974), a causal link between CS activation of primary reward circuits and reward-related learning has not been demonstrated. Furthermore, although a good number of studies have been conducted investigating neural mechanisms in terminal regions of the mesocorticolimbic DA system in relation to reward-related learning, only a handful of studies, recently conducted (Harris et al., 2004; Sharf and Ranaldi, 2006; Sharf et al., 2006; You et al., 2007), have looked at mechanisms in the VTA, the site of origin of this system, and these studies suggest a necessary role for VTA associative processes. In this article, we outline our VTA model of reward-related learning. This model is characterized as follows: (1) VTA DA neurons mediate unconditioned approach; (2) reward-related learning in the form of conditioned approach is dependent on a CS acquiring the ability to activate VTA dopamine (DA)

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neurons; (3) the acquisition of both conditioned approach and the ability of the CS to produce conditioned activation of VTA DA neurons is dependent on NMDA receptor stimulation and stimulation of another neurotransmitter receptor system mediating the effects of the primary reward [likely muscarinic acetylcholine (mACh) receptor stimulation in the case of food reward] in the VTA, (4) once acquired, the capacity of CSs to activate VTA DA neurons and cause conditioned approach no longer depends on NMDA receptor stimulation but depends on other VTA mechanisms, possibly α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor stimulation of DA cells.

Since the mid-1970s, a number of theories on the role of DA in reward have been put forth. These include a role in the hedonic effects of reward (Wise et al., 1978), reinforcement (Beninger, 1983; White, 1989; White and Milner, 1992; Di Chiara, 1999; Kelley, 1999), incentive-motivation (Wise and Bozarth, 1987; White, 1989; White and Milner, 1992; Robbins and Everitt, 1996; Zellner and Ranaldi, 2010), sensorimotor activation (Salamone, 1991; Salamone and Correa, 2002), reward prediction error (Schultz, 1997), reward learning (Horvitz et al., 2007), and incentive salience attribution (Berridge, 1995; Berridge and Robinson, 1998). Although the theories differ on the details of DA's role in reward, many (but not all) stipulate that DA is involved in what commonly is referred to as the approach-eliciting (or incentive motivational) effects of reward stimuli (Bozarth and Wise, 1986; Berridge and Robinson, 1998; Ikemoto and Panksepp, 1999; Wise, 2004; Zellner and Ranaldi, 2010). Our model is predicated on this latter view of DA's role in reward-related behavior.

Primary reward (approach and reinforcement) is mediated by VTA DA neuronal activity

Consumption of primary rewards is associated with enhanced DA neurotransmission in terminal regions of mesocorticolimbic DA systems (Hernandez and Hoebel, 1988; Radhakishun et al., 1988; Blackburn and Phillips, 1989). Blockade of DA neurotransmission in limbic, cortical, striatal, and midbrain regions reduces or eliminates the reinforcing effects of primary rewards such as food, brain stimulation, and drugs of abuse (Wise, 2004). A role for DA in the incentive-motivational (approach) effects of primary reward has also been demonstrated (Wise, 2004). In animals trained to self-administer psychostimulants and who have had the self-administration response extinguished, activation of mesolimbic DA reinstates the self-administration response (Stewart, 1984; Ranaldi et al., 1999), whereas blockade of DA neurotransmission attenuates response-reinstatement associated with enhanced DA neurotransmission (Khroyan et al., 2000; Ciccocioppo et al., 2001; Alleweireldt et al., 2002).

Conditioned stimuli cause and require DA release to function as such

Activation of VTA DA cells results in DA release in terminal regions. Food and drug reward-associated CSs cause DA release (Johnson et al., 1992; Phillips et al., 1993; Wilson et al., 1995; Bassareo and Di Chiara, 1999; Bassareo et al., 2007) in the nucleus accumbens (NAcc). NAcc depletions of DA impair conditioned approach (Parkinson et al., 1999), and NAcc lesions reduce cocaine self-administration maintained by periodic presentations of a CS (Ito et al., 2004). Intra-NAcc injections of DA D1 receptor antagonists (Wakabayashi et al., 2004; Yun et al., 2004a,b; Nicola et al., 2005), D2 receptor antagonists (Yun et al., 2004b), or mixed D1/D2 antagonists (Di Ciano et al., 2001) reduce responding maintained by a CS. Other DA terminal regions are also involved in CS processes (see, for example, Baxter and Murray, 2002, on reward processes and the amygdala). An important question that has remained largely unanswered is by what mechanisms do CSs cause DA release. We propose that these mechanisms include, at least in part, the activation of VTA DA neurons at the level of the VTA. We describe more fully how this mechanism may operate in the following.

Conditioned stimuli activate VTA DA neurons during acquisition of learning

The VTA and its terminal regions show progressive changes in activity as neutral stimuli are paired with rewards, indicating that associative processes are taking place. Midbrain DA neurons respond to novel events, in addition to rewards and CSs (see Horvitz, 2000, for a review). Various cells in the VTA increase their firing to auditory stimuli, tail pressing and pricks, and spontaneous movement (Miller et al., 1981; Kiyatkin and Rebec, 1998). Midbrain DA neurons receive visual information

through afferents from the superior colliculus (Comoli et al., 2003) and respond to the presentation of a light flash (Dommett et al., 2005). In awake primates, DA neurons habituate to non-reinforced stimuli to which they initially respond (Ljungberg et al., 1992; Schultz, 1998). In awake rats, a transient increase in NAcc DA to a novel stimulus disappears on subsequent sessions when not paired with a reward (Kiyatkin and Stein, 1996). Generally, midbrain DA neurons fire in response to reward receipt until animals are well trained at which time responding of DA cells comes primarily under the control of CSs (Ljungberg et al., 1992; Schultz et al., 1993; Pan et al., 2005; see Zellner and Ranaldi, 2010, for a review). It is precisely the neural mechanisms whereby CSs acquire control of this DA pathway that are not well understood and for which our model puts forth some possibilities.

We posit that at least some aspects of CS-unconditioned stimulus (US) associative learning occur in the VTA. In this model, the US consists of stimulation of mACh receptors on VTA DA cells and the CS consists of Glu stimulation of Glu receptors on VTA DA cells. Learning occurs through coincident stimulation of mACh (representing the US) and NMDA (representing the CS) receptors on VTA DA neurons, initiating intracellular NMDA-dependent calcium calmodulin protein kinase II (CaMKII) and protein kinase C (PKC) cascades that lead to long-term changes (e.g., long-term potentiation, or LTP) followed by neural plasticity in the VTA (described below) that leads to an increase in the strength of the Glu CS signal. This model will be presented in detail below, but first, we will review the attributes of the VTA that make it an appropriate candidate for these associative processes.

Acetylcholine excitatory afferents to the mesocorticolimbic DA system

The VTA receives cholinergic afferents from pedunculopontine (PPTg) and laterodorsal (LDTg) tegmental nuclei (Henderson and Sherriff, 1991; Oakman et al., 1995; Garzon et al., 1999). DA cells of the VTA possess mACh and nicotinic (nACh) receptors (Gronier and Rasmussen, 1998). Application of ACh or its agonists depolarizes VTA neurons *in vitro* (Lacey et al., 1990) and causes burst firing (Seutin et al., 1990; Gronier and Rasmussen, 1998) and DA release in prefrontal cortex (PFC) and NAcc (Westerink et al., 1998; Miller and Blaha, 2005).

VTA ACh is implicated in reward-related behavior. VTA ACh concentrations increase during eating, drinking, and lateral hypothalamic self-stimulation (Rada et al., 2000). Stimulation of mACh receptors in the VTA enhances brain stimulation reward, whereas mACh receptor antagonism reduces it (Yeomans et al., 1985, 1993; Kofman and Yeomans, 1988). Furthermore, mACh receptor antagonists in the VTA reduce eating and approach (Ikemoto and Panksepp, 1996; Rada et al., 2000). These findings suggest that VTA mACh receptor stimulation is involved in mediating the unconditional incentive motivational effects of rewards, including food. We have shown that intra-VTA microinjections of a mACh receptor antagonist prevents the acquisition of food-reinforced instrumental conditioning (Sharf et al., 2006), suggesting a necessary role for VTA mACh receptor stimulation in reward-related learning. Our studies demonstrate that VTA nACh receptors are not involved in acquisition of food-related operant learning or in food reward itself (Sharf and Ranaldi, 2006).

Glutamate excitatory afferents to the mesocorticolimbic DA system

The DA neurons of the VTA receive glutamate (Glu) afferents from the mPFC (Sesack and Pickel, 1992; Smith et al., 1996), various amygdala nuclei and the bed nucleus of stria terminalis (Hopkins and Holstege, 1978; Phillipson, 1979), the PPTg (Charara et al., 1996), and periaqueductal gray area (Omelchenko and Sesack, 2010). Glu acts on NMDA, AMPA, and metabotropic Glu (mGlu) receptors on DA cells (Albin et al., 1992) to excite these cells (Overton and Clark, 1992; Zhang et al., 1994; Christoffersen and Meltzer, 1995). The NMDA receptor conducts inward Ca^{2+} currents that activate CaMKII and PKC, protein kinases linked to gene transcription, and the proliferation and phosphorylation of AMPA receptors, resulting in LTP of the postsynaptic response to Glu. Thus, the NMDA receptor is linked to intracellular signaling cascades that are implicated in the development of LTP of Glu synapses.

VTA Glu is implicated in associative learning. Cocaine-conditioned locomotion fails to develop under VTA NMDA receptor antagonist treatment (Pert, 1998). Furthermore, simultaneous antagonism of VTA AMPA and NMDA receptors blocks the acquisition of cocaine-conditioned place preference (Harris and Aston-Jones, 2003), and NMDA receptor antagonism blocks the acquisition of morphine conditioned place preference (Harris et al., 2004). Context-induced cocaine seeking is correlated with VTA Glu release and reduced by VTA Glu receptor blockade (You et al., 2007). Moreover, inhibition of Glu release in the VTA during training of heroin self-administration later reduces context-induced reinstatement (Bossert et al.,

2004). We have shown that intra-VTA microinjections of AP-5, an NMDA receptor antagonist, prevents the acquisition of food-reinforced instrumental responding (Zellner et al., 2009), and we (Ranaldi et al., 2011) and another group (Stuber et al., 2008) have recently demonstrated that this treatment can inhibit the acquisition of a conditioned approach response. Thus, the VTA appears to be an important site for the synaptic modifications underlying the ability of environmental cues to become associated with food and drug reward.

The PPTg-VTA pathway is implicated in mediating effects of sensory stimuli and CSs. For instance, the PPTg-VTA pathway can convey information about sensory stimuli, CSs (Pan and Hyland, 2005) and food reward delivery (Kobayashi et al., 2002). However, it is still unclear what neurotransmitters (Glu or ACh) mediate these signals and whether they are necessary for the behavioral responses to CSs. Our previous studies have ruled out a role for cholinergic neurotransmission in the VTA in mediating CS information associated with food, as blockade of ACh neurotransmission here failed to affect responding maintained by a food-associated CS (Sharf and Ranaldi, 2006). However, injections of mACh receptor antagonists in the VTA attenuated the expression of a cocaine-conditioned place preference (Shinohara et al., 2014), indicating a possible role for VTA ACh in drug-associated CS effects on behavior. The VTA also receives norepinephrine (NE) and serotonin (5-HT) afferents from the locus ceruleus and raphe, respectively. Neurochemical studies demonstrate that VTA NE does not change and VTA 5-HT decreases (Fallon et al., 2007) during eating, indicating no role for NE and possibly a role for 5-HT in mediating a US signal. In addition, intra-VTA injections of a 5-HT2c agonist decreased food-reinforced responding on a progressive ratio schedule of reinforcement (Fletcher et al., 2004), again indicating a role for VTA 5-HT in food reward. A role for NE or 5-HT in mediating the effects of CSs has not been investigated. Given that VTA 5-HT inhibits DA cell activity (Kalivas, 1993), the nature of any possible role for VTA 5-HT in mediating the CS input would be to disinhibit DA cells.

LTP in the VTA

VTA DA cells demonstrate LTP that appears to be NMDA receptor dependent (Bonci and Malenka, 1999; Stuber et al., 2008). Generally, the induction of LTP appears to depend on NMDA receptor stimulation, but its expression does not (Muller et al., 1988), a distinction that suggests NMDA receptor-dependent acquisition, but not performance, of learning.

Our model of the neurobiology of reward-related learning

This model is predicated on the assumption that conditioned approach occurs when the CS acquires the ability to activate the same neural system that produces rewarding effects of the primary reward or US. For clarity and simplicity, we will illustrate the model based on conditioned approach learning. The model is a Pavlovian conditioning model (see Figure 1) consisting of the four components of classical conditioning: unconditioned stimulus (US), unconditioned response (UR), conditioned stimulus (CS), and the conditioned response (CR). The neural counterparts to these four components are the following: the US is the stimulation of mACh receptors on VTA DA cells; the UR is the activation of VTA DA cells (with DA release downstream); the CS is the stimulation of NMDA receptors on VTA DA cells; the CR is CS-produced VTA DA cell activation. The US-associated stimulus becomes a CS through concurrent stimulation of NMDA and mACh receptors on VTA DA cells. With concurrent stimulation, the mACh receptor stimulation strongly depolarizes the DA cells, dislodging the Mg^{2+} ion from the NMDA receptor channel, allowing this channel to conduct Ca^{2+} ions into DA cells. Intracellular Ca^{2+} initiates the CaMKII and PKC intracellular cascades, resulting in several long-term changes that can strengthen the CS signal; these neural changes can include proliferation and phosphorylation of AMPA receptors (Nicoll, 2003; Kessels and Malinow, 2009), growth of new synapses (Carlisle and Kennedy, 2005), increased presynaptic Glu release (Lisman and Raghavachari, 2006), and other gene transcription-related processes.

In this model then, the acquisition of conditioned approach is dependent on both NMDA and mACh receptor stimulation because blockade of either will prevent one or both of the necessary steps (NMDA conductance of Ca^{2+} and initiation of CaMKII and/or PKC) that initiates the neural plasticity. Once plasticity has occurred, the previously weak DA response to the CS-related Glu signal is now strong enough to activate DA cells to a level that produces approach. In this case, NMDA receptor stimulation is no longer necessary because the expression of this learning is now maintained by whatever neuronal changes have resulted in the increased CS signal, changes that could include proliferation and phosphorylation of AMPA receptors, growth of new synapses, increased

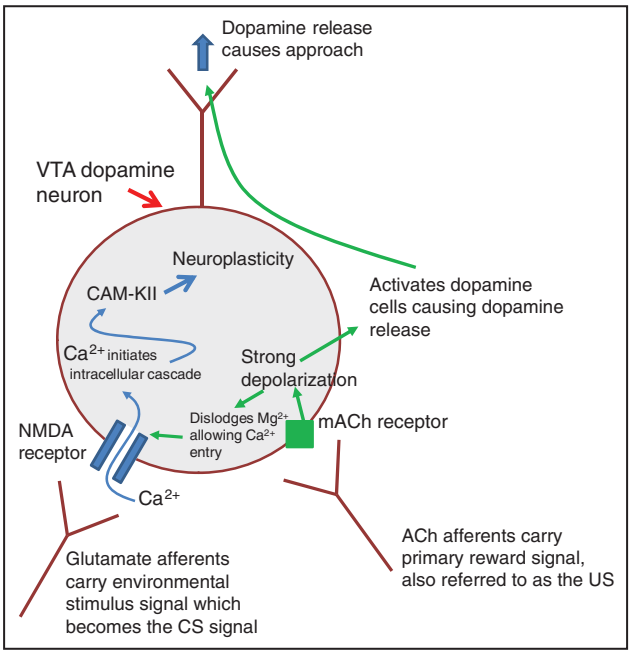

Figure 1 The proposed Hebbian-type model of neural plasticity in the VTA underlying reward-related learning.
Shown are the components proposed to be required for the acquisition by a previously neutral stimulus of the capacity to cause conditioned activation of the mesocorticolimbic DA system and to function as a conditioned stimulus. US, mACh receptor stimulation and activation of VTA DA neurons; UR, dopamine release in terminal regions causing approach, reward-directed behavior; CS, stimulation of NMDA receptors on VTA DA cells; CR, conditioned stimulus-induced activation of VTA DA cells causing dopamine release in terminal regions causing approach, reward-directed behavior. When there is coincident NMDA and mACh stimulation on VTA DA cells, the CA^{2+}-initiated intracellular cascades are activated resulting in one or more of several long-term changes in neural activity including proliferation and phosphorylation of AMPA receptors, growth of new synapses, increased presynaptic Glu release, and other gene transcription-related processes. It is proposed that these neural changes underlie the strengthening of CS-related synaptic activity on VTA dopamine cells resulting in the acquisition by the reward-associated stimulus of the capacity to activate VTA DA neurons and elicit conditioned approach.

presynaptic Glu release, and other gene transcription-related processes. The model stipulates that the enhanced CS signal is glutamatergic in nature. Our previous work has already ruled out the possibility that the CS signal is NMDA receptor stimulation alone (Zellner et al., 2009) and suggests that, at least in the case of conditioned approach, it may be combined NMDA and AMPA receptor stimulation (discussed below). (Note: This model is not aimed at explaining the acquisition of stimulus-response, stimulus-stimulus, stimulus-reward, or response-outcome associations that may involve neurochemical events and neural connections in forebrain regions such as the NAcc, caudate-putamen, amygdala, and mPFC.)

Predictions from the model and evidence supporting it

This model leads to the following predictions: (1) food (a US) should activate VTA DA cells, (2) a food-associated CS should activate VTA DA cells, (3) VTA DA cell activity is necessary for the expression of conditioned approach, (4) blockade of VTA NMDA receptor stimulation or mACh receptor stimulation should prevent the acquisition by a food-associated stimulus of the ability to activate VTA DA cells and to function behaviorally as a CS, (5) blockade of VTA NMDA receptor stimulation after acquisition of the conditioned approach response should not prevent the ability of a food-associated stimulus (CS) to activate VTA DA cells or to function behaviorally as a CS, (6) blockade of VTA AMPA receptors should prevent the performance of a learned conditioned approach response. Now we will review the evidence supporting these predictions.

We tested the effects of exposure to a US – eating food pellets – on the activity of DA cells in the VTA by comparing c-Fos activation in tyrosine hydroxylase (TH)-labeled cells (i.e., DA cells) in the VTA between rats eating food and rats not eating food. We found that in the group that ate the food pellets (exposed to the US), there was a significantly greater number of VTA TH-labeled cells expressing c-Fos than in the group that did not eat food (no US) (Kest et al., 2012). Thus, exposure to the US activates DA cells in the VTA, as the model predicts.

In the same study, we tested whether or not a CS can acquire the capacity to activate VTA DA cells. Here, animals were trained to associate light stimulus presentations with delivery of food pellets into a food trough. In one group, each food pellet was explicitly paired with each light presentation, and in another group, food pellets and light presentations were not explicitly paired. On a test day, all animals were exposed to only presentations of the light stimulus, with no food, and food trough head entries were measured for 6 s before each CS presentation (pre-CS head entries) and 6 s during CS presentations (CS head entries). The ratio of CS/pre-CS entries indicates the level of conditioned approach responding: the higher the ratio, the greater the conditioned approach response – that is, the more effectively the light stimulus is functioning as a CS. We found that in the explicitly paired animals CS/pre-CS ratios were significantly higher than in the not explicitly paired group (Kest et al., 2012). This demonstrates that the light stimulus came to function as an effective CS in the explicitly paired group. Furthermore, after conditioning, CS presentations were associated with a significantly greater number of c-Fos proteins expressed in TH-labeled

cells in the VTA in the explicitly paired group than in the not explicitly paired group. Thus, these findings suggest that the acquisition by a stimulus of the capacity to function as a CS is associated with the acquisition by that same stimulus of the capacity to activate DA cells.

In another study using the same conditioned approach paradigm described above, we tested whether or not NMDA receptor activation in the VTA is necessary for this type of reward-related learning to occur, as our model predicts it should be. We found that animals treated with microinjections of AP-5, a selective NMDA receptor antagonist, into the VTA before each conditioning session, showed a dose-related and significant reduction in the CS/pre-CS ratio during the CS-only test compared with animals treated with vehicle. In the group treated with the highest dose of the NMDA receptor antagonist, the CS/pre-CS ratios were indistinguishable from those in a group that received food and light presentations not explicitly paired during conditioning. Furthermore, in animals treated with intra-VTA AP-5 that blocked the acquisition of conditioned approach, we observed significantly less c-Fos activation in the terminal regions of the mesocorticolimbic DA system, suggesting a reduction in DA release (although this is an indirect indicator of such and remains speculative until direct tests can confirm it) (Ranaldi et al., 2011). Others also have found that NMDA receptor antagonism in the VTA prevents conditioned approach learning (Stuber et al., 2008) or acquisition of drug-conditioned place preference (Harris and Aston-Jones, 2003; Harris et al., 2004).

Our model also predicts that VTA NMDA receptor stimulation should not be necessary for the performance of an already-learned reward-related behavior. In the same study as described above, we tested the effects of intra-VTA AP-5 administered before the CS-only test session – after the animals learned the conditioned approach response. We found that the NMDA receptor antagonist had no effect on the learned conditioned approach response. Thus, as would be expected from LTP research, NMDA receptor stimulation is necessary for the learning of a rewarded behavior but not its performance. Others have reported similar findings (Stuber et al., 2008).

The findings described above extend to operant conditioning. We have observed similar effects when studying the acquisition of food-reinforced lever pressing. Intra-VTA microinjections of AP-5 during the initial food-reinforced lever-press conditioning sessions prevented the learning of this response, without effects on food reward *per se* (Zellner et al., 2009). Thus, although animals treated with intra-VTA NMDA receptor antagonists failed to acquire the lever-press response in comparison to control animals, they did, however, eat all the food they earned. Furthermore, in a separate experiment where animals could eat the same type of food pellets freely – without learning as a condition – intra-VTA NMDA receptor antagonist-treated animals ate the same amount of food as did vehicle-treated animals. Thus, VTA NMDA receptor blockade prevented learning but not eating or the experience of food reward. When intra-VTA AP-5 microinjections were made after the establishment of stable food-reinforced lever pressing (i.e., immediately before the tenth lever-pressing session), they had no effect on lever-pressing rates compared with vehicle controls. Thus, blockade of NMDA receptors in the VTA appears to prevent the learning of a reinforced lever-press response but has no effect on that response if it is already learned.

The model predicts that blockade of mACh receptors within the VTA should prevent the learning of reward-related behavior. Our research group was the first to demonstrate a role for VTA acetylcholine in reward-related learning. In a food-reinforced lever-pressing experiment, we observed that intra-VTA microinjections of scopolamine, a selective mACh receptor antagonist, prevented the acquisition of the lever-press response; when the injections were made after the acquisition of food-reinforced lever pressing (before the tenth session), they had no effect (Sharf et al., 2006). In an experiment where the task consisted of learning to eat food in a new environment, animals treated with scopolamine in the VTA failed to learn this, but when a similar intra-VTA scopolamine treatment was given after the acquisition of this behavior, it had no effect (Sharf and Ranaldi, 2006). Thus, antagonism of mACh receptors in the VTA inhibits the acquisition of reward-related behavior. This is consistent with the model's predictions.

In later studies, it was shown that intra-VTA injections of scopolamine before cocaine-conditioning sessions attenuated the acquisition of a cocaine-conditioned place preference (Shinohara et al., 2014). These authors also inactivated the cholinergic neurons of the LDTg, one of two sources of acetylcholine in the VTA, and observed the inhibition of cocaine-conditioned place preference (Shinohara et al., 2014). Interestingly, when the PPTg, the other source of acetylcholine in the VTA, was lesioned, it failed to affect the acquisition of cocaine-conditioned place preference (Steidl et al., 2014). In drug self-administration studies, it was reported that acetylcholine levels in the VTA increased in response to cocaine self-administration. Furthermore, injections of muscarinic antagonists in the VTA attenuated cocaine reward in trained cocaine self-administering animals (You et al., 2008). Both of these effects might be expected if the primary reward (i.e.,

cocaine) requires acetylcholine neurotransmission as a 'US' signal in the VTA, as predicted by our model. Thus, it appears that in most circumstances, acetylcholine neurotransmission at muscarinic receptors in the VTA is necessary for the mediation of the US effects of reward as well as in the acquisition of reward-related learning.

This model provides a mechanism for how CSs function as such and how they activate VTA DA cells. The model stipulates that Glu receptor stimulation in the VTA mediates these effects of CSs. If this is so, then the blockade of Glu receptors in the VTA should reduce the capacity of reward-associated stimuli to activate VTA DA cells. As indicated above, we found that NMDA receptor antagonism alone fails to affect conditioned approach or food-reinforced lever pressing. Currently, we are observing that conditioned approach can be significantly reduced with intra-VTA microinjections of a combination of an NMDA receptor antagonist (AP-5) and an AMPA receptor antagonist (NBQX) at doses of each that produce no effect when administered individually (unpublished data). Others have found that simultaneous blockade of NMDA and AMPA receptors in the VTA could block the expression of morphine (Harris et al., 2004)-conditioned place preference or context-induced cocaine seeking and DA release in NAcc (You et al., 2007).

Concluding remarks

Understanding the neural mechanisms underlying acquisition and maintenance of reward-related learning is crucial for achieving a full understanding of the etiology and perhaps development of effective treatment strategies for pathological behaviors such as drug addiction. Although much remains to be elucidated in this respect, we do know that reward-associated stimuli come to acquire the capacity to control reward-related behavior and also acquire the capacity to cause conditioned activation of the mesocorticolimbic DA system, the neurotransmitter system best implicated in reward. Thus, it seems that the capacity of CSs to control behavior may depend on its 'learned' capacity to activate VTA DA cells. The essential question is how does the mesocorticolimbic DA system come under the control of CSs? Put another way, how do the DA cells of the VTA 'come to know' when to activate in response to reward-related stimuli? We have proposed a Hebbian-type model stipulating that the ability of CSs to activate VTA DA cells is acquired when there is coincident activation of NMDA receptors (by reward-associated stimuli) and mACh receptors (by unconditioned rewarding stimuli) on these DA cells, resulting in the strengthening of glutamatergic synapses. We have also reviewed evidence that supports this model. However, whether or not this model is ultimately correct is secondary to the goal of delineating the precise neural mechanisms by which reward-associated stimuli come to control reward-directed behavior. In this respect, we hope this review clarifies some of what is known about this, and stimulates more research on this important topic.

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