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Reward and aversion in a heterogeneous midbrain dopamine system

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Abstract

The ventral tegmental area (VTA) is a heterogeneous brain structure that serves a central role in motivation and reward processing. Abnormalities in the function of VTA dopamine (DA) neurons and the targets they influence are implicated in several prominent neuropsychiatric disorders including addiction and depression. Recent studies suggest that the midbrain DA system is composed of anatomically and functionally heterogeneous DA subpopulations with different axonal projections. These findings may explain a number of previously confusing observations that suggested a role for DA in processing both rewarding as well as aversive events. Here we will focus on recent advances in understanding the neural circuits mediating reward and aversion in the VTA and how stress as well as drugs of abuse, in particular cocaine, alter circuit function within a heterogeneous midbrain DA system.

Keywords

dopamine; reward; aversion; mesocortical; mesolimbic; ventral tegmental area

1. Introduction

As investigators have delved deeper into the brain's neuronal networks underlying complex behaviors using innovative techniques such as optogenetics, it has become clear that previous notions about the functions and connectivity of individual cell types in the mammalian brain need to be revised. Nowhere is this more apparent than for midbrain dopamine (DA) cells. Over the past decade our view of the midbrain DA system has changed from a simple organization of anatomically separate DA neurons in the substantia nigra and ventral tegmental area (VTA) to a more complex system of DA neuron subtypes with different axonal projection and inputs, distinct anatomical, molecular and electrophysiological features (Ikemoto 2007; Lammel et al., 2008, 2011, 2012; Margolis et al., 2006, 2008) as well as additional co-transmitters such as GABA (Tritsch et al., 2012) and glutamate (El-Mestikawy et al., 2011). In addition, it is well established that midbrain DA neurons are intermingled and connected with different subpopulations of GABAergic and glutamatergic neurons (Hnasko et al., 2012; Margolis et al., 2012, Yamaguchi et al.,

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2011). Thus, perhaps not surprisingly, DA neuron activity has been associated with a variety of brain functions including the signaling of reward, aversion, salience, uncertainty and novelty (Bromberg-Martin et al., 2010; Schultz 2007; Ungless et al., 2010). Classic work demonstrated that midbrain DA neurons evince characteristic phasic responses to rewards and cues that predict rewards, and are inhibited by aversive events (Schultz 1997; Ungless et al., 2004). Furthermore, it has been suggested by many investigators that drugs of abuse hijack the brain's "reward system" and that among the most important neural circuit modifications that contribute to the development of addiction are changes in the properties of excitatory synapses on midbrain VTA DA neurons (Luscher and Malenka 2011). Here, we provide an overview of how different circuits involving distinct VTA DA neuron subpopulations may contribute to the role of DA in reward- and aversion-related behaviors. In addition, we highlight how these circuits may contribute to drug addiction.

2. Heterogeneity of the mesocorticolimbic dopamine system

Midbrain DA neurons are mainly located in three nuclei, the VTA (A10), the retrorubral field (RRF, A8) and substantia nigra pars compacta (SNc, A9) as well as sparsely scattered in the substantia nigra pars reticulata (SNp). The two major subnuclei of the VTA are the parabrachial pigmented (PBP) and paranigral (PN) nuclei. Furthermore, the caudal linear nucleus (CLi), interfascicular nucleus (IF), and rostral linear nucleus of the raphe (RLi) are often also considered VTA subregions (Oades and Halliday 1987; Swanson, 1982).

In the late 1980s and early 1990s using *in vitro* slice recordings researchers began to classify DA neurons as principal (mostly DAergic) and secondary (GABAergic) (Grace & Onn, 1989; Johnson & North, 1992) on the basis of distinct physiological and pharmacological properties as well as tyrosine hydroxylase (TH) immunohistochemistry (Grace & Onn, 1989; Johnson & North, 1992). Subsequent studies showed a third group of VTA neurons (tertiary neurons), which are hyperpolarized by serotonin and opioids but it appears that only one-third of these neurons are DAergic (Cameron et al., 1997). The neurochemical phenotype of the remaining two-third of the tertiary cells has not been clearly defined.

Based on these findings virtually all *in vitro* electrophysiological studies, many of them studying drug-induced synaptic adaptations, have considered VTA DA midbrain neurons as a single population (e.g. Argilli et al., 2008; Bellone and Luscher, 2006; Borgland et al., 2004; Chen et al., 2008; Dong et al., 2004; Engblom et al., 2008; Heikkinen et al., 2009; Liu et al., 2005; Saal et al., 2003; Stuber et al., 2008; Ungless et al., 2001). The identification of putative DA cells was based on low-frequency pacemaker activity, broad action potentials, hyperpolarization by DA via D2 receptors or the presence of the so-called Ih current, generated by hyperpolarization-activated cyclic nucleotide-regulated cation channels (HCN channels) (Kitai et al., 1999). The reliability of criteria for identification of DA neurons in slice recordings has generated some confusion (Ungless and Grace, 2012) because: (1) single-cell labeling studies have revealed that in the VTA the presence of Ih is not always consistent with a DAergic phenotype (Margolis, 2008; Zhang et al., 2010), (2) some VTA DA neurons do not respond to DA application (Bannon and Roth, 1983; Lammel et al., 2008) and (3) VTA DA neurons have been identified that have very small or no Ih (Ford et al., 2006; Hnasko et al., 2012; Jones and Kauer 1999; Lammel et al., 2008, 2011; Witten et al., 2011; Zhang et al., 2010). These findings likely account for the variability in using Ih as a reliable marker for the DA phenotype (Jones and Kauer 1999; Margolis et al., 2006; Ungless and Grace, 2012; Wanat et al., 2008; Zhang et al., 2010).

While species differences may contribute to this variability (Courtney et al., 2012) it is also likely that recordings have been performed in different subregions of the VTA (Zhang et al., 2010). Many studies that identified putative DA neurons based on their expression of a large

Ih performed patch clamp recording from horizontal slices and focused on a specific subregion of the VTA defined as the region medial to the MT (medial terminal nucleus of the accessory optical tract). While in this specific VTA subregion the correlation between Ih and DA phenotype might be high, other VTA subregions (such as the PN and medial PBP of the posterior VTA) have often been ignored and may contain DA neurons with a distinct electrophysiological profile (Lammel et al., 2008). For a more complete discussion of the criteria used to identify DA neurons in the VTA and SN *in vivo* and *in vitro*, we refer readers to a recent review on the topic (Ungless & Grace, 2012).

More recent studies began to classify VTA DA neurons based on their projection targets (Beckley et al., 2013; Ford et al., 2006; Ikemoto, 2007; Lammel et al., 2008; Margolis et al., 2006, 2008) since it is well established that midbrain DA neurons have widespread projections throughout the brain (Bjoerklund and Dunnett, 2007; Ikemoto, 2007). The vast majority of VTA DA neurons appear to send projections to non-overlapping target areas and thus these ascending VTA projections can be conceptualized as independent parallel lines (Fallon, 1981; Albanese and Minciacchi, 1983; Lammel et al., 2008). Combining retrograde tracing, brain slice patch clamp recordings and single cell mRNA quantification of various DAergic marker genes revealed that the mesocorticolimbic DA system consists of several DA neuron subpopulations with different properties (Lammel et al., 2008). Importantly, some VTA DA subpopulations did not exhibit some of the classic properties that previously have been used to identify DA neurons. Injection of fluorescent retrobeads into different brain areas of adult mice and subsequent anatomical analysis of coronal brain slices demonstrated that DA neurons in medial posterior VTA (PN and medial PBP) selectively project to the NAc medial shell and core, medial prefrontal cortex (PFC), and basolateral amygdala (BLA). These “non-conventional” VTA DA neurons had no or only very little Ih and also did not exhibit typical action potential waveforms and firing patterns (Figure 1). In contrast, DA neurons, which project to NAc lateral shell, were predominantly located in the lateral posterior and anterior VTA (lateral PBP). They exhibited a prominent Ih and firing patterns reminiscent of conventional VTA DA neurons (Hnsasko et al., 2012; Lammel et al., 2008; Ungless and Grace, 2012; Zhang et al., 2010) (Figure 2). Thus, it seems likely that the DA neurons in the lateral VTA (lateral PBP) projecting to NAc lateral shell are the same as those analyzed in studies that focused on the area medial of the MT using horizontal brain slices. The idea that “conventional” DA neurons are located in the lateral portions of the VTA is consistent with recent studies showing that VTA DA neurons have a differential latero-medial cellular expression of AADC, VMAT2, DAT, D2R and VGluT2 (Li et al., 2012). Interestingly, there is also a high degree of molecular and electrophysiological similarity between the populations of DA neurons within the lateral VTA and adjacent SN (Lammel et al., 2008; Li et al., 2012). The discovery of DA subpopulations with distinct anatomical, electrophysiological and molecular properties supports the idea that they also could be involved in mediating different behavioral responses to specific environmental stimuli (Bromberg-Martin et al., 2010).

Unlike VTA DA neurons, SNc DA neurons were thought to be a more homogenous cell population. Indeed, *in vivo* as well as *in vitro* criteria for identification of SNc DA neurons seem to be more reliable than for VTA DA neurons (Ungless and Grace, 2012). However, recent studies report that DA neurons in the SNc exhibit functional heterogeneity that may contribute to their diverse roles in behavior (Brown et al., 2009; Henny et al., 2012; Schiemann et al., 2012). Specifically, SNc DA cell functional heterogeneity appears to be correlated with differences in dendrite architecture and afferent connectivity (Henny et al., 2012). Further evidence for heterogeneity in SNc DA cells comes from the observation that K-ATP channels gate bursting selectively in medial SN DA neurons projecting to the dorsomedial striatum but not in lateral SN DA neurons, which project to the dorsolateral striatum as well as VTA DA neurons (Schiemann et al., 2012). DA neuronal signaling has

recently become even more complex with the demonstration that SNc DA cells release GABA resulting in the inhibition of dorsal striatal medium spiny neurons (Tritsch et al., 2012). Because this GABA release is dependent on the vesicular monoamine transporter, VMAT2, other DA neuron subpopulations may also co-release GABA although this prediction needs to be tested experimentally.

Although this review's focus is the heterogeneity of VTA DA neurons, it is important to mention that a significant proportion of cells in what are traditionally considered DA nuclei are GABAergic or glutamatergic (Nair-Roberts et al., 2008). Furthermore, there are considerable heterogeneities within these neuronal phenotypes (Margolis et al., 2012). Approximately 20–40% of VTA cells are GABAergic (Carr and Sesack, 2000a; Margolis et al., 2012; Nair-Roberts et al., 2008), which not only form local contacts onto both DA and non-DA VTA neurons (Omelchenko and Sesack, 2009) but also project to several forebrain regions (Fields et al., 2007). Recently, it has been reported that VTA GABA neurons projecting to the NAc inhibit accumbal cholinergic interneurons in a manner that enhances stimulus–outcome learning (Brown et al., 2012). However, activation of VTA GABA neurons projecting to NAc does not alter reward consumption (Van Zessen et al., 2012). Thus, VTA GABAergic neurons, like VTA DA neurons, may be a specialized and functionally heterogeneous cell population (Margolis et al., 2012).

VTA glutamate neurons selectively express VGLUT2 mRNA and can mainly be found in the medial VTA. It has been suggested that they represent approximately 2–3% of the total VTA cell population (Nair-Roberts et al., 2008). These cells rarely co–express mRNAs for tyrosine hydroxylase (TH) or glutamic acid decarboxylase (GAD) indicating that the vast majority are non-DAergic and non-GABAergic (Nair-Roberts et al., 2008; Yamaguchi et al. 2007). However, it has also been reported that a small subset of DA neurons co-release DA and glutamate (Chuhma et al. 2009; Hnasko et al. 2010; Stuber et al., 2010; Tecuapetla et al., 2010; but see Moss et al., 2011). Importantly, medial VTA glutamate neurons, which were identified in transgenic mice that express EGFP under control of the VGLUT2 promotor, exhibit a small Ih making them indistinguishable from medial VTA DA neurons, but not from lateral VTA DA neurons (Hnasko et al., 2012). VTA glutamate neurons, like VTA DA and GABAergic neurons, project to several different brain areas including NAc, lateral habenula, ventral pallidum, amygdala and medial PFC (Hnasko et al., 2012; Yamaguchi et al., 2011).

3. Dopamine neuron activity during reward and aversion

For decades investigators have been studying the relationship between midbrain DA neuron activity and reward (Schultz, 1997, 2007, 2012; Wise and Rompre, 1989). The central role of DA neuron activity in reward-related processes has been well-established by electrophysiological studies. Single unit recordings in primates performing an operant task showed that putative DA neurons are phasically excited (i.e. they exhibit burst discharges of action potentials) by unexpected food rewards (Schultz, 1997). An extensive and important body of work subsequently supported the heuristically attractive hypothesis that DA neurons phasic activity encodes reward prediction errors, the discrepancy between an expected reward and the actual outcome. Thus, the omission of an expected reward had the opposite effect on putative DA neuronal activity and silent periods were associated with negative reward prediction errors (Schultz, 1997). The reward prediction error hypothesis has been confirmed in humans using fMRI (D'Ardenne et al., 2008) and more recently using optogenetic single-cell identification of VTA DA neurons in mice (Cohen et al., 2012).

While there is strong evidence in support of the hypothesis that DA neurons signal reward prediction errors, it seems unlikely that this is their only function and that all DA neurons,

independent of their projection targets, subserve this single function. Indeed, it is well-established that aversive, stressful and salient events can excite midbrain DA neurons and cause DA release in target structures (Abercrombie et al., 1989; Anstrom and Woodward, 2005; Bassareo et al., 2002; Brischoux et al., 2009; Bromberg-Martin et al., 2010; Cohen et al., 2012; Guarraci and Kapp, 1999; Mantz et al., 1989; Matsumoto and Hikosaka, 2009; Ungless et al., 2010; Young, 2004). However, VTA DA neurons can also be inhibited by aversive stimuli (Mirenowicz and Schultz, 1996; Ungless et al., 2004) and it can be postulated that any increase in DA neuron activity caused by an acute aversive stimulus (e.g. foot shock) might be due to the rewarding effects of terminating the stimulus (Tanimoto et al., 2004). Indeed, most DA neurons that were inhibited by foot shocks also showed a phasic excitation at the termination of the foot shock (Brischoux et al., 2009). Studies in rats using juxtacellular labeling-recording techniques supported this idea and also suggested that the VTA neurons that were excited by aversive stimuli were non-DAergic (Ungless et al., 2004). However, subsequently the same group reported that DA neurons localized in the medioventral VTA were excited at the onset of aversive stimuli (Brischoux et al., 2009).

An unresolved question is what proportion of DA neurons are excited by aversive stimuli and whether they are a specific subpopulation that project to specific targets. While it has been argued that a relatively small proportion of DA neurons respond to aversive stimuli (Schultz, 2012), a recent study in mice found that roughly equivalent numbers of putative VTA DA neurons are activated or inhibited by tail pinch (Zweifel et al., 2011). Furthermore, the single unit activity of many optogenetically identified DA neurons in mice increased in response to an aversive stimulus (Cohen et al., 2012). It is also possible that through sampling biases VTA DA neurons that respond to aversive stimulation have been missed. Indeed, as mentioned earlier in this review, some DA subpopulations are located in a specific subregion of the caudal VTA (PN and medial PBP). Importantly, these cells have unconventional electrophysiological properties (Lammel et al., 2008) (Figure 1). Thus, in previous single unit recording studies they might not only have been missed but also could have been misinterpreted as non-DAergic if no verification of neurochemical phenotype of the recorded cells was performed.

4. Afferent control of dopamine neuron activity

The VTA receives both excitatory and inhibitory inputs from a broad distribution of brain areas (Geisler et al., 2007; Sesack and Grace 2010; Watabe-Uchida et al., 2012). Activation of postsynaptic NMDA receptors are particularly important for driving high-frequency bursts of action potentials (Korotkova et al., 2004) and are critical for reward-dependent learning (Zweifel et al., 2009). Clearly, an important line of research is to determine the anatomical connectivity of afferents to VTA neurons and their behavioral functions. While electrical stimulation has been used to activate known VTA inputs *in vivo* and this work has provided important information, with the advent of optogenetics and novel viral tracing strategies (Osakada et al., 2011; Tye and Deisseroth, 2012), it is now possible to determine the anatomical connectivity of specific inputs unequivocally and address their putative behavioral functions. Previous work demonstrated that electrical stimulation of the laterodorsal tegmentum (LDT) promotes burst firing of putative VTA DA neurons and increases DA release in the NAc (Forster and Blaha, 2000; Lodge and Grace, 2006). This is consistent with classical tracing work, which revealed that the LDT projects mainly to the VTA (Cornwall et al., 1990) and makes excitatory synapses on DA neurons projecting to the NAc (Omelchenko and Sesack, 2005). Interestingly, presumed inhibitory LDT inputs seems to be selective for mesoprefrontal but not mesolimbic DA neurons (Omelchenko and Sesack, 2005). In contrast, mesoprefrontal DA neurons receive excitatory input from the PFC (Carr and Sesack, 2000b).

Using *in vivo* rabies-virus mediated expression of channelrhodopsin-2 (ChR2) for optical control of LDT projection neurons combined with *in vitro* acute slice recordings from identified DA neurons with known projection targets, we found that LDT axons predominately make excitatory synaptic connections on DA neurons projecting to the NAc lateral shell (Lammel et al., 2012). Consistent with the idea that DA release in the NAc promotes reinforcement (Steinberg and Janak; 2012; Witten et al., 2011) phasic light activation of these LDT inputs within the VTA elicits conditioned place preference (CPP), which was prevented by infusion of DA receptor antagonists into the NAc (Lammel et al., 2012).

The same approach was taken to examine the connectivity and behavioral functions of the inputs to the VTA from the lateral habenula (LHb), a brain area thought to be critical for mediating behavioral responses to aversive stimuli and when expected rewards do not occur (Hikosaka, 2010). Consistent with previous findings (Christoph et al., 1986; Hong et al., 2011; Ji and Shepard, 2007), optogenetic activation of LHb axons in acute slices generated excitatory synaptic currents in GABAergic neurons in the rostromedial tegmental nucleus (RMTg) (Lammel et al., 2012; Stamatakis and Stuber, 2012), which is also known as the tail of the VTA (Kaufling et al., 2009). Surprisingly, these same inputs made excitatory synaptic connections onto DA neurons projecting to the mPFC but connections onto other DA neuron subpopulation were not detected (Lammel et al., 2012). Furthermore, the RMTg GABAergic neurons activated by LHb inputs generated inhibitory synaptic currents in DA cells projecting to NAc lateral shell. Thus, activation of LHb inputs to the VTA would be expected to activate DA neurons projecting to mPFC and inhibit DA neurons projecting to NAc lateral shell and therefore generate a profoundly different behavioral response than LDT axon stimulation. Indeed, phasic optogenetic activation of LHb inputs to the VTA elicited conditioned place aversion (CPA), which was prevented by infusion of a DA receptor antagonist into the mPFC (Lammel et al., 2012). Activation of LHb inputs to the VTA has also been shown to elicit both passive and conditioned behavioral avoidance (Stamatakis and Stuber, 2012).

These results suggest that different afferent inputs to the VTA influence distinct DA neuron subpopulations and VTA “microcircuits” and as a consequence influence behavior in profoundly different ways. Recent anatomical tracing studies using recombinant rabies viruses have revealed that the brain areas sending projections to the VTA are much more abundant than previously envisioned (Watabe-Uchida et al., 2012). The approaches taken in this recent work (Lammel et al., 2012) will be helpful in defining the synaptic connectivity of these inputs and their behavioral functions with the long-term goal of generating a much more comprehensive understanding of the circuits in which VTA cells are embedded.

5. VTA dopamine neurons and stress-induced depression

Chronic stress is an important diathesis for depression in humans and is used to generate rodent models of depression. It has long been postulated that malfunction of the brain’s reward circuitry may play an important role in mediating key symptoms of stress-elicited behaviors, including depression (Friedman et al., 2008; Nestler and Carlezon, 2006; Willner et al., 1991; Yadid and Friedman, 2008). Here we will briefly review the effects of stress on midbrain DA neurons. Both chronic restraint stress and repeated social defeat stress have been found to increase the spontaneous and burst firing of VTA DA neurons *in vivo* and *ex vivo* in brain slices (Anstrom and Woodward, 2005; Cao et al., 2010; Feder et al., 2009; Krishnan et al, 2007). Importantly, in the repeated social defeat stress model of depression, the increase in DA neuron activity only occurred in susceptible but not in resilient mice (Cao et al., 2010; Feder et al., 2009; Krishnan et al, 2007) and lasted for 3 weeks after the social defeat protocol (Razzoli et al., 2011). Moreover, the increased firing rates and bursting

events in VTA DA neurons were largely reversed by chronic administration of the antidepressant fluoxetine (Cao et al., 2010).

In the context of this review, a critical question is whether a specific subpopulation of VTA DA neurons exhibits this stress-induced change in firing patterns and which subpopulations contribute to the stress-induced behavioral changes. An *in vivo* microdialysis study in rats found that levels of DA in both NAc and PFC were elevated by a social defeat stress (Tidey and Miczek, 1996). Much more recently, optogenetic activation of VTA DA neuron phasic firing was shown to elicit a susceptible, depression-associated phenotype in mice undergoing a subthreshold social defeat protocol as well as in previously resilient animals that had been subjected to repeated social defeat stress (Chaudhury et al., 2012). Surprisingly, these dramatic behavioral effects appear to be mediated by release of DA in NAc as terminal field stimulation of DA fibers in NAc, but not mPFC, induced susceptibility to social defeat stress (Chaudhury et al., 2012). In marked contrast, another group found that optogenetic activation of VTA DA neurons reversed chronic stress-induced depression-associated behaviors while inhibition of these neurons promoted the same behaviors (Tye et al., 2012). Infusion of DA receptor antagonists into the NAc prevented the consequences of VTA DA neuron activation in one key depression-associated behavioral assay suggesting that DA release specifically in the NAc is required for the anti-depressant consequences of VTA DA neuron activation.

Providing an explanation for the discrepancies in these two studies is challenging. Both studies demonstrate that activity of VTA DA neurons projecting to NAc importantly influence depression-associated behaviors but one study concludes such activity is pro-depressant while the other concludes it is anti-depressant. These findings also need to be interpreted in the context of other studies reporting that optogenetic activation of VTA DA neurons can induce CPP and facilitate the development of positive reinforcement during reward seeking (Tsai et al., 2009; Adamantidis et al., 2011). It is possible, although disconcerting, that the different stress protocols used in the two studies (i.e. repeated social defeat stress versus chronic mild stress) caused dramatically different effects on the circuits being studied. Alternatively, there may have been differences in the specific subpopulations of VTA DA neurons that were manipulated. VTA DA neurons projecting to NAc can be subdivided based on their specific projection target (i.e. core versus medial shell versus lateral shell) and these differences may be behaviorally important (Ikemoto, 2007; Lammel et al., 2008, 2011, 2012). The potential involvement of the LHb in depression (Li et al., 2011; Morris et al., 1999; Shumake and Gonzalez-Lima, 2003) and the specific synaptic connectivity of LHb inputs in the VTA (Hikosaka, 2010; Lammel et al., 2012) provide additional evidence supporting the importance of further exploring the roles of specific VTA DA neuron subpopulations and the afferent inputs that drive them in depression-associated behaviors.

6. Effects of drugs of abuse on VTA dopamine neuron excitatory synapses

The mesocorticolimbic DA system has a central role in the acquisition of behaviors that are inappropriately reinforced by drugs of abuse. Drugs of abuse such as cocaine, morphine, nicotine and amphetamine have different pharmacological effects (for recent review see Luscher and Malenka, 2011) yet they all significantly impact reward and motivation at least in part by increasing DA release in the NAc. However, there is a growing body of evidence that motivational stimuli, stress, as well as drugs of abuse evoke DA release in anatomically and functionally distinct DA projections (Abercrombie et al., 1989; Aragona et al., 2008, 2009; Bassareo et al., 2002; Ikemoto 2007; Ito et al., 2000; Lammel et al., 2011; Lemos et al., 2012; Porter-Stransky et al., 2011; Roitman et al., 2008; Salamone and Correa, 2012; Young, 2004). Increased extracellular DA concentrations, such as that elicited by abused

drugs or motivational stimuli, likely facilitate learning and memory (Luscher and Malenka, 2011; Schultz, 2012). Thus, it is not surprising that an important research topic over the last decade has been to elucidate how motivational stimuli, stress, and drugs of abuse induce adaptations of VTA DA neuron excitatory inputs, which drive spiking of VTA DA neurons (Argilli et al., 2008; Bellone and Luscher, 2006; Borgland et al., 2004; ; Chen et al., 2008; Dong et al., 2004; Engblom et al., 2008; Heikkinen et al., 2009; Liu et al., 2005; Luscher and Malenka, 2011; Saal et al., 2003; Stuber et al., 2008; Ungless et al., 2001).

Briefly, these studies all support the hypothesis that all drugs of abuse as well as strong acute stress are sufficient to induce a potentiation of AMPA receptor-mediated synaptic transmission in VTA DA neurons. Natural rewards such as food can also cause LTP-like synaptic changes in VTA DA neurons but these are transient whereas the potentiation caused by self-administration of cocaine lasts at least 3 months after abstinence (Chen et al., 2008). Cocaine administration has also been reported to cause a decrease in NMDA receptor-mediated synaptic responses (Mameli et al., 2011). Importantly, these initial drug-induced synaptic adaptations in VTA DA neurons are thought to be critical for subsequent circuit modifications in downstream targets such as the NAc (Mameli et al., 2009; Wolf and Tseng, 2012).

A limitation of all of these studies is that the putative VTA DA neurons from which recordings were made were commonly identified by the presence of a large Ih, which, as reviewed above, is not present in important subpopulations of VTA DA neurons. To address this limitation, we examined the modification of excitatory synapses on identified VTA DA neuron subpopulations caused by cocaine administration as well as a strong aversive stimulus (Lammel et al., 2011). A single injection of cocaine strongly modified excitatory synapses on DA neurons projecting to the NAc medial shell but not synapses on DA neurons projecting to mPFC, as assayed by the ratio of AMPAR-mediated synaptic responses to NMDAR-mediated synaptic responses (see Kauer and Malenka, 2007 for discussion of the utility of this so-called AMPAR/NMDAR assay). An aversive stimulus (i.e. formalin injection into a single hindpaw) had the opposite effect; modifying the synapses on the medial VTA DA neurons projecting to mPFC but not those on DA neurons projecting to NAc medial shell. Both the rewarding and aversive experiences modified synapses on DA neurons projecting to NAc lateral shell. These results provide further evidence that different subpopulations of VTA DA neurons participate in related but independent circuits that contribute to the brain's response to reward, aversion and saliency.

Using optogenetics, a critical question that can now be addressed is whether distinct excitatory inputs making synapses on VTA DA neurons are modified differently by rewarding versus aversive stimuli. And if so, what are the circuit and behavioral consequences of such input-specific synaptic modifications. VTA DA neurons also receive inhibitory synaptic inputs from NAc medium spiny neurons, the RMTg as well as from local interneurons within the VTA, which themselves receive projections from the NAc. A more definitive elucidation of this inhibitory circuitry within the VTA and how these inhibitory synapses on VTA DA neuron are modified by experience will also be important for a more comprehensive understanding of the neural circuit mechanisms mediating reward- and aversion associated behaviors.

7. Conclusions

We have attempted to concisely summarize the evidence supporting the idea that VTA DA neurons are heterogeneous not only in regard to their anatomical, molecular and electrophysiological properties but also in their response to salient appetitive and aversive stimuli. Heterogeneity in DA neurons and their behavioral functions has also been observed

in *Drosophila* (Claridge-Chang et al., 2009; Krashes et al., 2009; Liu et al., 2012), suggesting strong evolutionary pressure to conserve such heterogeneity as nervous systems evolved. Consistent with this view, many recent findings challenge the view that DA release in target structures has a primary or exclusive role in reward processing (Bromberg-Martin et al., 2010; Salamone and Correa, 2012) although arguments can be made that the primary role of DA neuron phasic activity remains the generation of reward prediction error signals (Schultz, 2012). Given DA's prominent role as a neuromodulator of neuronal excitability and synaptic function, intuitively it seems likely that DA release in different target structures containing complex neural networks composed of diverse cell populations (e.g. mPFC, dorsal and ventral striatum) will have different behavioral consequences. Recent studies investigating the contribution of DA subsystems in rodent models of depression, addiction, aversive behavior and Parkinson's disease (Aragona et al., 2009; Beckley et al., 2013; Brischoux et al., 2009; Chaudhury et al., 2012; Lammel et al., 2011, 2012; Schieman et al., 2012; Tye et al., 2012) provide further evidence supporting the importance of a heterogeneous midbrain DA system in mediating the pathophysiology of prominent neuropsychiatric disorders. Over the next decade, we anticipate that further work on elucidating the organization, function and modification of midbrain DA circuits will importantly contribute to understanding how motivational systems are organized in the brain and mediate adaptive and pathological behaviors.

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Abbreviations

DA	dopamine
VTA	ventral tegmental area
RRF	retrorubral field
SN	substantia nigra
SNc	substantia nirgra pars compacta
SNr	substantia nigra pars reticulata
ml	medial lemniscus
IPN	interpeduncular nucleus
CM	mammillary body
mVTA	medial VTA
lVTA	lateral VTA
fr	fasciculus retroflexus
PBP	parabrachial pigmented nucleus
PN	paranigral nucleus
CLi	caudal linear nucleus
IF	interfascicular nucleus
RLi	rostral linear nucleus of the raphe

MT	medial terminal nucleus of the accessory optical tract
PFC	prefrontal cortex
NAc	nucleus accumbens
BLA	basolateral amygdala
AADC	amino acid decarboxylase
VMAT2	vesicular monoamine transporter 2
DAT	dopamine transporter
D2R	dopamine D2 receptor
VGluT2	vesicular glutamate transporter 2
KATP	ATP sensitive potassium channel
TH	tyrosine hydroxylase
GAD	glutamic acid decarboxylase
LDT	laterodorsal tegmentum
LHb	lateral habenula
RMTg	rostromedial tegmental nucleus
ChR2	channelrhodopsin 2
AMPAR	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor
NMDAR	N-methyl-D-aspartate
MSN	medium spiny neuron

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Highlights

- Midbrain dopamine neurons have heterogeneous molecular and physiological properties
- DA neurons may have distinct behavioral functions based on their projection targets
- Inputs to the ventral tegmental area synapse on different neuron subpopulations
- DA neurons participate in distinct circuits mediating partially distinct functions

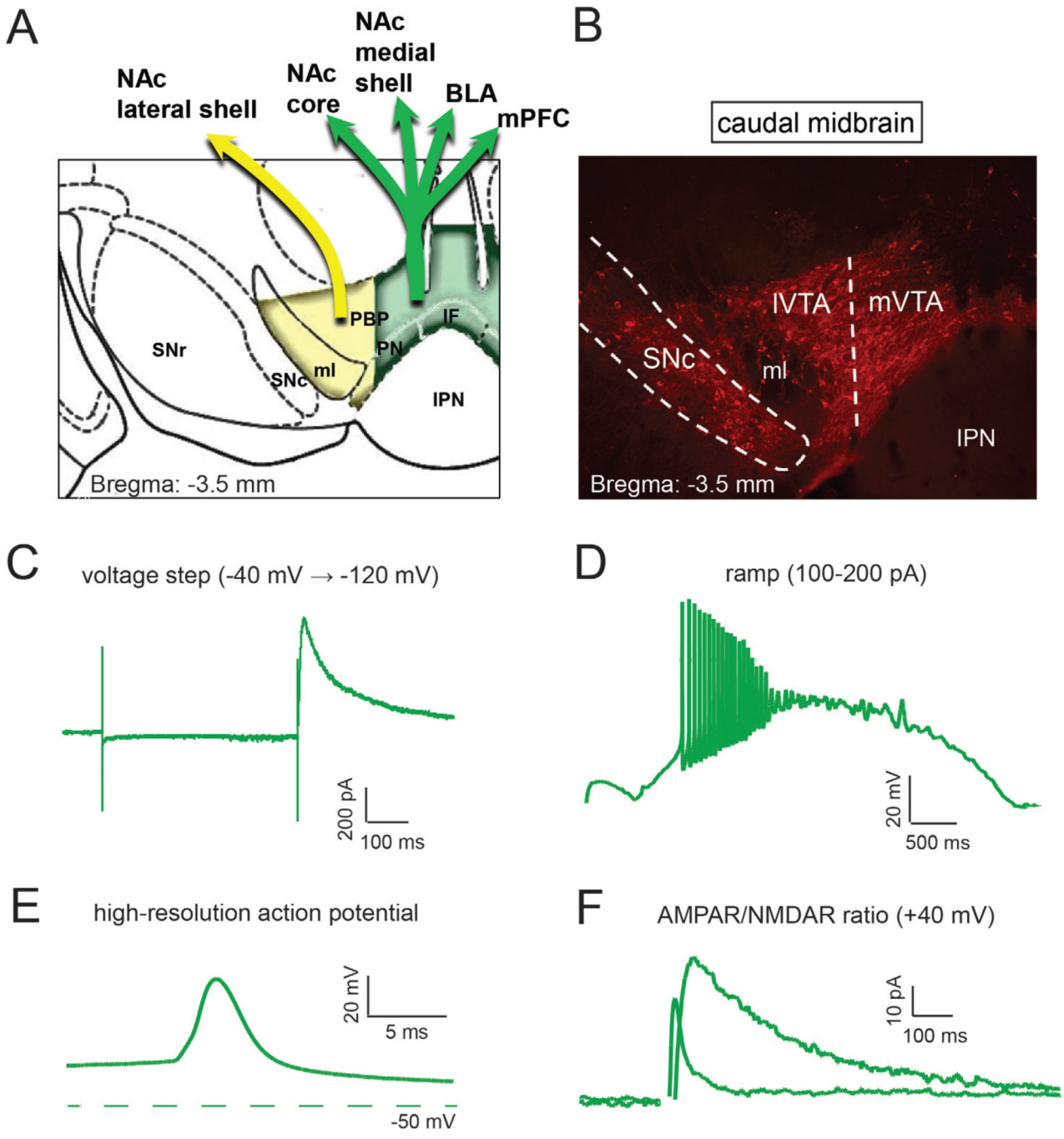

Figure 1. Dopamine neurons with unconventional electrophysiological properties are mainly located in the medial VTA of the caudal midbrain

(A) Schematic drawing showing that dopamine (DA) neurons projecting to medial prefrontal cortex (mPFC), basolateral amygdala (BLA), nucleus accumbens (NAc) core, and NAc medial shell are predominantly located in the medial VTA (IF, medial PN and medial PBP nuclei) of the caudal midbrain. These VTA subregion and projections are highlighted in green. DA neurons projecting to NAc lateral shell can be found in the lateral VTA (lateral PBP nucleus) of the caudal midbrain (highlighted in yellow). (SNr, substantia nigra pars reticulatae; SNc, substantia nigra pars compacta; ml, medial lemniscus; PBP, parabrachial pigmented nucleus; PN, paranigral nucleus; IF, interfascicular nucleus.) **(B)** Fluorescence

microscope image (TH-immunocytochemistry in red) showing the location of DA neurons in lateral (lVTA) and medial (mVTA) VTA of the caudal midbrain. **(C, D, E, F)** DA neurons located in the mVTA projecting to mPFC, NAc medial shell, NAc core and BLA have unconventional electrophysiological properties including: **(C)** lack of a prominent Ih current, **(D)** high maximal firing frequencies (~20–30 Hz), **(E)** broad action potentials and small afterhyperpolarization and **(F)** a high AMPAR/NMDAR ratio under basal conditions (~0.6).

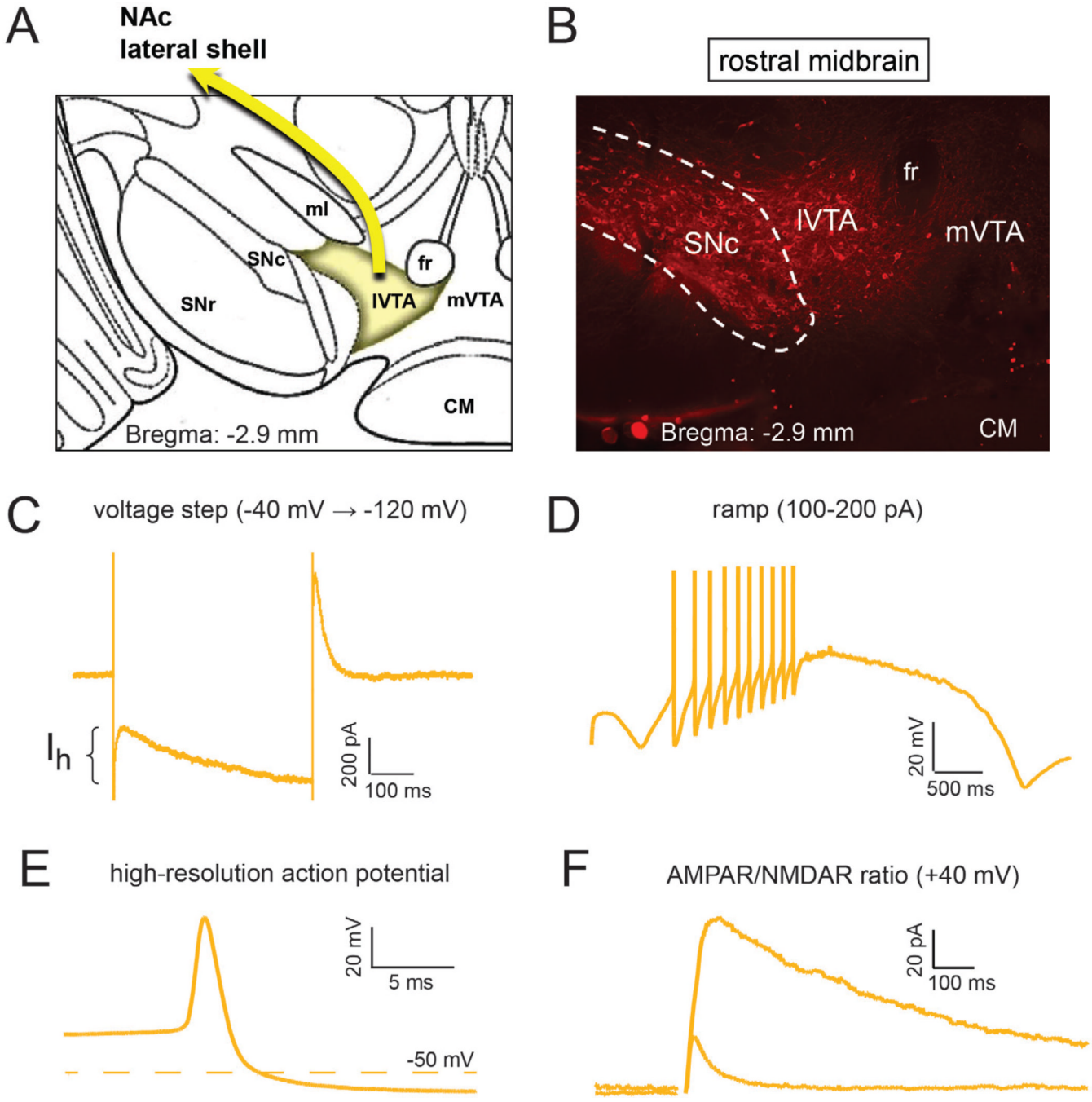

Figure 2. Many dopamine neurons with classical electrophysiological properties are located in the lateral VTA of the rostral midbrain

(A) Schematic drawing showing that dopamine (DA) neurons projecting to nucleus accumbens (NAc) lateral shell are located in the lateral VTA of the rostral midbrain. The VTA subregion and projection is highlighted in yellow. Note, that DA neurons projecting to NAc lateral shell can also be found in the caudal midbrain (see figure 1). (Abbreviations are the same as in Figure 1. fr, fasciculus retroflexus; CM, mammillary body.) **(B)** Fluorescence microscope image (TH-immunocytochemistry in red) showing that in the rostral midbrain the lateral VTA contains many more TH-immunopositive cells than the medial VTA (compare to figure 1B; see Lammel et al., 2008 for a detailed anatomic analysis of DA

projection neurons). **(C, D, E, F)** DA neurons located in the lVTA projecting to NAc lateral shell have classical electrophysiological properties including: **(C)** a prominent Ih current, **(D)** firing frequencies up to 10 Hz, **(E)** short action potentials with prominent afterhyperpolarizations and **(F)** a low AMPAR/NMDAR ratio under basal conditions (~0.4).