Ventral Tegmental Area Neurons in Learned Appetitive Behavior and Positive Reinforcement

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Annu. Rev. Neurosci. 2007. 30:289–316

First published online as a Review in Advance on March 15, 2007

The *Annual Review of Neuroscience* is online at neuro.annualreviews.org

This article's doi: 10.1146/annurev.neuro.30.051606.094341

0147-006X/07/0721-0289$20.00

Key Words

opioids, dopamine, reward, nucleus accumbens, prefrontal cortex

Abstract

Ventral tegmental area (VTA) neuron firing precedes behaviors elicited by reward-predictive sensory cues and scales with the magnitude and unpredictability of received rewards. These patterns are consistent with roles in the performance of learned appetitive behaviors and in positive reinforcement, respectively. The VTA includes subpopulations of neurons with different afferent connections, neurotransmitter content, and projection targets. Because the VTA and substantia nigra pars compacta are the sole sources of striatal and limbic forebrain dopamine, measurements of dopamine release and manipulations of dopamine function have provided critical evidence supporting a VTA contribution to these functions. However, the VTA also sends GABAergic and glutamatergic projections to the nucleus accumbens and prefrontal cortex. Furthermore, VTA-mediated but dopamine-independent positive reinforcement has been demonstrated. Consequently, identifying the neurotransmitter content and projection target of VTA neurons recorded in vivo will be critical for determining their contribution to learned appetitive behaviors.

Contents

INTRODUCTION

Neurons in the midbrain ventral tegmental area (VTA) contribute to the acquisition and expression of learned appetitive behaviors and to the addictive power of drugs such as opioids, psychostimulants, nicotine, and alcohol (Everitt & Robbins 2005; Kalivas & Volkow 2005; Schultz 1998, 2002; Wise 2002, 2005). Despite a large and methodologically diverse effort, detailed knowledge of how VTA neurons contribute to these behaviors is hampered by a lack of general agreement on a conceptual framework for relating findings across disciplinary lines. One consequence of this lack of consensus is the limited number of experiments explicitly designed to determine how the actions of VTA neurons at specific target sites contribute to learning and performance of appetitive behaviors. This problem is often compounded by the difficulty of applying constructs used in the behavioral literature to the interpretation of electrophysiological data. This is particularly the case for the construct of reward. In this review we follow Ikemoto & Panksepp (1999) and use the term reward in an operational sense, as an unconditioned stimulus that elicits appetitive approach behavior (e.g., food, water).

The probability of performing a specific appetitive behavior is influenced by internal and external factors. Environmental factors such as olfactory and visual cues can initiate and guide these behaviors. If successful, the receipt of a reward increases the future probability of the behaviors when the conditions are similar to those occurring just prior to and during reward receipt (see Sugrue et al. 2005 for an in-depth review). The process by which reward receipt enhances the future probability of successful actions is called positive reinforcement. VTA neurons contribute to both positive reinforcement and to the selection, initiation, and invigoration of learned appetitive behaviors. This review focuses on how VTA neurons participate in these distinct but interrelated functions. Our emphasis is on circuit analysis, which requires determining how neurons at each relay contribute to circuit operation and how the behaviorally relevant synaptic connections change with learning.

Among VTA neurons, the dopaminergic subgroup has drawn the greatest experimental effort. Many studies have used behavioral pharmacology to determine the contribution of dopamine. In interpreting these behavioral experiments some researchers have tended to make the simplifying assumption that midbrain dopaminergic neurons operate as a single functional unit. Often, different proposals for the functions of dopaminergic neurons are set up as competing, as if their participation in one function would preclude their participation in a different function. In fact, experimental data do not support the idea that midbrain dopamine neurons have a single general function. To the contrary, VTA neurons can be divided into distinct subpopulations that participate in different circuits mediating different behaviors. As with any group of neurons defined by their location and neurotransmitter content, their function is determined by the circuit of which they are a part and the behaviors that depend on the operation of that circuit.

THE ANATOMY OF THE VTA: MULTIPLE PARALLEL CIRCUITS

MFB: medial forebrain bundle

LH: lateral hypothalamus

The VTA lies medial to the substantia nigra and ventral to the red nucleus in the midbrain. It is not well-defined cytoarchitectonically, and its boundaries are determined largely by those of adjacent structures. Although the VTA is positioned strategically between the caudal hypothalamus and brainstem reticular formation, its physiological function was largely ignored until the discovery that it includes dopaminergic neurons (the A10 group; Dahlstroem & Fuxe 1964) and that these neurons project widely to limbic areas implicated in motivation and positive reinforcement (Fallon & Moore 1978, Swanson 1982, Ungerstedt 1971a) (**Figure 1**). A decade earlier, Olds & Milner (1954) showed that effective sites for intracranial electrical self-stimulation included what was later discovered to be the major rostral efferent pathway of the mesocorticolimbic dopamine system, the medial forebrain bundle (MFB) (Olds &

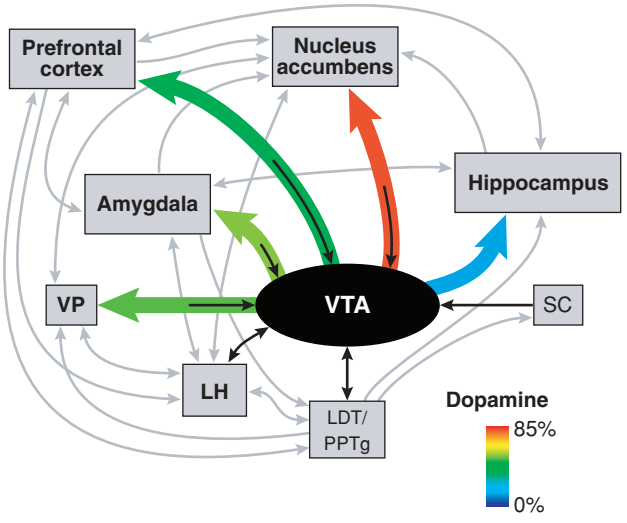

Figure 1

Major efferent and afferent projections of the VTA. Direct connections to and from the VTA are shown in black or color. Other connections are shown in gray. If known, the approximate percentage of projecting neurons that are cytochemically identified as dopaminergic is indicated by the color scale shown in the lower right. Abbreviations: LDT, laterodorsal tegmental nucleus; PPTg, pedunculopontine tegmental nucleus; LH, lateral hypothalamus; VP, ventral pallidum; SC, superior colliculus.

SNc: substantia nigra pars compacta

PFC: prefrontal cortex

NAc: nucleus accumbens

MSN: medium spiny neuron

KOP: κ opioid

Milner 1954; Olds & Olds 1963, 1969). Together, these findings attracted broad interest, and many investigators were quick to attribute to dopamine neurons the powerful motivational effects elicited by manipulations of this pathway (see Stellar & Stellar 1985 for an excellent review of early research in this field). This conclusion was supported by the observation that destruction of the midbrain dopamine system produced profound motivational deficits (Ungerstedt 1971b). Furthermore, the behavioral effectiveness of intracranial self-stimulation could be reduced or abolished by dopaminergic blockade or lesions, including selective blockade of VTA target sites (reviewed in Wise 2005). Interest broadened further with initial reports that the reinforcing and motivational properties of opioids and psychostimulant drugs depend on actions in the mesocorticolimbic dopamine pathway (Lyness et al. 1979, Roberts et al. 1980, Wise 2005). Thus by the early 1980s, the VTA and its dopaminergic projections to the limbic forebrain were well established as critical for performance and positive reinforcement of appetitive behaviors.

Neurotransmitter Content of VTA Neurons

Although commonly identified as a dopaminergic region, fewer than 60% of VTA neurons in the rat are dopaminergic (Margolis et al. 2006b, Swanson 1982). This contrasts with the adjacent substantia nigra pars compacta (SNc) where ~90% of neurons are dopaminergic (Margolis et al. 2006b). A large population of VTA neurons can be cytochemically identified as GABAergic neurons (Carr & Sesack 2000, Margolis et al. 2006a, Van Bockstaele & Pickel 1995). Some evidence also indicates that some VTA neurons release glutamate (Chuhma et al. 2004, Lavin et al. 2005).

Inputs

The VTA receives inputs from a large number of CNS sites (Geisler & Zahm 2005, Phillipson 1979) (**Figure 1**). It receives glutamatergic inputs from the prefrontal cortex (PFC) (Sesack & Pickel 1992), LH (Rosin et al. 2003), bed nucleus of the stria terminalis (Georges & Aston-Jones 2002), and the superior colliculus (Geisler & Zahm 2005, McHaffie et al. 2006). The input from the LH also includes afferents that contain the peptides orexin (Fadel & Deutch 2002) or α-melanocyte stimulating hormone (Semba & Fibiger 1992).

Two groups of mesopontine tegmental area neurons provide a major input to the VTA: the pedunculopontine tegmental nucleus (PPTg) and the more lateral and slightly more posterior laterodorsal tegmental nucleus (LDT) (Paxinos & Watson 1998, Semba & Fibiger 1992). Although these two nuclei receive largely overlapping inputs, including those from the LH, the LDT receives a heavier input from PFC, whereas the PPTg has a larger input from the amygdala (Semba & Fibiger 1992). Both nuclei provide significant numbers of glutamatergic and cholinergic as well as GABAergic projections to the VTA (Cornwall et al. 1990, Oakman et al. 1995, Semba & Fibiger 1992). Whereas the LDT projects primarily to the VTA, the PPTg projects to both the VTA and the SNc (Oakman et al. 1995). In vivo recordings using PPTg inactivation have shown that the PPTg is a major route by which short latency sensory input reaches VTA neurons (Pan & Hyland 2005).

Other, presumably GABAergic, inputs arise from the ventral pallidum (Geisler & Zahm 2005) and the nucleus accumbens (NAc) (Conrad & Pfaff 1976). The striatal projections arise from medium spiny neurons (MSNs) that co-contain the peptides substance P and dynorphin, the endogenous κ opioid (KOP) receptor-selective peptide (Fallon et al. 1985, Lu et al. 1998). Orexin-containing neurons in the LH that project to the VTA also contain dynorphin (Chou et al. 2001). The VTA also receives projections from the noradrenergic locus coeruleus and the serotonergic dorsal raphe nucleus

(Geisler & Zahm 2005, Phillipson 1979). Additional VTA inputs arise from the amygdala (Wallace et al. 1992), the diagonal band of Broca, the preoptic area of the hypothalamus (Phillipson 1979), and several pontine, cerebellar, and medullary nuclei (Geisler & Zahm 2005).

VTA Projections: Diversity of Neurotransmitter Content and Projection Targets

The VTA projects densely and largely ipsilaterally to the ventromedial striatum, primarily the NAc core and shell. Other limbic areas receiving large inputs from VTA are the pregenual and subgenual PFC, the amygdala, and the LH (Berger et al. 1974; Fuxe et al. 1974; Lindvall & Bjorklund 1974; Lindvall et al. 1974, 1978; Ungerstedt 1971b). The hippocampus, entorhinal cortex, and lateral septal area receive smaller projections (Beckstead et al. 1979, Swanson 1982). Studies using different retrograde markers injected into pairs of VTA forebrain target sites demonstrate a relatively small percentage of double labeled neurons (Fallon et al. 1984, Margolis et al. 2006a, Swanson 1982), indicating that each target receives input from a distinct group of VTA neurons. Although attention has focused on dopaminergic neurons, Swanson (1982) first demonstrated that VTA projections to different targets consist of variable proportions of dopaminergic neurons (**Figure 1**). The projection to the NAc is richest in dopamine neurons (65%–85% dopaminergic), followed by those to the lateral septal area (72%), amygdala (53%), entorhinal cortex (46%), PFC (30%–40%), and hippocampus (6%–18%) (Fallon et al. 1984, Gasbarri et al. 1994, Margolis et al. 2006a, Swanson 1982).

A significant proportion of VTA afferents to both the NAc and PFC contain GABA (Carr & Sesack 2000, Margolis et al. 2006a, Van Bockstaele & Pickel 1995). Electrophysiological studies indicate that there are also glutamatergic projections from the VTA to both the PFC and the NAc. Lavin et al. (2005) showed in adult rats that electrical or chemical stimulation of the VTA in vivo evokes glutamatergic excitatory postsynaptic potentials (EPSPs) in PFC neurons. Likewise, in a mouse in vitro preparation, Chuhma et al. (2004) showed that VTA stimulation evoked glutamatergic EPSCs in NAc neurons. Because glutamate acting at ligand-gated channels can produce fast EPSPs, the presence of these glutamatergic projections increases the likelihood that temporally precise information is conveyed by phasic activity of VTA neurons.

One intriguing possibility raised by these studies is that glutamate is a cotransmitter in some dopaminergic VTA projection neurons. Cultured VTA neurons form autapses, thus permitting selective stimulation and intracellular recording of synaptic responses in the same neuron. Under these conditions, glutamatergic EPSPs were evoked and enhanced by the D2 antagonist sulpiride (which presumably blocked autoreceptor-mediated inhibition of transmitter release). This result indicates that dopamine and glutamate were released by the same neuron (Sulzer et al. 1998). However, whether dopaminergic neurons release glutamate in vivo in adult animals is uncertain. In fact, direct colabeling for vesicular glutamate transporters and TH yields virtually no (Yamaguchi et al. 2007) or a low rate of colocalization, limited to the most medial parts of the VTA (Kawano et al. 2006). Furthermore, ultrastructural evidence indicates that dopaminergic synapses are largely symmetrical (inhibitory) in the PFC and NAc (e.g., Seguela et al. 1988, Totterdell & Smith 1989). These anatomical results demonstrate that, in addition to GABA and dopamine neurons, there is a separate and significant population of glutamatergic VTA neurons.

Afferents Differentially Target Subpopulations of VTA Neurons

Afferents to the VTA are of diverse origin and neurotransmitter content, and they

TH: tyrosine hydroxylase

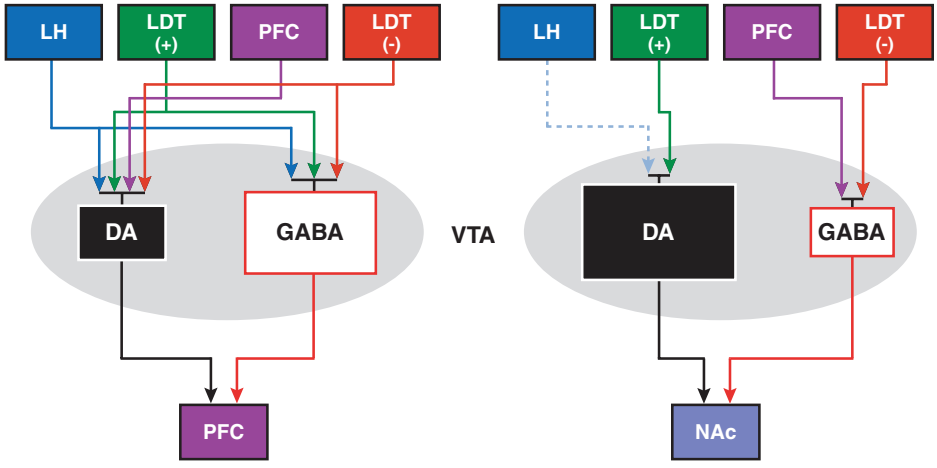

Figure 2

Inputs to VTA neurons depend on their neurotransmitter content and projection target. The few VTA inputs that have been examined in detail show selectivity for different subpopulations of neurons. Prefrontal cortex (PFC)- and nucleus accumbens (NAc)-projecting VTA neurons receive different patterns of excitatory (+) and inhibitory (−) inputs, and these patterns differ depending on whether the VTA neuron is GABAergic or dopaminergic. Although the projection to the NAc is larger than that to the PFC in total number and in percentage of dopaminergic neurons (DA), fewer of its inputs have been determined. The dashed lateral hypothalamic (LH) projection to dopaminergic NAc-projecting neurons indicates that less than 10% of the LH projections synapse onto this subset of VTA neurons. Whether LH inputs to PFC-projecting neurons segregate on the basis of VTA neuron neurotransmitter content has not yet been determined. See **Figure 1** caption for abbreviations.

differentially target subpopulations of VTA neurons on the basis of their projection target and neurotransmitter content (**Figure 2**). Recent ultrastructural studies (Carr & Sesack 2000, Omelchenko & Sesack 2005, 2006) have begun to parse these different circuits. Excitatory afferents from the PFC target VTA dopamine neurons that project back to the PFC, but not those that project to the NAc. Conversely, excitatory PFC afferents target GABAergic neurons that project to the NAc, but not those projecting back to the PFC. Projections from the LH synapse onto VTA projections to the PFC, but not those projecting to the NAc (Balcita-Pedicino & Sesack 2005). Afferents from the LDT to the VTA have been studied in detail (Omelchenko & Sesack 2005). Both dopaminergic and GABAergic VTA neurons that project to the PFC receive excitatory as well as inhibitory LDT inputs. Presumed excitatory (asymmetric synapses) cholinergic inputs from LDT selectively target NAc-projecting VTA neurons that contain dopamine, but not those that contain GABA. Infusion of the cholinergic drug carbachol into the VTA increases NAc dopamine release (Westerink et al. 1996). Furthermore, using an acetylcholinesterase inhibitor, Blaha et al. (1996) showed that Ach released from LDT but not PPTg terminals in the VTA increased NAc dopamine release. Conversely, Ach released in the SNc from PPTg but not LDT terminals increased dopamine release in the NAc. This observation is important because it shows two parallel dopaminergic pathways from the mesopontine cholinergic neurons to the NAc. In contrast, inhibitory (and possibly cholinergic) inputs from the LDT selectively target GABAergic VTA neurons projecting to the NAc. Sesack and colleagues (1989) have proposed that the LDT, which receives a direct projection from PFC,

may provide an indirect pathway by which the PFC can excite VTA dopamine neurons projecting to the NAc (**Figure 2**).

The idea that subsets of VTA dopamine neurons participate in separate circuits is also supported by studies showing different patterns of dopamine release in different VTA projection targets. For example, following intra-VTA administration of KOP receptor agonists, dopamine levels in the PFC decrease, whereas they are unchanged in the NAc (Margolis et al. 2006a). Conversely, local delivery of orexin into the VTA increases dopamine levels in the PFC, but not in the NAc (Vittoz & Berridge 2006). Infusion of the AMPA/kainate receptor antagonist LY293558 into the VTA increases the dopamine level in the NAc but decreases it in the PFC (Takahata & Moghaddam 2000). These studies clearly show that VTA dopaminergic neurons projecting to different targets are regulated by different afferents.

Intra-VTA Circuitry

VTA GABAergic neurons make local inhibitory connections including some to dopaminergic neurons. Johnson & North (1992a) showed in vitro that the frequency of spontaneous inhibitory potentials in putative dopamine neurons was decreased in the presence of tetrodotoxin, indicating that they arise from impulse activity in neurons within the slice. That local μ opioid (MOP) receptor agonists also decreased this frequency suggests that local, spontaneously active GABAergic interneurons expressing the MOP receptor tonically inhibit dopamine neurons. Consistent with this microcircuit, Steffensen et al. (2006) found a population of GABAergic neurons inhibited by systemic MOP receptor agonists. However, most of these GABAergic neurons were just dorsal to the A10 dopaminergic group and were antidromically activated from the internal capsule (Steffensen et al. 2006). Thus, although they could still contribute to the VTA microcircuit through local collaterals, they are projection neurons, not interneurons. Furthermore, a significant number of VTA neurons with properties similar to proposed VTA interneurons project to the PFC (Margolis et al. 2006a). Whether, in fact, any VTA neurons make only local connections remains an open question.

MOP: μ opioid

Locally released dopamine also plays an important regulatory role within the VTA. Both dopaminergic and nondopaminergic VTA neurons are directly hyperpolarized by dopamine acting at D2 dopamine receptors (Johnson & North 1992b, Margolis et al. 2006b). Local stimulation of VTA neurons in vitro produces a TTX-sensitive and calcium-dependent slow IPSP via these D2 receptors (Beckstead et al. 2004). Although the source of dopamine released locally in the SNc does not appear to be synapse-like axon terminal structures (Juraska et al. 1977, Wassef et al. 1981), both axodendritic and dendrodendritic dopaminergic appositions are found in the VTA (Bayer & Pickel 1990). D2 receptors are strategically located in proximity to dopamine-containing vesicles (Pickel et al. 2002). In addition, in culture, stimulation-dependent dopamine release from VTA neurons occurs at presynaptic axon varicosities that express a vesicular monoamine transporter (Pothos et al. 1998).

PHYSIOLOGY AND PHARMACOLOGY OF VTA NEURONS

In Vivo Electrophysiology

Establishing that a population of neurons contributes to a specific aspect of behavior typically requires an accurate temporal correlation of their spike activity with discrete behavioral events. If a neuron is to contribute to the selection and initiation of an action, a change in its activity must precede the onset of that action. Conversely, a neuron proposed to encode a function of reward magnitude should exhibit a change in firing that follows reward receipt. Single-unit resolution is essential to obtain such information in brain regions such

as the VTA, which have intermixed populations of neurons that vary in their connectivity and neurotransmitter content. In fact, in vivo single-unit recordings have strongly influenced current views of the function of midbrain dopamine neurons (Schultz 1998, 2002). Most of these studies have involved recordings in the SNc of awake behaving primates; however, similar responses have been reported in rat SNc and in the VTA of both rats and monkeys (Pan et al. 2005, Schultz 1998). Neurons in the SNc and VTA show transient changes in discharge under a variety of conditions including novel or otherwise salient sensory stimuli such as unexpected rewards, stress, and noxious stimuli (Anstrom & Woodward 2005, Horvitz 2000, Ungless 2004).

Most relevant to the topic of this review, SNc and VTA neurons exhibit transient increases in firing on receipt of an unpredicted reward (e.g., fruit juice to a thirsty monkey) (Bayer & Glimcher 2005, Mirenowicz & Schultz 1994, Schultz et al. 1993). After training in a paradigm in which sensory cues are consistently presented prior to reward delivery or availability, these neurons show robust responses to the reward-predictive cue (Ljungberg et al. 1992, Schultz et al. 1993, Takikawa et al. 2004). These firing patterns are consistent with two distinct functions: signaling delivery of a reward that is better than expected, and signaling available reward. Reward-predictive activity could promote appetitive behavior, and activity encoding unexpected reward magnitude could contribute to reinforcement.

The evidence implicating VTA neurons in promoting and reinforcing appetitive behaviors is based on the correlation of VTA neuronal firing with the relevant behaviors, as well as on studies of dopamine release and of dopamine agonist and antagonist effects on behavior. The assumption that VTA neurons recorded in vivo are dopaminergic is very attractive because, if true, it would allow the activity in those neurons to be related in a causal manner to the behavior of interest using pharmacological methods (e.g., using dopamine receptor antagonists or depletion in target nuclei). However, drawing such a conclusion requires that the VTA neurons in question be correctly identified with respect to both their dopamine content and their projection target. These issues are both currently problematic (Margolis et al. 2006b and see below). Because of this, prior to discussing specific functions attributed to VTA neurons we review some of the major difficulties that limit our ability to relate in vivo VTA recordings to behavioral studies that manipulate dopamine.

Identifying Dopaminergic Neurons

The electrophysiological properties of dopamine neurons were first characterized in the SNc, where about 90% of neurons are dopaminergic (Margolis et al. 2006b). The initial assignment of such properties to dopamine neurons was based on indirect in vivo studies in rat SNc and SNr (substantia nigra, pars reticulata) using the dopamine neuron-selective neurotoxin, 6-hydroxydopamine (6-OHDA) (Guyenet & Aghajanian 1978). Two distinct electrophysiological profiles were observed: type I neurons with wide action potentials, slow axonal conduction velocity, and a slow firing rate with intermittent burst-like activity; and type II neurons expressing a briefer action potential, higher firing rate, and faster conduction velocity. After 6-OHDA lesions of the MFB, the proportion of antidromically stimulated type I cells decreased greatly, whereas the proportion of type II cells was preserved. These data indicated that type I neurons were dopaminergic. Grace & Bunney confirmed the neurotransmitter content of SNc neurons directly using intracellular recordings both in vivo (1980) and in vitro (1983). After intracellular recording, SNc neurons were labeled and then processed cytochemically. SNc type I neurons were confirmed to be dopaminergic. Type II neurons recorded in the SNr were subsequently confirmed as GABAergic (Richards et al. 1997). Later in vitro studies in the SNc yielded another physiological

marker: a hyperpolarization-activated, nonspecific cation current (I_h). The I_h was found in type I, but not type II, SNc neurons (Lacey et al. 1989).

In many electrophysiological studies, VTA neurons are identified as dopaminergic using the criteria developed for the SNc. However, in vitro work utilizing immunocytochemistry for tyrosine hydroxylase (TH), a marker of dopaminergic neurons, has clearly shown that many VTA neurons with these properties are not dopaminergic. In the VTA, neurons lacking an I_h are nondopaminergic (but see Jones & Kauer 1999), and all dopaminergic neurons express an I_h. However, a significant number of nondopaminergic neurons in the VTA also express an I_h (Johnson & North 1992b; Jones & Kauer 1999; Margolis et al. 2003, 2006a,b). Furthermore, the long action potential duration characteristic of dopamine neurons in the SNc does not reliably distinguish dopamine-containing from other neurons in the VTA (Johnson & North 1992b; Margolis et al. 2006a,b). Although Ungless et al. (2004), using juxta-cellular recording and TH immunocytochemistry in vivo, reported a tendency for dopaminergic neurons to have longer action potential durations compared with nondopaminergic VTA neurons, the two populations in that study were overlapping. In contrast, using whole cell recording in vitro, we found no differences in action potential duration between TH positive and TH negative VTA neurons (Margolis et al. 2006b).

Another problematic criterion used to identify VTA dopaminergic neurons is inhibition by a dopamine D2 agonist (Bunney et al. 1973). We found that some VTA dopamine neurons were not inhibited by a dopamine D2 receptor agonist, and many nondopamine VTA neurons were inhibited (Margolis et al. 2006b). On the other hand, postsynaptic hyperpolarization of VTA neurons by the KOP receptor agonist U69593 is restricted to a subpopulation of TH-positive neurons (Margolis et al. 2003, 2006a). Whereas this criterion is useful in vitro, it may be less so in vivo because KOP receptor agonists also inhibit glutamate release onto nondopaminergic VTA neurons (Margolis et al. 2005).

In summary, in the VTA the absence of an I_h reliably identifies a neuron as nondopaminergic, and direct hyperpolarization by KOP receptor agonists identifies a subset of dopamine neurons, but there are currently no physiological or pharmacological criteria that can be used to identify all dopaminergic neurons. Until this problem is solved, in vitro studies should use a cytochemical marker such as TH to identify a neuron as dopaminergic. Where feasible, a similar approach should be used in vivo, although this is a major technical challenge, especially in awake behaving animals. The best approach for in vivo VTA recordings is to include all detected neurons in the analysis and accept that any firing patterns observed may be derived from either dopaminergic or nondopaminergic neurons.

Regulation of Extracellular Dopamine Concentration in Target Sites

Even if the assumption that VTA neurons with particular firing patterns are dopaminergic turns out to be correct, correlating these patterns of activity to behavioral events is not straightforward. This is because the relatively slow kinetics of dopamine's clearance make its postrelease regulation a major determinant of the time course of its synaptic action.

In fact, dopamine transients are slow and can act on cellular targets distant from the dopamine terminals before being removed from the extracellular space. Single pulse stimulation of the VTA or MFB results in dopamine transients in the NAc lasting several seconds, and similar transients occur in behaving animals either spontaneously, after stimulus presentation, or just prior to operant behavior (Phillips et al. 2003; Robinson et al. 2001, 2002; Roitman et al. 2004). Investigators have detected relatively small "spontaneous" dopamine events that last on the order of 500 ms in the dorsal striatum, NAc and the olfactory tubercle (Robinson et al. 2002).

In comparison, the time course of glutamate concentration following its release is on the order of 1 ms (Clements 1996). The volume and time course of dopamine concentration after synaptic release has been mathematically modeled as a diffusion system with a single constant reuptake rate for the dopamine transporter (Garris et al. 1994). This model suggests that even a single vesicle of dopamine can result in dopamine diffusion to extrasynaptic sites. Thus, compared with neurotransmitters such as glutamate and GABA, that act at ligand-gated channels and are rapidly removed from the synaptic cleft, the dopamine signal is both spatially and temporally diffuse. This conclusion is confirmed by comparisons of voltammetric measurements of dopamine in behaving animals with the firing of putative dopamine neurons in similar situations. For instance, although the bursts exhibited by rat VTA neurons during free-run operant or cue-responding tasks rarely exceed 200 ms in duration (Hyland et al. 2002, Pan et al. 2005), the dopamine transients measured in the NAc during similar tasks last several seconds (Phillips et al. 2003, Roitman et al. 2004) (although it is possible that briefer or smaller signals occur below the limit of detection). Presumably, then, the firing of a single dopamine neuron can produce a dopamine signal that affects many neurons by volume transmission and has a prolonged time course.

Several factors further complicate analysis of the temporal relationship between dopamine neuron firing and the action of the dopamine released. First, the amount of dopamine release depends critically not just on the instantaneous firing rate, but also on the neuron's recent firing history (Montague et al. 2004b). Second, the density of dopamine transporters is highly variable in different brain regions. For instance, they are much lower in the PFC than in the NAc (Sesack et al. 1998), which may explain why dopamine signals tend to be shorter in the NAc than in the PFC (Cass & Gerhardt 1995). Finally, dopamine release can be modulated by activating receptors on dopamine terminals (**Figure 3**). Dopamine terminals express D2 autoreceptors that inhibit release of dopamine (e.g., Kennedy et al. 1992). Also, dynorphin released from axon collaterals of MSNs within the NAc acts at KOP receptors to inhibit release from dopamine axon terminals (Di Chiara & Imperato 1988, Spanagel et al. 1992). Ultrastructural studies have also demonstrated NMDA receptors on TH-positive varicosities in the NAc (Gracy & Pickel 1996), and local application of glutamate enhances NAc dopamine release (Imperato et al. 1990, Youngren et al. 1993). Consistent with these observations, electrical stimulation of the basolateral amygdala (BLA), which sends glutamatergic projections to the NAc, increases dopamine levels in the NAc. This effect is not blocked by inactivation of the VTA but is blocked by infusion of glutamate receptor antagonists within the NAc (Howland et al. 2002). These data suggest that beyond the temporal pattern of action potentials in VTA neurons, release of dopamine from their terminals can be regulated by a variety of mechanisms. This complicates the interpretation of in vivo VTA recordings and emphasizes the critical importance of temporally precise measurements of dopamine at its site of action.

Synaptic Actions of Dopamine in VTA Target Nuclei

Because the cellular effects of dopamine receptor activation in the NAc and PFC have been reviewed extensively (Nicola et al. 2000, 2004a; O'Donnell 2003; Seamans & Yang 2004), we give only a brief overview here. In the NAc, dopamine presynaptically inhibits the release of glutamate as well as GABA onto MSNs (Harvey & Lacey 1996, Nicola et al. 1996, Nicola & Malenka 1997, Pennartz et al. 1992). The net effect of this presynaptic modulation by dopamine depends critically on the firing frequency of the MSN's afferents (Hjelmstad 2004). In addition, dopamine postsynaptically excites NAc MSNs by inhibiting a slow A-type potassium current that

is active when the neuron is depolarized (Hopf et al. 2003). How these actions of dopamine integrate to alter the firing of NAc MSNs in vivo is unclear, but its function depends critically on the level of synaptic inputs and resting membrane potential of the neuron.

In the PFC, dopamine's effect depends on the neuron's activation state (Seamans & Yang 2004). Intracellular in vivo recordings show that some PFC neurons are bistable, switching between a depolarized and a hyperpolarized membrane potential. These neurons show no change in spontaneous firing rate after VTA stimulation, but they do show dopamine-dependent enhanced firing to depolarizing pulses (Lavin et al. 2005). The enhancement lasts for at least 35 min following a VTA stimulation protocol that raises dopamine levels in the PFC for less than 10 s. In contrast, PFC neurons that are not bistable show a decrease in spontaneous activity following this VTA stimulation protocol.

One important conclusion from these studies is that in contrast to classical amino acid neurotransmitters such as glutamate and GABA, there are apparently no dopamine-mediated rapid transient postsynaptic potentials. Rather, dopamine's action on target neurons is critically dependent on concomitant activity in other afferents to those neurons. Furthermore, interpreting the impact of VTA neuron activity on neurons in target nuclei is limited by uncertainties about the temporal characteristics of dopamine kinetics and the time course and sign of its synaptic action. Thus, determining how the firing of

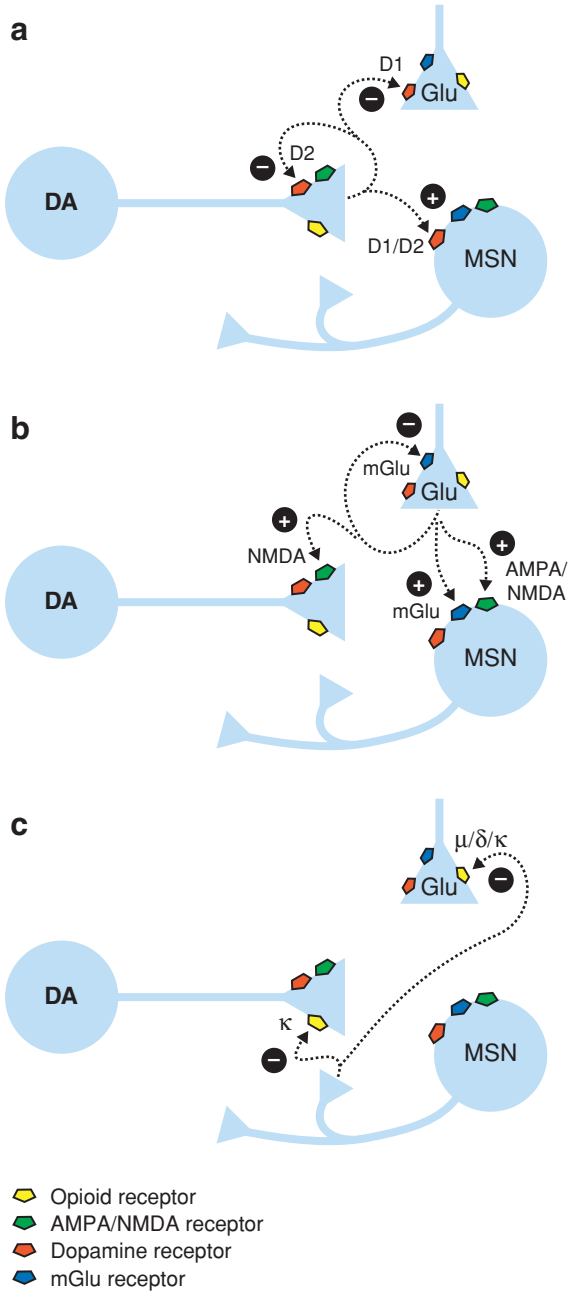

Figure 3

Interactions between dopamine and glutamate release in the NAc. *A*: Dopamine, released from dopaminergic VTA neurons (DA), inhibits glutamate (Glu) release through presynaptic D1-like receptors. It acts on postsynaptic dopamine receptors on medium spiny neurons (MSNs) and feeds back onto D2 autoreceptors to inhibit dopamine release. *B*: Glutamate release not only excites MSNs, but also regulates its own release through metabotropic glutamate receptors (mGlu) and can enhance dopamine release through NMDA receptors. *C*: Both glutamate and dopamine release can be modulated by opioid peptides released from MSN axon collaterals. An action on κ opioid receptors, presumably by dynorphin from MSNs, inhibits dopamine release. A different population of MSNs releases enkephalin, which can act at both μ and delta δ receptors to inhibit glutamate release.

dopamine neurons contributes to behavior presently requires an integrated approach. This involves electrophysiology in both VTA and target nuclei, the use of receptor-selective antagonists, and the measurement of local dopamine concentration using methods having sufficient temporal resolution.

CPP: conditioned place preference

THE VTA AND POSITIVE REINFORCEMENT

Consistent with a role in positive reinforcement, in vivo recordings show that VTA neurons fire when animals receive an unexpected reward (Mirenowicz & Schultz 1994, Schultz et al. 1993). Direct evidence supporting this view is derived largely from two types of behavioral experiments. In one type, sensory stimuli paired with passive reward delivery acquire the power to promote appetitive behaviors. One example of this is the conditioned place preference paradigm (CPP), in which animals acquire approach behavior to a location previously paired with reward. A variety of manipulations that increase VTA dopamine neuron activity or dopamine concentration in the NAc can produce CPP. In the other experimental paradigm, the probability of behavioral responses that the animal "spontaneously" emits is increased if that behavior results in reward receipt. A widely used example of this type is drug self-administration, in which animals learn to carry out an operant action (e.g., press a lever) prior to reward delivery. Animals will self-administer drugs that activate VTA neurons and that raise dopamine levels in VTA target sites.

In Vivo Recordings

In vivo recording data are consistent with the idea that SNc and VTA neurons contribute to positive reinforcement (Schultz 1998, 2002). SNc and VTA neurons fire transiently immediately following receipt of an unpredicted reward (Mirenowicz & Schultz 1994, Schultz et al. 1993). This increase in firing scales with the magnitude of reward received (e.g., volume of juice; Tobler et al. 2005). No excitation is observed if the reward is fully predicted by a cue. Furthermore, if the predicted reward is withheld, the same neurons show a pause in firing at the time of the expected reward. If the predicted reward occurs earlier or later than expected, reward also causes a transient increase in the firing of these neurons (Hollerman & Schultz 1998). This pattern of firing suggests that the neuron encodes a reward prediction error, defined as the difference between the predicted reward value (probability x magnitude) and the magnitude of the reward received. This firing pattern meets the formal requirements of the temporal difference model developed for machine learning (Montague & Berns 2002, Montague et al. 2004a, Schultz 2002) and is consistent with a role for these neurons in signaling positive reinforcement. For example, if one expects a reward of a certain value following behavior X, and the value received is greater than predicted by the recent history of outcome values for behavior X in the same context, the future probability of behavior X increases (positive reinforcement). In one test of this idea, Fiorillo et al. (2003) trained monkeys with different visual cues indicating different probabilities of juice delivery. They found that the response of SNc and VTA neurons to juice delivery varies inversely with the probability of reward predicted by the preceding visual cue. The less certain the reward (i.e., the lower its predicted value), the greater is the discharge of these neurons immediately following reward delivery.

Although confirming the increased firing for rewards that exceed predicted value, more recent studies have not demonstrated a strong relation between inhibition of VTA and SNc neurons and the degree to which the received reward falls below its predicted value (Bayer & Glimcher 2005, Satoh et al. 2003). Furthermore, in rodents, Pan et al. (2005) found that during learning, VTA neurons continued to show excitations to delivery of rewards that were fully predicted. In summary, although researchers disagree about the

existence of populations of VTA neurons with firing patterns that fulfill the specific requirements of the reward prediction error hypothesis and temporal difference model, they generally agree that populations of VTA neurons encode some aspect of the magnitude of a received reward and how unexpected it is. This pattern is consistent with a role for VTA and SNc neurons in positive reinforcement.

Behavioral Pharmacology

Much of the behavioral pharmacology literature relevant to reinforcement has focused on the contribution of the dopaminergic projection from the VTA to NAc. Early experiments showed that nonselective dopamine receptor antagonists affected simple operant tasks such as fixed ratio 1 (FR1) for food reward (i.e., one lever press required for reward). Systemically administered dopamine antagonists reduced these previously learned responses (Wise 1982). Because reinforcement is considered necessary to prevent extinction of operant behavior, this result argues that dopamine is necessary for reinforcement. However, whereas dopamine may be required for positive reinforcement in these tasks, the dopaminergic projection to the NAc is not. For example, FR1 performance for food reward in trained animals is unaffected by dopamine depletion or microinjection of dopamine antagonists into the NAc (Aberman & Salamone 1999, Hernandez et al. 2005, Roberts et al. 1977, Smith-Roe & Kelley 2000, Yun et al. 2004a).

Although the VTA-to-NAc dopaminergic projection is not required to sustain appetitive behaviors by reinforcement, it is required for learning novel tasks in some paradigms. Indeed, blockade of NAc D1 receptors during training slows FR1 task acquisition (Hernandez et al. 2005, Smith-Roe & Kelley 2000). One critical caveat in the interpretation of this type of experiment is that if the D1 receptor antagonist prevents the motor performance of the action it cannot be reinforced. One approach to address this issue is to inject dopamine antagonists into the NAc just after the training session is complete. Although FR1 performance is unaffected by immediate post-training infusion of D1 receptor antagonists (Hernandez et al. 2005), post-training injection of dopamine antagonists into the NAc impairs learning of some maze tasks (Setlow & McGaugh 1998, 2000) and Pavlovian approach behavior (Dalley et al. 2005). Because in these studies the animal is never under the influence of the antagonist during its interactions with the training environment and reward receipt, the positive result implicates mesolimbic dopamine in positive reinforcement and avoids the problem of impaired performance.

The motor-impairment problem does not apply to the CPP paradigm. In this paradigm, the animal experiences a drug while confined to one room of a two- or three-room apparatus. Vehicle administration is paired with one of the other rooms as a control for novelty, handling, and manipulation. After conditioning, the animal is allowed to explore all rooms freely. If it spends more time in the drug-paired room, this is evidence for reinforcement of an association between the drug and the sensory stimuli in that room. Thus, reinforcement occurs without the requirement that the animal take any action during training. Administration of a variety of dopamine antagonists either systemically or into the NAc during conditioning significantly impairs the acquisition of CPP, including CPP induced by opioids such as morphine and psychostimulants (reviewed by Tzschentke 1998). These data support a role for NAc dopamine in reinforcement that is independent of an effect on motor performance.

μ Opioid Receptor Agonists Activate VTA Dopamine Neurons and Produce Positive Reinforcement via the NAc

Systemic administration of MOP receptor agonists produces CPP and disinhibits a subset of VTA neurons (Gysling & Wang 1983,

Matthews & German 1984) (but see Kiyatkin & Rebec 2001). Microinjection of MOP receptor agonists directly into the VTA also elicits robust CPP (Hand et al. 1989, Shippenberg & Herz 1987), and rodents will learn to self-administer both MOP and δ opioid receptor agonists directly into the VTA (reviewed in McBride et al. 1999, Wise 2004). Dopamine is likely involved in these forms of reinforcement because both systemic opioid administration and enkephalin microinjection directly into the VTA produce CPP that is blocked by systemic dopamine antagonists (Ashby et al. 2003, Phillips et al. 1983, Tzschentke 1998).

This evidence demonstrates that a population of VTA dopaminergic neurons can contribute to positive reinforcement but does not specify the relevant projection targets of these neurons. At least some of the CPP-relevant VTA dopaminergic neurons project to the NAc because systemic and intra-VTA MOP receptor agonists increase NAc dopamine levels (Chefer et al. 2005, Devine et al. 1993, Di Chiara & Imperato 1988, Latimer et al. 1987, Leone et al. 1991, Spanagel et al. 1992), and local activation of dopamine receptors in the NAc (using psychostimulants or dopamine agonists) can elicit CPP (Chefer et al. 2005, Tzschentke 1998). Accordingly, we recently demonstrated in vitro that MOP receptor agonists directly activate (disinhibit) VTA dopamine neurons that project to the NAc (Margolis et al. 2004). Furthermore, in rats pre-exposed to MOP receptor agonists and in a withdrawal state, the acquisition of CPP induced by MOP receptor agonists in the VTA is blocked by a nonselective dopamine receptor antagonist microinjected into the NAc (Laviolette et al. 2002). More recently, using a single trial conditioning paradigm in opioid-naïve rats, acquisition of morphine CPP was blocked by the selective dopamine D1 receptor antagonist SCH 39,166 injected into the NAc shell (Fenu et al. 2006). These findings strongly argue that the VTA dopaminergic projection to the NAc is sufficient to produce reinforcement; however, they leave open the question of whether it is necessary.

Alternative VTA Circuits for Positive Reinforcement

VTA dopaminergic neurons projecting to targets other than the NAc can produce positive reinforcement. MOP agonist microinjection into the VTA activates dopaminergic neurons that project to the PFC and increases dopamine levels in the PFC (Chefer et al. 2005, Latimer et al. 1987, Margolis et al. 2004). Furthermore, animals self-administer cocaine directly into the PFC, an effect blocked by local dopamine depletion (see Tzchentke 2000 for review).

Systemic or intra-VTA morphine can also produce dopamine-independent CPP (Hnasko et al. 2005, Mackey & van der Kooy 1985, Nader & van der Kooy 1997, Olmstead & Franklin 1997). Although the projection target for the relevant VTA neurons is uncertain, one possibility is the PFC. We have demonstrated in vitro that a local action of MOP receptor agonists in the VTA inhibits a population of nondopaminergic neurons projecting to the PFC (Margolis et al. 2004). In addition, although systemic morphine CPP is unaffected by PFC dopamine depletion, it is blocked by excitotoxic lesions of PFC, consistent with a role for a nondopaminergic VTA-to-PFC projection (Tzchentke 2000). Furthermore, in opioid-naïve rats, CPP produced by injection of morphine into the VTA can be insensitive to dopamine antagonists and is instead blocked by lesions of the PPTg (Nader & van der Kooy 1997). Because the PPTg receives a major input from PFC, the nondopaminergic VTA projection to the PFC might contribute to this positive reinforcement via the PPTg.

In addition to the NAc and PFC, self-administration and place preference studies indicate that other VTA projection targets, including the olfactory tubercle, ventral pallidum, central nucleus of the amygdala, and dorsal hippocampus, can contribute to

positive reinforcement (McBride et al. 1999, Wise 2004, Zarrindast et al. 2003). Much less information is available about the relevant circuitry underlying these actions, and they are not reviewed further here.

Dopamine and Synaptic Plasticity

Given that the VTA to NAc projection can contribute to positive reinforcement, one possibility is that VTA neurons facilitate a change in synaptic strength in the NAc and that that change underlies positive reinforcement. In fact, glutamatergic synapses onto NAc MSNs do exhibit both long-term potentiation (LTP) and long-term depression (LTD). High-frequency stimulation of excitatory afferents that produce depolarization sufficient to activate NMDA receptors can potentiate the AMPA receptor–mediated EPSP (Pennartz et al. 1993). The voltage-dependent blockade of NMDA receptors can also be overcome by directly depolarizing the postsynaptic neuron. Pairing this depolarization with afferent stimulation also results in LTP (Kombian & Malenka 1994, Li & Kauer 2004). Multiple forms of LTD have been observed in the NAc. Synaptic stimulation associated with moderate postsynaptic depolarization results in a form of LTD that is dependent on NMDA receptors and postsynaptic calcium (Thomas et al. 2001). Another NMDA receptor-independent form of LTD can be induced by high-frequency synaptic stimulation through the activation of group 2 metabotropic glutamate receptors (Robbe et al. 2002). This form of LTD is presynaptic, resulting in a reduction in the probability of glutamate release.

Despite the demonstration of synaptic plasticity in the NAc, currently no evidence links these changes to activity in VTA neurons, or for that matter to positive reinforcement as measured at the behavioral level. Dopamine antagonists do not alter tetanus-induced LTP in the NAc (Pennartz et al. 1993), and dopamine itself has no effect on either tetanus-induced (Pennartz et al. 1993) or pairing-induced (Li & Kauer 2004) LTP. Likewise, neither the NMDA receptor–dependent nor metabotropic glutamate receptor–dependent forms of LTD are affected by dopamine receptor antagonists (Robbe et al. 2002, Thomas et al. 2000). Although these data argue against the idea of a dopamine-dependent modulation of synaptic plasticity in the NAc, dopamine could modulate activity-dependent synaptic plasticity by suppressing inhibitory inputs (Hjelmstad 2004, Nicola & Malenka 1997), thus allowing sufficient depolarization to activate NMDA receptors. In this regard, it is relevant that in the negative experiments discussed above, $GABA_A$ receptors were pharmacologically blocked.

As mentioned above, VTA projections to target regions other than the NAc can contribute to positive reinforcement, and in some of these regions dopamine modulation of synaptic plasticity has been demonstrated. In the hippocampus, LTP is modulated by D1 receptors (reviewed in Lisman & Grace 2005). Within the amygdala, dopamine gates LTP by depressing feed-forward inhibition produced by local interneurons (Bissiere et al. 2003). Finally, in the PFC, dopamine can enhance LTP (Huang et al. 2004, Otani et al. 2003) and D1 receptor antagonists reduce LTP (Huang et al. 2004).

In summary, dopaminergic VTA neurons projecting to the NAc can promote positive reinforcement. Other subpopulations of VTA neurons can also mediate both dopamine-dependent and -independent positive reinforcement through projections to targets other than the NAc. The synaptic mechanism by which VTA neurons contribute to positive reinforcement is unknown. Although dopamine-dependent changes in synaptic strength have been demonstrated in the PFC, hippocampus, and amygdala, there are no experimental results that link these changes to positive reinforcement observed at the behavioral level. Future progress in understanding how VTA neurons contribute to reinforcement of appetitive behaviors will

require attention to these specific questions of circuitry and synaptic function.

VTA NEURONS AND EXPRESSION OF LEARNED APPETITIVE BEHAVIORS

VTA Neurons Promote Performance of Learned Appetitive Behaviors

Subpopulations of SNc and VTA neurons in primate and rat are transiently excited by reward-predictive sensory cues, and these excitations are larger when the predicted reward is either larger or more likely (Fiorillo et al. 2003, Kawagoe et al. 2004, Morris et al. 2006, Pan et al. 2005, Satoh et al. 2003, Schultz et al. 1998, Tobler et al. 2003). This pattern of firing is consistent with a causal role for these neurons in promoting learned appetitive behavior (McClure et al. 2003).

NAc Dopamine and Appetitive Behavior

Some of the neurons responding to reward-predictive cues are likely dopaminergic neurons that project to the NAc because voltammetric measurements have demonstrated transient elevations in NAc dopamine in response to such cues (Phillips et al. 2003, Richardson & Gratton 1996, Roitman et al. 2004). Appetitive behavioral responses (lever press or nose poke) to a reward-predictive discriminative stimulus (DS) are significantly reduced by infusion of dopamine antagonists into the NAc or by reversible inactivation of the VTA (Yun et al. 2004a,b). These results directly tie the firing of VTA dopaminergic neurons to behaviors initiated by reward-predictive cues. They are consistent with other experiments showing that responding to salient sensory cues is impaired by disruption of NAc dopamine function in several tasks, including Pavlovian approach (Di Ciano et al. 2001, Parkinson et al. 2002) and conditioned avoidance (Jackson et al. 1977, Wadenberg et al. 1990). These dopamine effects may be specific to responding elicited by sensory cues. In well-trained animals, performance on tasks such as FR1, which involve apparently identical motor actions (e.g., lever pressing and reward consumption) but without the requirement to respond to cues, are unimpaired by reduction of NAc dopamine function (Aberman & Salamone 1999, Hernandez et al. 2005, Roberts et al. 1977, Smith-Roe & Kelley 2000, Yun et al. 2004a). Taken together, these experiments provide powerful support for the idea that the VTA dopaminergic neurons projecting to the NAc promote responding to salient outcome-predictive cues (see Ikemoto & Panksepp 1999, Nicola 2007 for further discussion).

Consistent with the idea that the VTA to NAc dopamine projection facilitates cue responding, in a modified DS task, increasing NAc dopamine levels increased the percentage of reward-predictive cues to which the animal responds (Nicola et al. 2005). Raising dopamine levels using amphetamine injected into the NAc shell also increased responding to reward-predictive cues during Pavlovian-instrumental transfer. In these experiments, rats were first trained to press a lever for sucrose reward (instrumental conditioning) in the absence of an explicit conditioned sensory stimulus (CS). They were then separately trained to associate a CS with noncontingent sucrose delivery (Pavlovian conditioning). Following this training, CS presentation increased lever pressing. Both systemic dopamine antagonist injection (Dickinson et al. 2000) and inactivation of the VTA (Murschall & Hauber 2006) reduced lever pressing in response to the CS. Conversely, lever pressing to the CS was potentiated by NAc amphetamine injection (Wyvell & Berridge 2000). Finally, injection of amphetamine into the NAc increased cue responding during conditioned reinforcement. Training in these experiments is similar to the training used in Pavlovian-instrumental transfer experiments, but in the test session, the CS is presented contingent on the animal's instrumental response. The

response-promoting effect of the CS was greatly increased by NAc amphetamine injection, and the increase was blocked by disruption of NAc dopamine function (Taylor & Robbins 1986, Wolterink et al. 1993). Thus, increasing NAc dopamine is sufficient to increase the rate of responding to reward-associated cues.

VTA Dopaminergic Neurons Modulate the Firing of NAc Neurons that Promote Responding to Reward-Predictive Cues

Recordings of NAc neurons during tasks dependent on NAc dopamine provide insight into how VTA neurons promote appetitive behaviors (e.g., the DS task and Pavlovian approach) (Day et al. 2006, Ghitza et al. 2003, Nicola et al. 2004b, Wan & Peoples 2006). In these and other tasks, subpopulations of NAc neurons are either excited or inhibited just before and during operant behavior (Carelli 2002) and during reward consumption (Janak et al. 1999, Nicola et al. 2004c, Roitman et al. 2005, Taha & Fields 2005, Wilson & Bowman 2004). Reversible inactivation of the VTA by microinjection of the $GABA_B$ agonist baclofen reduces NAc dopamine levels (Westerink et al. 1996) and selectively and reversibly abolishes both the behavior and the NAc neuronal excitations and inhibitions evoked by reward predictive sensory cues (DSs) (Yun et al. 2004b). DS-elicited behaviors are also blocked by dopamine antagonists injected directly into the NAc. Taken together, these results strongly support the idea that cue-evoked appetitive behavior is promoted by transient dopamine-dependent changes in NAc neuronal firing in response to the reward-predictive cue.

Pharmacological studies provide some insight into the mechanism by which mesolimbic dopamine promotes learned cue–elicited appetitive behavior. Although NAc dopamine antagonists block DS-elicited behavioral responses, these responses persist when the NAc is inactivated with either TTX or glutamate antagonists. Furthermore, NAc dopamine antagonists decrease, whereas TTX or glutamate antagonists increase, responses to an unreinforced sensory cue and increase the number of responses made on an unreinforced lever (Yun et al. 2004a). One explanation for this paradox is that distinct subpopulations of NAc neurons promote one behavior while inhibiting competing behaviors. If such subpopulations exist, dopamine released in the NAc could promote the behavior elicited by the most salient reward-predictive cue by enhancing activity in the subpopulation excited by that cue while inhibiting other subpopulations that mediate competing behaviors (e.g., exploration, grooming, etc.) (Nicola 2007, Yun et al. 2004a). The finding that the reward-predictive DS elicits both excitations and inhibitions in different subpopulations of NAc neurons, and that both are abolished by VTA inactivation (Yun et al. 2004b), is consistent with this view.

SUMMARY AND CONCLUSIONS

The SNc and VTA are the sole source of dopamine innervation for the striatum and limbic forebrain. In these target regions, measurements of dopamine release and pharmacological or molecular genetic manipulation of dopamine or its receptors have implicated midbrain dopaminergic neurons in positive reinforcement and in the expression of learned appetitive behaviors. These two functions are distinct conceptually, but they can be difficult to separate experimentally because the performance of a learned motor act is a prerequisite for it to be reinforced and, conversely, reinforcement is typically expressed as a change in the probability and vigor of the motor act that is reinforced. Single-unit electrophysiology provides an essential analytical tool to separate these two functions. To implicate a neuron in the selection and initiation of an action, a change in activity must precede the onset of that action. To implicate a neuron in positive reinforcement

(or extinction) of an action, one must demonstrate that a change in its activity occurs at a short interval after the outcome of that action. In fact, in vivo recordings of midbrain (SNc and VTA) neurons in behaving animals demonstrate activity changes consistent with both functions.

Although SNc and VTA neurons show similar patterns of activity in awake rodent and primate, the SNc consists largely of dopaminergic neurons whereas the VTA consists of distinct subpopulations of neurons that are heterogeneous in both neurotransmitter content and projection target. More than 40% of rat VTA neurons are not dopaminergic, and VTA neurons project to a variety of targets including the NAc, PFC, hippocampus, and amygdala. Furthermore, the proportion of the VTA projection that is dopaminergic varies greatly with projection target, and GABA and glutamate are present in VTA projections to both PFC and NAc. Subpopulations of VTA neurons, defined by neurotransmitter and projection target, receive different afferent input and can be independently controlled. Although a VTA dopaminergic projection to the NAc can promote positive reinforcement, other VTA projections, including to the PFC and amygdala, can also contribute to this function. Furthermore, VTA-dependent but dopamine-independent positive reinforcement can be produced, possibly through a circuit that includes a VTA-to-PFC-to-LDT/PPTg projection.

In addition to mediating positive reinforcement, VTA dopaminergic neurons projecting to the NAc excite neurons that initiate and promote behaviors elicited by reward-predictive cues. VTA neuron firing and dopamine release in target nuclei also increase with stress, novelty, and noxious stimulation. Clearly, future studies of the VTA will need to acknowledge the fact that its constituent neurons are heterogeneous in their anatomical connections, neurotransmitter content, and contributions to behavior.

ACKNOWLEDGMENTS

This work was supported by funds provided by the State of California for medical research on alcohol and substance abuse through the University of California, San Francisco, by the UCSF Wheeler Center for the Neurobiology of Addiction, by Department of the Army award DAMD17-03-1-0059 to H.L.F., and by NIH grants DA015686 to G.O.H. and DA019473 to S.M.N. The content of the information does not necessarily reflect the position or the policy of the Government, and no official endorsement should be inferred.

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