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ETHANOL ACTION ON DOPAMINERGIC NEURONS IN THE VENTRAL TEGMENTAL AREA: INTERACTION WITH INTRINSIC ION CHANNELS AND NEUROTRANSMITTER INPUTS

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Abstract

The dopaminergic system originating in the midbrain ventral tegmental area (VTA) has been extensively studied over the past decades as a critical neural substrate involved in the development of alcoholism and addiction to other drugs of abuse. Accumulating evidence indicates that ethanol modulates the functional output of this system by directly affecting the firing activity of VTA dopamine neurons, whereas withdrawal from chronic ethanol exposure leads to a reduction in the functional output of these neurons. This chapter will provide an update on the mechanistic investigations of the acute ethanol action on dopamine neuron activity and the neuroadaptations/plasticities in the VTA produced by previous ethanol experience.

I. Introduction

In addressing the neurobiological mechanisms underlying alcohol drinking and the development of alcohol addiction (alcoholism), one can broadly ask the following two types of questions: (1) Why do we drink and why do we keep drinking? (2) How do we get drunk/intoxicated? For the latter question, studying the action of ethanol in the cerebellum, for example, would shed light on the neurobiology of motor incoordination, which causes potentially life-threatening impairment, as occurs when driving under the influence of alcohol. However, depending on the dosage and rate of consumption, ethanol affects diverse brain structures subserving different behavioral and cognitive functions, leading to a wide array of drunken/intoxicated states ranging from sedation and impaired memory to euphoria, which could drive alcohol drinking, especially during the initial stage. Conversely, withdrawal from chronic ethanol consumption results in a “negative affective state” characterized by dysphoria, anhedonia, and depression, which persists even after somatic withdrawal symptoms have subsided (Koob, 2009; Trevisan *et al.*, 1998). Thus one might simply argue that part of the answer to the first question can be found in that to the second question (“We drink because we like the state of being drunk and we keep drinking because we want to avoid the affective symptoms of withdrawal”). But how so in neurobiological terms? In this review chapter, we will focus on the mesolimbic dopamine (DA) system, which comprises DA neurons in the midbrain ventral tegmental area (VTA) and their projection targets (the nucleus accumbens (NAc) and other limbic structures), in order to provide insights into the critical components of these two interrelated questions.

II. The Mesolimbic DA System and Alcohol Drinking

Multiple lines of evidence implicate the mesolimbic DA system in the behavioral actions of ethanol. For example, neurotoxin lesioning of DA terminals in the NAc has been shown to suppress the acquisition of ethanol-taking behaviors, measured with a two-bottle choice or self-administration paradigms (Ikemoto *et al.*, 1997; Rassnick *et al.*, 1993). A number of studies

have demonstrated that DA receptor blockade, either systemically or locally in the NAc, suppresses ethanol consumption/self-administration (Dyr *et al.*, 1993; Gonzales *et al.*, 2004; Hodge *et al.*, 1997; Myers and Robinson, 1999; Pfeffer and Samson, 1986, 1988; Rassnick *et al.*, 1992; Samson *et al.*, 1993). Furthermore, intra-VTA infusion of the DA D2 receptor agonist quinpirole, which would inhibit the firing activity of DA neurons, has been shown to attenuate ethanol consumption/self-administration (Hodge *et al.*, 1993; Nowak *et al.*, 2000).

Interestingly, expression of ethanol-induced conditioned place preference is also decreased by inhibition of DA neurons via intra-VTA infusion of a gamma-aminobutyric acid B receptor (GABA_B receptor) agonist, suggesting the involvement of DA neurons in the memory of ethanol-associated environmental stimuli (Bechtholt and Cunningham, 2005). More direct evidence for the role of VTA DA neurons in ethanol reinforcement comes from a series of studies demonstrating self-administration of ethanol directly into the VTA, which is blocked by co-administration of the D2 agonist quinpirole (Gatto *et al.*, 1994; Rodd *et al.*, 2005c, 2004; Rodd-Henricks *et al.*, 2003). Finally, a human study has reported that pharmacological blockade of DA synthesis can reduce ethanol-induced euphoria and behavioral stimulation (Ahlenius *et al.*, 1973).

It is well established that systemic administration of ethanol, either passively or by self-administration, produces an increase in DA levels in the NAc (Imperato and Di Chiara, 1986; Weiss *et al.*, 1993). This effect is thought to be caused by ethanol action in the VTA and not because of DA uptake inhibition in the NAc (Budygin *et al.*, 2001; Yim and Gonzales, 2000). Indeed, administration of ethanol directly into the VTA leads to DA release in the NAc (Ding *et al.*, 2009a; but see Ericson *et al.*, 2003). Consistent with these observations, it has been shown that ethanol stimulates the firing activity of VTA DA neurons both *in vivo* (Foddai *et al.*, 2004; Gessa *et al.*, 1985; Mereu *et al.*, 1984) and *in vitro* (Brodie *et al.*, 1999b, 1990). Accordingly, it is widely believed that ethanol-induced DA neuron stimulation and the resulting DA release in the NAc provide the neural substrate mediating the reinforcing and rewarding actions ethanol, that is, learning of the behaviors and environmental stimuli associated with ethanol consumption and, perhaps, perception of an aspect of the ethanol-induced interoceptive state ("euphoria").

In the following sections, we will first describe the regulation and function of VTA DA neurons and DA output in general. Then, we will discuss the neural mechanisms underlying ethanol actions on DA neuron activity and the neuroadaptations/plasticities in DA neurons produced by previous ethanol exposure. It should be noted that ethanol also produces various effects in the NAc and other DA projection areas that play important roles in the behavioral actions of ethanol. The readers of this chapter should refer to some recent reviews on this subject (Ron and Jurd, 2005; Zhang *et al.*, 2006).

III. Intrinsic and Synaptic Mechanisms Controlling DA Neuron Activity

A. Two Modes of DA Neuron Firing and Their Functions

DA neurons display two distinct modes of action potential (AP) firing *in vivo*: (1) tonic, regular/irregular single-spike activity at 1–5 Hz, and (2) phasic bursts comprising 2–10 APs at 10–30 Hz, which are often followed by a depression of activity (Fig. 1A) (Grace and Bunney, 1984a,b; Hyland *et al.*, 2002; Overton and Clark, 1997). In contrast, DA neurons show uniform pacemaker-like firing (0.5–5 Hz) in an *in vitro* brain slice preparation or even in an acutely dissociated preparation, indicating that it reflects the intrinsic activity of these neurons removed from the influence of active synaptic inputs (Grace and Onn, 1989; Hainsworth *et al.*, 1991; Lacey, 1993; Puopolo *et al.*, 2007). However, recent studies demonstrate that phasic burst firing can be reproduced in brain slices by electrical stimulation of glutamatergic afferents or by rapid and focal application of glutamate or aspartate onto the recorded DA neuron (Fig. 1B and C) (Blythe *et al.*, 2009; Deister *et al.*, 2009; Morikawa *et al.*, 2003).

In awake, behaving animals (primates and rodents), unexpected presentation of primary rewards or reward-predicting stimuli triggers a switch from tonic single-spike activity to transient bursts (~200 ms) in a large population of DA neurons (55–80%) in both the VTA and SNc (Pan *et al.*, 2005; Schultz, 1998). These DA neuron bursts are thought to generate phasic DA signals in projection areas. In line with this idea, intra-VTA infusion of the *N*-methyl-D-aspartate (NMDA) receptor antagonist AP-5, which would suppress DA neuron burst firing (see below), has been shown to inhibit rapid DA transients (~2–3 s) in the NAc detected with voltammetry (Somers *et al.*, 2009). These phasic DA signals will promote the induction of long-term synaptic plasticity in DA projection areas (Arbuthnott and Wickens, 1996; Mahon *et al.*, 2004; Wickens *et al.*, 2003; Wolf *et al.*, 2004), thereby providing the “teaching signal” for animals to learn the valence of environmental stimuli and behaviors associated with rewards (Montague *et al.*, 2004; Schultz, 1998). In addition, phasic DA transients may also act as an incentive salience signal to temporarily direct cognitive and behavioral resources toward biologically significant environmental stimuli that elicit DA neuron burst firing, thereby attracting attention and triggering approach behavior toward those stimuli (Berridge, 2006; Nicola, 2007; Niv, 2007). This effect of phasic DA is most likely achieved through its immediate short-term actions on synaptic transmission and neuronal excitability in DA projection areas, resulting in an enhanced signal-to-noise ratio in the firing activity among neurons (Horvitz, 2002; Nicola *et al.*, 2004).

The tonic, asynchronous single-spike activity of DA neurons gives rise to global DA tone in the projection areas (Venton *et al.*, 2003). It has been suggested that there are “silent” DA neurons *in vivo* and that a proportion of active DA neurons, termed the “population activity,” regulate tonic DA levels measured with microdialysis (Floresco *et al.*, 2003; but see Dai and Tepper, 1998). Interestingly, intra-VTA infusion of the NMDA receptor antagonist AP-5, which inhibits phasic DA signals in the NAc (Somers *et al.*, 2009), can also reduce tonic DA levels in the NAc (Karreman *et al.*, 1996; Kretschmer, 1999) and attenuate the slow rise (~1 min) in NAc DA levels following intravenous injection of cocaine (Somers *et al.*, 2009). These observations raise the possibility that tonic DA may, at least partially, reflect integration of phasic DA transients over time (Niv *et al.*, 2007; Stuber *et al.*, 2005). Although the precise role of tonic DA signals remains to be determined, they may act to motivate and energize the performance of already-learned goal-directed and habitual behaviors via short-term modulatory effects on synaptic transmission and neuronal excitability (Cagniard *et al.*, 2006; Goto and Grace, 2005; Niv, 2007; Salamone *et al.*, 2007; Yin *et al.*, 2009).

Intriguingly, animals that have learned to self-administer cocaine regulate their operant behavior (e.g., lever pressing) as if to maintain tonic DA levels in the NAc within a certain range (Pettit and Justice, 1989; Stuber *et al.*, 2005; Weiss *et al.*, 1992a; Wise *et al.*, 1995). Hence, decline in DA levels below a “threshold” will trigger operant responding, while animals will stop responding when DA levels are elevated beyond a certain upper limit. This is also supported by the well-known bell-shaped dose–response function of drug self-administration. Here, animals will compensate for an increase in unitary dose, where each operant response would produce a larger increase in DA levels, by a decrease in the rate of responding if the unitary doses are on the descending limb of the bell-shaped dose–response function (Gonzales *et al.*, 2004; Pettit and Justice, 1991; Piazza *et al.*, 2000). Furthermore, certain doses of DA receptor antagonists can cause an increase in operant responding in a manner analogous to a reduction in unitary dose (Bergman *et al.*, 1990; Caine and Koob, 1994; Gonzales *et al.*, 2004; Maldonado *et al.*, 1993). Therefore, there seems to be a mechanism to titrate DA levels, or more specifically the degree of DA receptor activation, in the NAc. This idea apparently cannot be accounted for by the behavior-facilitating function of DA described in the previous paragraphs. Although one might argue that animals are attempting to maintain an optimal “hedonic state” encoded by tonic DA levels (Wise, 2008), a further mechanistic explanation, including the actual neural substrate being sensed in maintaining tonic DA levels, seems

warranted (Ahmed and Koob, 2005; Goto *et al.*, 2007; Tsibulsky and Norman, 1999). In this regard, it is well documented that tonic DA levels correlate with the self-report of “high” or “euphoria” in humans (Volkow *et al.*, 2004), although whether tonic DA actually mediates the subjective experience of pleasure (“hedonia hypothesis of DA”) remains a matter of debate (Berridge, 2006; Wise, 2008).

B. Ionic Mechanisms Underlying Spontaneous Pacemaker Activity OF DA Neurons

Numerous studies have investigated the roles of various ionic conductances in the generation of intrinsic pacemaker activity of DA neurons. It should be noted that the vast majority of these studies has focused on SNc DA neurons in the past, and it has been generally assumed that DA neurons possess rather homogeneous properties in both the SNc and the VTA. However, it has become increasingly evident in recent years that certain populations of VTA DA neurons, depending on their location and projection targets, exhibit electrophysiological and pharmacological profiles that do not conform to the conventional description of DA neurons (Cameron *et al.*, 1997; Ford *et al.*, 2006; Lammel *et al.*, 2008; Margolis *et al.*, 2006a, 2008b; Neuhoff *et al.*, 2002; Wolfart *et al.*, 2001). The three major criteria used by *in vitro* electrophysiologists to identify DA neurons are (1) low-frequency (<5 Hz) pacemaker firing with long-duration APs (~2–3 ms); (2) the presence of hyperpolarization-activated slow inward current (I_h), which mediates the characteristic depolarizing sag in response to membrane hyperpolarization; and (3) hyperpolarization/outward current produced by activation of D2-type DA autoreceptors (Grace and Onn, 1989; Johnson and North, 1992b; Lacey, 1993). Although most DA neurons in the lateral part of the VTA, likely projecting to the core and lateral shell regions of the NAc and to the basolateral amygdala (BLA), appear to display these properties (Ford *et al.*, 2006; Ikemoto, 2007; Labouebe *et al.*, 2007; Lammel *et al.*, 2008; Riegel and Williams, 2008; Wanat *et al.*, 2008; but see Margolis *et al.*, 2006b, 2008b), the readers of this chapter should be cautioned that the description of ionic mechanisms of DA neuron firing in this section would not be entirely valid for all VTA DA neurons, especially those in the medial VTA that projects to the prefrontal cortex (PFC) (Lammel *et al.*, 2008). In the following subsections, we will describe four major ion channels that have been shown to play critical roles in generating/controlling intrinsic DA neuron pacemaker activity.

1. L-Type Voltage-Gated Ca^{2+} Channel—Studies in the late 1980s have demonstrated that the spontaneous pacemaker firing of DA neurons can be completely suppressed by cadmium or cobalt, which inhibit all voltage-gated Ca^{2+} channels (Fujimura and Matsuda, 1989; Grace and Onn, 1989; Harris *et al.*, 1989). This is in sharp contrast to the Ca^{2+} -independent pacemaking mechanisms in other central nervous system (CNS) neurons (Bevan and Wilson, 1999; Llinas and Alonso, 1992; Raman and Bean, 1999). It has been further shown that dihydropyridine compounds that selectively inhibit L-type Ca^{2+} channels can block DA neuron pacemaker activity (Fig. 2) (Chan *et al.*, 2007; Mercuri *et al.*, 1994; Nedergaard *et al.*, 1993; Puopolo *et al.*, 2007). L-type Ca^{2+} channels expressed in DA neurons, encoded by the $\text{Ca}_v1.3$ gene, are unique in that they can be activated at relatively hyperpolarized membrane potentials (~–60 mV) and exhibit little inactivation (Chan *et al.*, 2007; Durante *et al.*, 2004), making them ideally suited to drive the subthreshold membrane potential oscillation underlying the pacemaker activity of these neurons. A modeling study has demonstrated that depolarization caused by L-type Ca^{2+} conductance combined with hyperpolarization mediated by Ca^{2+} -sensitive K^+ conductance is sufficient to produce intrinsic pacemaker activity (Wilson and Callaway, 2000). However, recent evidence calls this model into question and suggests that certain Ca^{2+} -independent mechanisms, for example, persistent Na^+ currents active at subthreshold membrane potentials, may also be able to sustain DA neuron pacemaking (Chan *et al.*, 2007; Guzman *et al.*, 2009; Puopolo *et al.*, 2007; Putzier *et al.*, 2009).

2. Small-Conductance Ca^{2+} -Sensitive K^+ Channel—The bee venom peptide toxin apamin, which selectively blocks small-conductance Ca^{2+} -sensitive K^+ (SK) channels, reduces the large afterhyperpolarization (AHP), which follows each AP and dominates the first part of the interspike interval, in DA neurons (Ji *et al.*, 2009; Shepard and Bunney, 1991; Wolfart *et al.*, 2001). Here, Ca^{2+} entry during the subthreshold depolarization preceding each AP, together with Ca^{2+} influx triggered by the large, brief depolarization of the AP itself, elicits activation of SK-mediated outward K^+ current causing large hyperpolarization, that is, the AHP. There appear to be multiple sources of Ca^{2+} responsible for SK channel activation, including L-type Ca^{2+} channels (Chan *et al.*, 2007; Wilson and Callaway, 2000), T-type Ca^{2+} channels (Wolfart and Roeper, 2002), P/Q-type Ca^{2+} channels (Puopolo *et al.*, 2007), and Ca^{2+} -induced Ca^{2+} release from intracellular Ca^{2+} stores (Cui *et al.*, 2007; Wolfart and Roeper, 2002). It has been reported that SK channels do not play a major role in controlling the firing activity of VTA DA neurons, which have relatively low levels of SK channel expression compared to SNc neurons (Wolfart *et al.*, 2001).

3. Hyperpolarization-Activated Cation (I_h) Channel— I_h was first described as a current driving the pacemaker activity of cells in the sinoatrial node of the heart (Brown and DiFrancesco, 1980; Noma and Irisawa, 1976). Since then, numerous studies have investigated the expression and function of I_h in neurons, where I_h contributes to the autonomous firing (Chan *et al.*, 2004; Luthi and McCormick, 1998; Maccaferri and McBain, 1996) and is also involved in setting the resting membrane potential and resting membrane conductance, thereby controlling the postsynaptic response to synaptic inputs (George *et al.*, 2009; Magee, 1998; Poolos *et al.*, 2002). Four genes encoding I_h channels, termed HCN (for hyperpolarization-activated cyclic nucleotide-gated) 1–4, have been identified, each having distinct biophysical properties and expression profiles (Kaupp and Seifert, 2001; Robinson and Siegelbaum, 2003). I_h channels have a mixed Na^+/K^+ conductance and are activated by hyperpolarization beyond -50 to -60 mV, producing slowly developing inward currents. As described earlier in this section, the presence of I_h has been widely used as an electrophysiological marker to identify DA neurons in the VTA/SNc. The activation of I_h takes place over hundreds of milliseconds in DA neurons, which express HCN2–4 but lack fast-activating HCN1 (Franz *et al.*, 2000; Notomi and Shigemoto, 2004; Santoro *et al.*, 2000). In spontaneously firing DA neurons, the AHP following each AP activates I_h , which accelerates depolarization back to the AP threshold during the interspike interval. Accordingly, inhibiting I_h with ZD7288, a relatively specific I_h blocker (Chevalleyre and Castillo, 2002; Harris and Constanti, 1995), prolongs AHPs and reduces the frequency of spontaneous DA neuron firing in both the VTA and SNc (Beckstead and Phillips, 2009; Okamoto *et al.*, 2006; Seutin *et al.*, 2001). It should be noted that the role of I_h in controlling DA neuron pacemaker activity may vary depending on the age and species of animals used, as well as on the location and neurochemical characteristics of DA neurons recorded (Chan *et al.*, 2007; McDaid *et al.*, 2008; Neuhoff *et al.*, 2002).

4. A-Type K^+ (I_A) Channel—A number of studies have characterized the voltage-gated transient K^+ current, termed I_A , in DA neurons (Koyama and Appel, 2006a; Liss *et al.*, 2001; Liu *et al.*, 1994; Silva *et al.*, 1990). I_A is a rapidly inactivating, transient K^+ current that is activated by depolarization (above -50 to -60 mV) from hyperpolarized membrane potentials where inactivation is removed. Activation is extremely rapid (<10 ms), whereas inactivation and the recovery from inactivation occur in the order of tens of milliseconds. I_A generally acts to exert negative control over the frequency of relatively slow, repetitive firing (1–100 Hz) (Connor and Stevens, 1971; Rudy, 1988). Indeed, blocking I_A with 4-aminopyridine (4-AP) has been shown to increase the firing frequency of DA neurons (Hahn *et al.*, 2003; Koyama and Appel, 2006a; Liss *et al.*, 2001; Yang *et al.*, 2001). It is thought that the large AHP removes the inactivation of I_A , which becomes inactivated by the end of each AP. Subsequently, I_A will

be transiently activated during the depolarizing phase of AHP. The resulting outward (hyperpolarizing) current will slow down the return of the membrane potential to the AP threshold, thereby prolonging the interspike interval. Therefore, I_A and I_h , which are both active in the subthreshold voltage range during the interspike interval, play opposing roles in controlling tonic DA neuron firing.

C. The Influence of Neurotransmitter Inputs on DA Neuron Activity

The firing pattern of DA neurons *in vivo* significantly deviates from the uniform pacemaker activity observed in brain slices. In particular, DA neurons exhibit phasic bursts, that is, transient increases in the firing frequency (~10–30 Hz) for ~200 ms, in response to unexpected presentation of primary rewards or reward-predicting stimuli. These reward-predicting stimuli that elicit DA neuron bursts can be of a variety of sensory modalities and characteristics, which illustrates the wide divergence of neurotransmitter inputs that are integrated to regulate the activity of DA neurons (Geisler and Zahm, 2005). Here, we will summarize the sources and potential roles of various neurotransmitter inputs in the VTA.

1. Glutamate—Feedback input from the PFC provides the sole major cortical projection to the VTA (Geisler *et al.*, 2007; Sesack and Pickel, 1992). Interestingly, it has been shown that excitatory PFC afferents preferentially target DA neurons that project back to the PFC and GABAergic neurons that project to the NAc, demonstrating considerable target specificity of PFC input into the VTA (Carr and Sesack, 2000).

Previous studies have identified a limited number of subcortical structures providing glutamatergic afferents into the VTA. These include the pedunculopontine (PPTg) and laterodorsal tegmental (LDTg) nuclei (Charara *et al.*, 1996; Clements *et al.*, 1991; Lavoie and Parent, 1994a), the bed nucleus of the stria terminalis (BNST) (Georges and Aston-Jones, 2002), and the superior colliculus (SC) (Comoli *et al.*, 2003; Dommett *et al.*, 2005). However, a recent thorough analysis using retrograde tract tracing combined with *in situ* hybridization for vesicular glutamate transporters has revealed a large number of previously unknown subcortical sources of glutamatergic inputs into the VTA, such as the lateral hypothalamic and preoptic areas, periaqueductal gray, and the dorsal and median raphe (Geisler *et al.*, 2007). Furthermore, glutamatergic synapses from these subcortical structures outnumber those from the PFC (Geisler *et al.*, 2007; Omelchenko and Sesack, 2007). These diverse sources of glutamatergic afferents may enable DA neurons to respond to a wide range of environmental stimuli. For example, electrophysiological evidence implicates glutamatergic inputs primarily from the SC and PPTg in mediating DA neuron burst responses to visual and auditory cues, respectively (Comoli *et al.*, 2003; Pan and Hyland, 2005).

Glutamatergic inputs activate α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)- and NMDA-type ionotropic glutamate receptors in DA neurons. Accordingly, iontophoretic application of both AMPA and NMDA receptor agonists can stimulate DA neuron firing (Christoffersen and Meltzer, 1995; Zhang *et al.*, 1997). A number of *in vivo* electrophysiological studies in anesthetized animals have reported that DA neuron bursts occurring spontaneously or those triggered by electrical stimulation of the PFC, PPTg, or subthalamic nucleus are suppressed by local application of NMDA receptor antagonists but not by AMPA receptor antagonists (Chergui *et al.*, 1994, 1993; Christoffersen and Meltzer, 1995; Overton and Clark, 1992; Tong *et al.*, 1996). In line with this, it has been shown recently that genetic inactivation of NMDA receptors selectively in DA neurons impairs spontaneous bursts as well as those evoked by PPN stimulation (Zweifel *et al.*, 2009). Furthermore, DA neuron bursts triggered by electrical stimulation of glutamatergic afferents or iontophoretic application of glutamate/aspartate in brain slices are also blocked by NMDA receptor antagonists (Deister *et al.*, 2009; Morikawa *et al.*, 2003). These converging lines of evidence

indicate the critical role of NMDA receptors in the generation of DA neuron bursts. However, it should be noted that AMPA receptor antagonists have also been shown to attenuate DA neuron burst firing evoked by stimulation of glutamatergic inputs both *in vivo* and *in vitro* (Blythe *et al.*, 2007; Di Loreto *et al.*, 1992; Georges and Aston-Jones, 2002). Therefore, the exact contribution of these two types of ionotropic glutamate receptors to DA neuron bursting under different conditions, and maybe in different populations of DA neurons, remains to be fully determined.

Glutamate also activates metabotropic glutamate receptors (mGluRs), mainly type 1 mGluR (mGluR₁), in DA neurons (Hubert *et al.*, 2001; Testa *et al.*, 1994). Tonic activation of mGluRs produces membrane depolarization that is mediated, at least partly, by opening of a nonselective cationic conductance (Guatteo *et al.*, 1999; Tozzi *et al.*, 2003). In contrast, rapid activation of mGluRs leads to membrane hyperpolarization via opening of SK channels following Ca²⁺ release from intracellular stores (Fiorillo and Williams, 1998; Morikawa *et al.*, 2003). This mGluR-induced hyperpolarization mediates the transient pause of activity following NMDA-induced bursting observed in brain slices (Fig. 1B and C), and is desensitized by tonic mGluR activation through a mechanism likely involving Ca²⁺ store depletion. It has recently been reported that tonic mGluR activation causes amplification of AP-evoked Ca²⁺ signals, thereby driving activity-dependent synaptic plasticity of NMDA receptor-mediated transmission (Cui *et al.*, 2007; Harnett *et al.*, 2009).

2. GABA—The powerful influence of GABAergic inhibition on DA neuron activity is illustrated by the observation that over 70% of synapses onto SNc DA neurons are GABAergic (Bolam and Smith, 1990; Tepper and Lee, 2007), although the proportion of GABAergic synapses seems to be lower in the VTA (Smith *et al.*, 1996). There are major GABAergic feedback projections from the NAc and the ventral pallidum into the VTA (Conrad and Pfaff, 1976; Kalivas *et al.*, 1993; Walaas and Fonnum, 1980; Zahm, 1989). Interestingly, the feedback loops between the VTA/SNc and NAc/striatum form a cascading spiral of projections (Haber *et al.*, 2000, 1990; Heimer *et al.*, 1991). Here, projections from the NAc shell, that is, the medial part of the NAc, to the VTA influence DA neurons that in turn project to the more lateral core of the NAc, which provides projections to the SNc that projects to the dorsal striatum. This anatomical arrangement has formed a conceptual framework to account for the transition from a goal-directed action to a stimulus-response habit during the progression of addiction, in which more lateral part of the VTA/SNc complex, together with more dorsal part of the striatum, are gradually recruited (Belin and Everitt, 2008; Everitt and Robbins, 2005).

DA neurons also receive GABAergic inputs from local GABA neurons within the VTA (Johnson and North, 1992b; Omelchenko and Sesack, 2009; Phillipson, 1979a). Furthermore, a region located at the caudal tail of the VTA, termed rostromedial tegmental nucleus (RMTg), has been recently shown to be an important source of GABAergic input to the VTA and SNc (Jhou *et al.*, 2009a, b; Kaufling *et al.*, 2009).

Both GABA_A and GABA_B receptors mediate the inhibitory action of GABA on DA neurons (Brazhnik *et al.*, 2008; Johnson and North, 1992b). GABA_B receptor-mediated inhibition is achieved through activation of G protein-gated inwardly rectifying K⁺ (GIRK) channels (Labouebe *et al.*, 2007; Lacey *et al.*, 1988). Furthermore, activation of presynaptic GABA_B receptors inhibits glutamate and GABA release onto DA neurons (Hausser and Yung, 1994; Manzoni and Williams, 1999). GABAergic inhibition via both GABA_A and GABA_B receptors can suppress burst firing of DA neurons (Erhardt *et al.*, 2002; Paladini *et al.*, 1999), and recent evidence indicates that a disinhibitory mechanism, that is, transient removal of GABAergic inhibition, may contribute to the generation of phasic DA neuron bursts in behaving animals (Jhou *et al.*, 2009a; Matsumoto and Hikosaka, 2007; Tepper and Lee, 2007).

3. Glycine—The VTA receives sparse glycinergic input (Rampon *et al.*, 1996). Exogenous application of glycine has been shown to activate Cl⁻-mediated currents in dissociated VTA neurons, indicating the presence of functional ionotropic glycine receptors (Ye, 2000; Ye *et al.*, 1998). Furthermore, glycine-induced presynaptic inhibition of GABA release has been reported in VTA DA neurons, an effect that may lead to disinhibition of the firing activity (Ye *et al.*, 2004).

4. Dopamine—Dendrodendritic synapses, as well as a small number of axodendritic synapses, formed between DA neurons have been observed with electron microscopy in the VTA (Bayer and Pickel, 1990). Vesicular monoamine transporter 2 (VMAT2)-positive vesicles, which would underlie Ca²⁺-dependent vesicular DA release from the somatodendritic area (Chen and Rice, 2001; Kita *et al.*, 2009; Rice *et al.*, 1997), have also been detected in DA neuron dendrites (Nirenberg *et al.*, 1996). Released DA exerts feedback inhibition of DA neuron activity via activation of D2 autoreceptors causing GIRK-mediated hyperpolarization (Groves *et al.*, 1975; Lacey *et al.*, 1987). Indeed, D2 receptor-mediated IPSCs elicited by electrical stimulation of dendrodendritic synapses have been recently reported in DA neurons of the SNc and VTA (Beckstead *et al.*, 2007, 2004). In addition, DA inhibits glutamate release and facilitates GABA release onto DA neurons via activation of presynaptic D2 and D1 receptors, respectively (Cameron and Williams, 1993; Koga and Momiyama, 2000). Postsynaptic D5 receptor-mediated slow potentiation of NMDA EPSCs has also been reported recently, suggesting that DA may promote burst firing of DA neurons (Schilstrom *et al.*, 2006).

5. Norepinephrine—The locus coeruleus and other noradrenergic nuclei in the medulla give rise to norepinephrine (NE)-containing terminals in the VTA (Mejias-Aponte *et al.*, 2009; Phillipson, 1979b). Overall, NE exerts excitatory control over DA neuron activity via activation of α 1 adrenergic receptors causing membrane depolarization or suppressing the mGluR-induced pause (Grenhoff *et al.*, 1995; Paladini *et al.*, 2001). However, transient activation of α 1 adrenergic receptors produces SK-mediated membrane hyperpolarization (Paladini and Williams, 2004). NE can also bind to D2 DA receptors and cause membrane hyperpolarization in DA neurons (Grenhoff *et al.*, 1995; Guiard *et al.*, 2008).

6. Serotonin—Serotonergic neurons originating in the midbrain dorsal and median raphe nuclei provide dense projections to the VTA (Azmitia and Segal, 1978; Herve *et al.*, 1987; Parent *et al.*, 1981; Phillipson, 1979b). Different subtypes of serotonin (5-HT) receptors are present in the VTA, such as 5-HT_{1B}, 5-HT_{2A}, and 5-HT_{2C} receptors (Bubar and Cunningham, 2007; Doherty and Pickel, 2000; Pazos and Palacios, 1985; Waeber *et al.*, 1989a). It has been shown that 5-HT application produces membrane depolarization and suppresses the mGluR-mediated pause in DA neurons, most likely via activation of 5-HT_{2A} receptors (Paolucci *et al.*, 2003; Pessia *et al.*, 1994). Furthermore, activation of presynaptic 5-HT_{1B} receptors on GABAergic terminals leads to a reduction in GABA release, an effect that may also contribute to the excitatory action of 5-HT on DA neuron activity (Johnson *et al.*, 1992; Morikawa *et al.*, 2000). In contrast, activation of 5-HT_{2C} receptors on local GABAergic neurons is thought to mediate inhibition of DA neuron output (Di Matteo *et al.*, 2001; Pessia *et al.*, 1994). 5-HT-induced depression of glutamate release onto DA neurons has also been reported (Jones and Kauer, 1999).

Numerous studies have implicated 5-HT₃ receptors, the only ionotropic receptor among 5-HT receptor subtypes, in the VTA in stimulation of the functional output of the mesolimbic DA system (reviewed by Engleman *et al.*, 2008). However, previous radioligand binding studies have failed to detect 5-HT₃ receptor expression in the VTA (Gehlert *et al.*, 1991; Waeber *et al.*, 1989b). Interestingly, 5-HT₃ receptors appear to be preferentially localized on nerve

terminals (Miquel *et al.*, 2002). The exact mechanism underlying 5-HT₃ receptor-mediated stimulation of DA neurons (e.g., presynaptic vs. postsynaptic) remains to be determined.

7. Acetylcholine—Cholinergic projections to the VTA mainly derive from the PPTg and LDTg nuclei (Mena-Segovia *et al.*, 2008; Oakman *et al.*, 1995; Omelchenko and Sesack, 2005; Semba and Fibiger, 1992). It has been shown that glutamate or GABA is co-released with acetylcholine (ACh) from these projections (Clements *et al.*, 1991; Jia *et al.*, 2003; Lavoie and Parent, 1994b; Omelchenko and Sesack, 2006), although whether there is co-release from the same terminals appears to remain controversial (Mena-Segovia *et al.*, 2008). Cholinergic terminals in the VTA have been shown to preferentially innervate DA neurons that project to the NAc (Omelchenko and Sesack, 2006). DA neurons generally exhibit excitatory responses to ACh via activation of nicotinic and muscarinic ACh receptors.

Different subtypes of nicotinic ACh receptors (nAChRs), including $\alpha 3-7$ and $\beta 2-4$, are expressed in DA neurons, although $\beta 2$ -containing nAChRs mediate the majority of postsynaptic nicotinic responses (Azam *et al.*, 2002; Champtiaux *et al.*, 2003; Wooltorton *et al.*, 2003). Activation of $\beta 2$ -containing nAChRs by the levels of nicotine experienced in smokers transiently excites DA neurons followed by desensitization in seconds to minutes (Pidoplichko *et al.*, 1997). Recent evidence highlights the important roles of presynaptic nAChRs in regulating DA neuron activity, where persistent activation of presynaptic $\alpha 7$ -containing nAChRs enhances glutamatergic inputs onto DA neurons, while desensitization of presynaptic $\beta 2$ -containing nAChRs on GABAergic terminals may lead to disinhibition (Mansvelder and McGehee, 2002).

Activation of postsynaptic M5 muscarinic ACh receptors causes excitation of DA neurons (Gronier and Rasmussen, 1998; Lacey *et al.*, 1990; Yamada *et al.*, 2003; Yeomans *et al.*, 2001), although rapid activation of these receptors can evoke SK-mediated membrane hyperpolarization via intracellular Ca²⁺ release (Fiorillo and Williams, 2000). Presynaptic inhibition of glutamate and GABA release by activation of mAChRs has also been reported (Grillner *et al.*, 2000, 1999).

8. Opioids— β -Endorphin-containing fibers originating from the arcuate nucleus of the hypothalamus constitute the major opioid input to the VTA (Mansour *et al.*, 1988). Enkephalin-containing terminals have also been detected in the VTA (Garzon and Pickel, 2002). Endorphins and enkephalins bind to both μ - and δ -opioid receptors. It is generally believed that μ -opioid receptor-mediated inhibition of local GABAergic neurons, which leads to excitation of DA neurons via a disinhibitory mechanism, is the predominant mode of action of these endogenous opioids, as well as exogenous opiate drugs, within the VTA (Johnson and North, 1992a,b). In line with this, both systemic and intra-VTA administrations of μ -opioid receptor agonists stimulate VTA DA neuron firing and NAc DA release (Di Chiara and Imperato, 1988; Latimer *et al.*, 1987; Leone *et al.*, 1991; Matthews and German, 1984; Spanagel *et al.*, 1992).

The VTA also contains terminals that release the κ -opioid receptor-selective opioid, dynorphin (Fallon *et al.*, 1985; Pickel *et al.*, 1993). These terminals originate from the NAc, the central nucleus of the amygdala, and the lateral hypothalamus (Fallon *et al.*, 1985; Meredith, 1999). Recent studies demonstrate that activation of κ -opioid receptors can directly inhibit a subset of VTA DA neurons (Margolis *et al.*, 2003, 2006a), an effect that may contribute to the aversive action of κ -opioid receptor agonists in the VTA (Bals-Kubik *et al.*, 1993).

A number of studies have demonstrated that activation of all three opioid receptors can produce presynaptic inhibition of glutamate and/or GABA release onto VTA DA neurons (Ford *et al.*,

2006; Manzoni and Williams, 1999; Margolis *et al.*, 2008a, 2005; Shoji *et al.*, 1999). These presynaptic opioid receptors may also play a significant role in regulating DA neuron activity.

9. Other Neuropeptides—Recent evidence highlights the importance of inputs containing corticotropin-releasing factor (CRF) and orexins in driving drug-seeking behavior and preference for drug-associated stimuli (Aston-Jones *et al.*, 2009; Narita *et al.*, 2006; Wise and Morales, 2009).

The BNST, the central nucleus of the amygdala, and the paraventricular nucleus of the hypothalamus are sources of CRF input to the VTA (Rodaros *et al.*, 2007), where CRF is co-released with glutamate or GABA (Tagliaferro and Morales, 2008). CRF also appears to be produced in certain DA neurons in the VTA (Korotkova *et al.*, 2006). Recent studies demonstrate that CRF regulates glutamatergic and GABAergic transmission onto DA neurons and their intrinsic pacemaker activity via multiple signaling cascades and receptor subtypes (Beckstead *et al.*, 2009; Korotkova *et al.*, 2006; Riegel and Williams, 2008; Ungless *et al.*, 2003; Wanat *et al.*, 2008).

Orexin input to the VTA arises from the lateral hypothalamus (Balcita-Pedicino and Sesack, 2007; Fadel and Deutch, 2002; Milevskiy *et al.*, 2005), which may co-release glutamate with orexins (Rosin *et al.*, 2003). The two types of orexins (orexin A and orexin B) can both directly activate VTA DA neurons (Korotkova *et al.*, 2003). Furthermore, orexin A has been shown to produce prolonged potentiation of NMDA receptor-mediated excitatory transmission onto DA neurons (Borgland *et al.*, 2006). These effects may underlie the increased release of DA in projection areas caused by intra-VTA orexin injection (Narita *et al.*, 2006; Vittoz and Berridge, 2006).

10. Cannabinoids—Recent evidence indicates that endocannabinoids released from DA neurons themselves inhibit both glutamate and GABA release onto DA neurons via activation of presynaptic CB1 receptors (Melis *et al.*, 2004a,b; Riegel and Lupica, 2004). Release of endocannabinoids appears to be triggered by a rise in cytosolic Ca^{2+} levels due to increased firing and bursting activity or activation of mGluRs causing Ca^{2+} mobilization from intracellular stores.

IV. Acute Ethanol Action on DA Neuron Activity

The original studies in the 1980s found that i.v. administration of ethanol (0.5–2 g/kg) dose-dependently increases the firing frequency of VTA and SNc DA neurons recorded *in vivo* using awake (unanesthetized), but pharmacologically paralyzed, rats (Gessa *et al.*, 1985; Mereu *et al.*, 1984). It was subsequently shown that ethanol (10–200 mM) increases the spontaneous firing frequency of VTA DA neurons in a concentration-dependent manner using an *in vitro* brain slice preparation (Brodie *et al.*, 1990). This stimulatory effect of ethanol can be observed when the influence of synaptic inputs are blocked by low Ca^{2+} /high Mg^{2+} solution (Brodie *et al.*, 1990) or by a cocktail of ionotropic neurotransmitter receptor antagonists (Okamoto *et al.*, 2006; but also see Xiao *et al.*, 2007), and even when DA neurons are dissociated into individual cells (Brodie *et al.*, 1999b). These observations demonstrate that ethanol can influence the intrinsic mechanisms underlying the spontaneous pacemaker activity of these neurons. However, several lines of evidence indicate that the effects of ethanol on neurotransmitter inputs, either via its action on synapses at DA neurons or that on the activity of the neurons providing those inputs, should also play a significant role in ethanol stimulation of DA neuron firing observed *in vivo*. First, although not quantified, Mereu *et al.* (1984) reported that ethanol promoted the occurrence of bursting in SNc DA neurons *in vivo* (Figs. 3A and 4). This has been confirmed in a recent study demonstrating that ethanol increases the percentage of spikes occurring in bursts in VTA DA neurons (Foddai *et al.*, 2004). Furthermore,

it has been shown that i.v. administration of ethanol in awake rats leads to an increase in the frequency of phasic DA transients in the NAc detected with voltammetry (Cheer *et al.*, 2007; Robinson *et al.*, 2009), supporting the idea that ethanol promotes the occurrence of DA neuron bursts.

Second, the magnitude of the ethanol effect appears to be significantly larger *in vivo* than *in vitro*. For example, i.v. injection of 1 g/kg ethanol, which would produce 60–70 mM peak ethanol concentrations in the blood and brain (Robinson *et al.*, 2002, 2000), increases DA neuron firing frequency by ~60–70% *in vivo* (Gessa *et al.*, 1985; Mereu *et al.*, 1984), whereas similar concentrations of ethanol cause only modest increases (~10–40%) in pacemaker frequency recorded *in vitro* (Brodie and Appel, 2000; Brodie *et al.*, 1999b, 1990; Okamoto *et al.*, 2006). Finally, it has been shown that VTA DA neurons are significantly more sensitive to ethanol than SNc DA neurons, especially at low ethanol doses (Gessa *et al.*, 1985). However, DA neurons in these two areas appear to be equally affected by ethanol in brain slices (Okamoto *et al.*, 2006), although full concentration dependence was not determined in this study. Taken together, it is most likely that ethanol stimulates DA neuron firing through its action on both the intrinsic pacemaker mechanisms of these neurons and the neurotransmitter inputs they receive.

A. Acute Ethanol Action on Intrinsic Pacemaker Activity of DA Neurons

How does ethanol stimulate the pacemaker activity of DA neurons? The first clue to this question comes from the effect of ethanol on the membrane potential trajectory in spontaneously firing DA neurons, where ethanol reduces the size and duration of the AHP and accelerates the depolarizing ramp toward AP threshold during the interspike interval (Fig. 3B) (Brodie and Appel, 1998; Brodie *et al.*, 1999a; Okamoto *et al.*, 2006). As described in the previous section, there are a number of ion channel species (L-type Ca^{2+} channel, SK channel, I_h , I_A , and others) that become activated in the subthreshold voltage range and shape the membrane potential trajectory during the interspike interval. Therefore, we will next discuss the potential involvement of these ion channels in ethanol stimulation of DA neuron firing.

1. L-Type Voltage-Gated Ca^{2+} Channel—It has been shown that acute ethanol exposure inhibits L-type Ca^{2+} channels via modulation of voltage gating in several neuronal preparations (Walter and Messing, 1999; Wang *et al.*, 1994); however, the effect of ethanol on L-type Ca^{2+} currents has not been tested in DA neurons. Reduction in L-type Ca^{2+} channel conductance would (1) slow down the depolarizing ramp preceding APs and (2) decrease the size of SK-dependent AHPs due to a reduction in Ca^{2+} influx required for SK channel activation. Recent evidence indicates that DA neurons may be able to maintain their pacemaker activity through a mechanism dependent on I_h and persistent Na^+ conductance active during the interspike interval even when L-type Ca^{2+} channels are completely blocked by dihydropyridine antagonists (Chan *et al.*, 2007; Guzman *et al.*, 2009). Although L-type Ca^{2+} channels are unlikely to be the major target mediating ethanol excitation of DA neurons, analyzing the effect of ethanol on pacemaker activity under L-type Ca^{2+} channel blockade might help elucidate the roles of other ionic conductances in ethanol stimulation of DA neuron firing.

2. Small-Conductance Ca^{2+} -Sensitive K^+ Channel—Ethanol (≥ 10 mM) has been shown to inhibit SK channel (SK2 subtype)–mediated currents in a heterologous expression system (Dreixler *et al.*, 2000). Reduction in SK conductance may well lead to an enhancement of DA neuron pacemaker firing by suppressing AHPs. However, blockade of SK channels with apamin failed to reduce the magnitude of ethanol excitation of DA neuron firing, ruling out a major role of SK channels in the ethanol effect (Brodie *et al.*, 1999a). It is possible that SK3

channel, which is the major SK channel subtype expressed in DA neurons (Wolfart *et al.*, 2001), might be less sensitive to ethanol compared to the SK2 channel described above.

3. Hyperpolarization-Activated Cation (I_h) Channel—A recent study has demonstrated that acute ethanol exposure (50–100 mM) enhances I_h in DA neurons of the VTA and SNc (Okamoto *et al.*, 2006). Consistent with this, ethanol increases the size of the I_h -dependent depolarizing sag response to membrane hyperpolarization (Brodie and Appel, 1998). Ethanol enhances I_h partly via augmenting cyclic adenosine monophosphate (cAMP)-dependent facilitation of voltage gating (Okamoto *et al.*, 2006). There appears to be species difference in the contribution of I_h to the effect of ethanol. Thus, ZD7288, an I_h blocker, significantly suppresses ethanol stimulation of DA neuron firing in mice but not in rats (Appel *et al.*, 2003; McDaid *et al.*, 2008; Okamoto *et al.*, 2006). It has been reported lately that SNc DA neurons from a line of mice bred for high sensitivity to the locomotor-stimulating effect of ethanol possess large I_h density and high sensitivity to ethanol stimulation of firing, suggesting the potential role of I_h in locomotor activation caused by ethanol (Beckstead and Phillips, 2009). It is interesting to note that a similar ethanol-induced enhancement of I_h has been implicated in ethanol stimulation of spontaneous pacemaker firing in hippocampal interneurons (Yan *et al.*, 2009).

4. A-Type K^+ (I_A) Channel—The effect of ethanol on I_A has been tested in non-mammalian neurons or in a heterologous expression system using high ethanol concentrations (≥ 200 mM) (Aleksiev *et al.*, 1997; Anantharam *et al.*, 1992; Treisman and Wilson, 1987a,b). In these investigations, ethanol produced a reduction in peak I_A amplitude and/or deceleration of inactivation without much change in the voltage dependence of activation, although the ethanol effect appears to vary depending on the cell type examined. A recent study reported that low concentrations (≤ 1 μ M) of acetaldehyde, the first metabolite of ethanol, increased I_A partly via facilitating the voltage dependence of activation in VTA DA neurons (Melis *et al.*, 2007). The effect of ethanol itself on I_A was not examined in this study. However, treatment of the slice with the catalase inhibitor 3-aminotriazole completely prevented ethanol stimulation of DA neuron pacemaker activity, suggesting that local conversion of ethanol to acetaldehyde by catalase might be a necessary step for ethanol actions on DA neurons. The involvement of acetaldehyde in the pharmacological and behavioral actions of ethanol has been extensively reviewed recently (Melis *et al.*, 2009).

5. Other Ion Channels—It has been shown that ethanol inhibits a type of delayed rectifier K^+ current, termed M-current (I_M), in VTA DA neurons (Koyama *et al.*, 2007). I_M is a non-inactivating K^+ current that is active at subthreshold membrane potentials and has been implicated in dampening repetitive firing and general neuronal excitability (Brown and Passmore, 2009). Pharmacological blockade of I_M has a marginal effect on the pacemaker firing of DA neurons recorded in brain slices, although I_M appears to have a significant role in controlling the firing of dissociated DA neurons, where dendrites are mostly severed from the soma (Koyama and Appel, 2006b; Koyama *et al.*, 2007). Accordingly, pharmacological blockade of I_M has little effect on the magnitude of ethanol stimulation of DA neuron firing in brain slices, ruling out I_M as a major ethanol target mediating stimulation of firing (Koyama *et al.*, 2007). It has also been reported that quinidine reduces the size of the early component of the AHP and suppresses the ethanol excitation of DA neurons (Appel *et al.*, 2003); however, the exact ion channel species affected by quinidine remains to be determined.

Another type of ethanol-sensitive ion channel that should be mentioned here is the GIRK channel, which is an important mediator of inhibitory neurotransmitter action that works through $G_{i/o}$ subtype of G proteins (Dascal, 1997). In DA neurons, GIRK-mediated hyperpolarization is responsible for the inhibition produced by activation of certain G protein-coupled receptors, such as D2 DA receptors and GABA_B receptors (Beckstead *et al.*, 2004;

Labouebe *et al.*, 2007; Lacey *et al.*, 1987). Ethanol has been shown to directly bind to GIRK channels and activate them without the involvement of G proteins (Aryal *et al.*, 2009; Kobayashi *et al.*, 1999; Lewohl *et al.*, 1999). In line with this, it has been reported recently that barium, which blocks GIRK channels, suppresses ethanol-induced inhibition of DA neuron firing uncovered after pharmacological blockade of I_h (McDaid *et al.*, 2008). This observation suggests that ethanol-induced activation of GIRK channels may mediate an inhibitory component of ethanol action on DA neuron pacemaker activity.

It is increasingly clear that ethanol acts on multiple ion channel species to influence the intrinsic pacemaker firing of DA neurons. Furthermore, the ionic mechanisms of ethanol action may well vary depending on the location and projection targets of individual DA neurons. Therefore, some of the discrepancies among different studies may be partly due to sampling of different populations of DA neurons, in addition to the difference in the age and species/strains of animals used. It should also be noted that the plane of brain slices (e.g., coronal vs. horizontal), in which different dendritic branches would be severed from the soma, might affect the ionic mechanisms of DA neuron firing and ethanol action.

B. Acute Ethanol Action on Neurotransmitter Inputs onto DA Neurons

As described earlier in this chapter, ethanol action on neurotransmitter inputs clearly plays a pivotal role in regulating VTA DA neuron activity *in vivo*. As throughout the mammalian CNS, glutamate and GABA form the major excitatory and inhibitory synaptic inputs onto VTA DA neurons. Indeed, many of the pharmacological effects of ethanol are mediated by its actions on these neurotransmitter systems in the brain (Lobo and Harris, 2008; Lovinger, 1997; Vengeliene *et al.*, 2008; Weight *et al.*, 1992). Although previous mechanistic investigations on ethanol stimulation of DA neuron activity have mostly, and curiously, focused on the effects of ethanol on the intrinsic properties of these neurons, a number of recent studies have examined the interactions of ethanol with neurotransmitter inputs in the VTA.

1. Glutamate—It has been reported recently that acute ethanol exposure can indirectly enhance glutamatergic transmission onto VTA DA neurons, likely via promoting somatodendritic release of DA (Deng *et al.*, 2009; Xiao *et al.*, 2009). Direct ethanol action on postsynaptic AMPA receptors was not detected in these studies. In the proposed scenario, released DA would subsequently activate presynaptic D_1 DA receptors on glutamatergic terminals, resulting in enhanced glutamate release. However, it should be noted that small *inhibition* of glutamate release onto VTA DA neurons via presynaptic D_1 -like receptors has been reported in another study (Schilstrom *et al.*, 2006). Although it is well established that ethanol inhibits NMDA receptor-mediated currents in a variety of neurons/preparations (Lovinger *et al.*, 1989; Maldve *et al.*, 2002; Ronald *et al.*, 2001; Valenzuela *et al.*, 1998), the acute effect of ethanol on NMDA receptors in DA neurons is currently unknown.

Regarding the mGluR-mediated transmission, a preliminary study has 2^+ release shown that ethanol suppresses the mGluR- and IP_3 -induced Ca from intracellular stores, thereby inhibiting the SK-mediated hyperpolarization resulting from rise in Ca^{2+} (Bernier *et al.*, 2007). This ethanol effect may promote the occurrence of DA neuron burst firing by suppressing the pause in firing (Fig. 4).

2. GABA—An early *in vivo* recording study demonstrated that systemic administration of ethanol suppresses the firing of neurons in the substantia nigra pars reticulata, which provide GABAergic afferents to SNc DA neurons (Mereu and Gessa, 1985). This study raised the possibility that ethanol might stimulate DA neuron firing via a disinhibitory mechanism. In recent years, a series of work performed by Steffensen and colleagues have investigated the effect of ethanol on GABAergic neurons in the VTA recorded *in vivo* (Gallegos *et al.*, 1999;

Steffensen *et al.*, 2000, 2009; Stobbs *et al.*, 2004). In their initial study, systemic administration of ethanol (0.2–2.0 g/kg, i.p.) dose-dependently inhibited VTA GABA neuron firing recorded in freely behaving rats (Gallegos *et al.*, 1999). This inhibitory effect can also be observed with local application of ethanol directly into the VTA, and likely involves ethanol inhibition of NMDA receptor-mediated excitation of these GABA neurons (Steffensen *et al.*, 2000; Stobbs *et al.*, 2004). Additionally, systemic administration of a DA D2 receptor antagonist has been shown to block ethanol inhibition of VTA GABA neuron firing (Ludlow *et al.*, 2009), although the location of D2 receptors involved is not clear. Interestingly, i.v. administration of low-dose ethanol (0.01–0.1 g/kg) stimulates VTA GABA neuron firing (Steffensen *et al.*, 2009), demonstrating dual effects of ethanol on the activity of these neurons depending on dosage/concentration. However, the contribution of ethanol effects on GABA neurons to the regulation of DA neuron firing *in vivo* has not been directly examined in these studies. It should also be noted that no change in VTA GABA levels, monitored with *in vivo* microdialysis, was detected after acute ethanol administration (1–2 g/kg, i.p.) in alcohol-preferring and non-preferring rat lines (Kempainen *et al.*, 2010).

In line with these *in vivo* recording studies, Xiao *et al.* have recently reported ethanol concentration (20–80 mM)-dependent inhibition of GABA neuron firing, together with enhancement of DA neuron firing, recorded from the VTA in brain slices (Xiao *et al.*, 2007). In this study, ethanol stimulation of DA neuron firing was significantly reduced by application of GABA_A receptor antagonists, consistent with the major role of a disinhibitory mechanism in ethanol excitation of DA neurons. Interestingly, the nonselective opioid receptor antagonist naloxone largely abolished the ethanol inhibition of GABA neuron firing. Furthermore, the stimulatory effect of ethanol on DA neuron firing was attenuated by naloxone and was also substantially occluded by the μ -opioid receptor agonist DAMGO. It is well known that activation of μ -opioid receptors on VTA GABA neurons causes membrane hyperpolarization and suppression of firing (Johnson and North, 1992a). Therefore, ethanol may disinhibit VTA DA neurons via promoting μ -opioid receptor-mediated suppression of GABAergic inputs onto DA neurons.

Direct recordings of GABAergic synaptic transmission onto VTA DA neurons have revealed some contradictory results. Theile *et al.* have shown that ethanol increases the frequency of GABA_A miniature IPSCs (mIPSCs) recorded in the presence of tetrodotoxin (TTX) in VTA DA neurons, demonstrating that ethanol directly facilitates GABA release from presynaptic terminals (Theile *et al.*, 2008, 2009). No changes in mIPSC decay kinetics or baseline holding currents were observed following ethanol application, suggesting that DA neurons do not express slowly inactivating, tonically active GABA_A receptors that are highly sensitive to ethanol (Wallner *et al.*, 2003; but also see Borghese and Harris, 2007). Theile *et al.* have further shown that ethanol-induced presynaptic facilitation of GABA release involves 5-HT_{2C} receptor activation causing release of Ca²⁺ from intracellular stores (Theile *et al.*, 2009). Similar enhancement of GABAergic transmission onto VTA DA neurons by acute ethanol exposure has been noted previously as an unpublished observation (Melis *et al.*, 2002), and has also been reported in substantia nigra reticulata neurons (Criswell *et al.*, 2008). In contrast, Xiao and Ye have reported that ethanol “inhibits” evoked and spontaneous GABA_A IPSCs, most likely via reduction in VTA GABA neuron excitability, as evidenced by the lack of ethanol effect on AP-independent mIPSCs (Xiao and Ye, 2008). This observation is consistent with the previous finding by this group described above (Xiao *et al.*, 2007). However, the same group further detected presynaptic “enhancement” of GABA_A IPSCs by ethanol in the presence of the μ -opioid receptor agonist, DAMGO, which would silence VTA GABA neuron activity (Xiao and Ye, 2008). Therefore, different levels of endogenous opioids present in the slice might, at least partially, account for the discrepancy in the observed ethanol effects on GABAergic transmission. In this regard, it is of note that Theile *et al.* used horizontal midbrain slices, while Xiao *et al.* used coronal slices.

Recently, more complex interactions involving purinergic type 2 (P2) receptors have been reported in VTA DA neurons (Xiao *et al.*, 2008). Here, ethanol appears to attenuate P2X₁- and P2X₃-mediated increase in GABAergic transmission, while enhancing P2Y-mediated inhibition of GABAergic transmission. Both of these ethanol effects would lead to inhibition of GABAergic inputs onto DA neurons.

Concerning GABA_B receptor-mediated transmission, it has been shown that ethanol potentiates GABA_B receptor-induced inhibition of VTA DA neurons (Federici *et al.*, 2009), most likely via enhancement of GIRK channels, as reported previously in expression systems and cerebellar granule cells (Kobayashi *et al.*, 1999; Lewohl *et al.*, 1999). Interestingly, GABA_B receptor-induced inhibition of VTA GABA neuron firing recorded *in vivo* can also be augmented by acute ethanol administration (Steffensen *et al.*, 2000).

3. Glycine—Acute effects of ethanol on glycine-induced currents have been reported in immature VTA neurons freshly dissociated from neonatal rats (PND 5–14) (Ye *et al.*, 2001a,b). These studies have shown that ethanol either potentiates or inhibits glycine-induced currents in 35 and 45%, respectively, of VTA neurons tested. Both of these effects appear to involve changes in glycine receptor affinity through a protein kinase C-dependent mechanism (Jiang and Ye, 2003; Tao and Ye, 2002). The contribution of these actions on glycine-induced currents to ethanol stimulation of DA neuron firing has not been addressed in these studies.

4. Dopamine—Ethanol, either administered systemically or applied locally, has been shown to increase DA levels in the VTA (Kohl *et al.*, 1998; Yan *et al.*, 1996). Yan *et al.* have further reported that the ability of local ethanol application to increase DA levels in the VTA is not blocked by co-infusion of Ca²⁺-free medium or TTX, suggesting that a simple mechanism involving ethanol excitation of DA neuron firing and subsequent Ca²⁺-dependent somatodendritic DA release may not entirely account for the effect of ethanol on VTA DA release. The involvement of other neurotransmitter systems in ethanol-induced DA release in the VTA will be described below.

5. Serotonin—As serotonergic antidepressants may be therapeutic for alcoholism (Johnson, 2008; Uzbay, 2008), there is a strong impetus to investigate the involvement of serotonergic systems in the pharmacological and behavioral actions of ethanol. Indeed, local microinfusion of ethanol directly into the VTA induces release of 5-HT along with DA (Yan *et al.*, 1996). Thus, it would be important to understand how 5-HT interacts with ethanol to regulate VTA DA neuron activity. Brodie *et al.* have reported that, whereas 5-HT itself has no consistent effect on VTA DA neuron firing recorded in midbrain slice preparations, 5-HT, as well as the selective 5-HT₂ agonists 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) and α -methyl-5-HT, markedly potentiate ethanol enhancement of DA neuron firing (Brodie *et al.*, 1995). Furthermore, a moderate concentration of the 5-HT reuptake inhibitor clomipramine has also been shown to significantly enhance ethanol- and 5-HT-induced increases in VTA DA neuron firing (Trifunovic and Brodie, 1996). In line with the role of 5-HT₂ receptors in ethanol stimulation of DA neuron activity, a recent study has demonstrated that self-administration of ethanol directly into the VTA is attenuated by coinfusion of a 5-HT_{2A} antagonist (Ding *et al.*, 2009b). Furthermore, stimulation of 5-HT_{2C} receptors on GABAergic terminals have been shown to play a role in ethanol-induced facilitation of GABA release onto VTA DA neurons (Theile *et al.*, 2009).

Other types of 5-HT receptors have also been implicated in ethanol actions in the VTA. It has been shown that local intra-VTA administration of a 5-HT_{1B} antagonist, but not antagonists of 5HT_{1A} or 5-HT_{1D} receptors, significantly diminishes DA release in the VTA and NAc produced by systemic administration of ethanol (1–2 g/kg, i.p.) (Yan *et al.*, 2005). Furthermore, intra-VTA administration of a 5-HT_{1B} agonist also prolongs ethanol-induced DA release in

the NAc. Ethanol-induced 5-HT release in the VTA might activate 5-HT_{1B} receptors located on GABAergic terminals, thus causing disinhibition of DA neurons via presynaptic inhibition of GABA release (Johnson *et al.*, 1992; Morikawa *et al.*, 2000).

The 5-HT₃ subtype is unique among 5-HT receptors in that it forms a ligand-gated cation channel as opposed to a G protein-coupled receptor. McBride and colleagues have demonstrated that intra-VTA infusion of the selective 5-HT₃ receptor agonist chlorophenylbiguanide (CPBG) via reverse microdialysis produces Ca²⁺-dependent somatodendritic DA release in the VTA in a concentration-dependent manner (Campbell *et al.*, 1996). In this study, a maximal concentration of CPBG (100 μM) increased VTA DA concentrations by ~400% above baseline levels, and this effect was completely blocked by a 5-HT₃ antagonist (ICS 205–930). Meanwhile, ethanol (2 g/kg, i.p.) stimulated DA release in the VTA by only ~50% over baseline levels, and, most importantly, this stimulatory action of ethanol on somatodendritic DA release was completely blocked by the 5-HT₃ antagonist. These findings indicate that the potentiating effect of ethanol on 5-HT₃ receptor function within the VTA may be critically involved in the activation of mesolimbic DA circuits (Lovinger, 1991). In support of this idea, it has been shown that both the acquisition and maintenance of intra-VTA self-administration of ethanol can be completely prevented by co-administration of a 5-HT₃ antagonist (Rodd *et al.*, 2005c; Rodd-Henricks *et al.*, 2003).

6. Acetylcholine—Clearly, there exists a unique interaction between the reinforcing actions of ethanol and nicotine (Dani and Harris, 2005); thus, it is logical to investigate how co-administration of nicotine and ethanol may interact to modulate VTA DA release. Söderpalm and colleagues first demonstrated that systemic blockade of nAChRs completely prevented the ability of systemic ethanol to increase DA concentration in the NAc (Blomqvist *et al.*, 1993). It has been further shown that intra-VTA, but not intra-NAc, infusion of nAChR antagonists abolishes the NAc DA release produced by systemic ethanol administration (Blomqvist *et al.*, 1997; Tizabi *et al.*, 2002) and even that elicited by intra-NAc administration of ethanol (Ericson *et al.*, 2003). The latter study raises the possibility that ethanol action in the NAc might stimulate cholinergic inputs into the VTA, thereby activating DA neuron activity. The potent effects of nAChR antagonists within the VTA, together with those of 5-HT₃ antagonists described previously, provide evidence that modulation of ligand-gated ion channels operated by ACh or 5-HT can play a permissive role in ethanol excitation of VTA DA neurons.

Tizabi *et al.* (2002) have extended these works and demonstrated that direct intra-VTA administration of a low dose of nicotine alone induces NAc DA release and enhances the ability of a low systemic dose of ethanol (0.5 g/kg, i.p.) to increase NAc DA levels in an additive manner. Essentially similar additive effects of ethanol and nicotine on VTA DA neuron firing have also been reported in brain slices (Clark and Little, 2004), which may contribute to the positive association between alcohol drinking and cigarette smoking in humans (Dani and Harris, 2005).

7. Opioids—As the general opioid antagonist, naloxone, is FDA approved for alleviating alcohol craving, it is important to understand opioid regulation of the mesolimbic DA output. Behaviorally relevant doses of ethanol have been shown to increase β-endorphin levels in the VTA, which appears to be particularly sensitive to ethanol compared to other brain areas in terms of β-endorphin release (Jarjour *et al.*, 2009; Rasmussen *et al.*, 1998). Furthermore, a significant reduction (~40%) in the binding of [³H]DAMGO, a μ-opioid receptor-selective radioligand, was observed in the VTA within 30 min of *in vivo* administration of ethanol (2.5 g/kg, i.g.), likely due to displacement by released β-endorphin and/or rapid internalization of μ-opioid receptors (Mendez *et al.*, 2001). As discussed previously, ethanol may stimulate VTA DA output via μ-opioid receptor-mediated inhibition of local GABA neuron firing (Xiao *et al.*, 2007). Indeed, systemic injection of the nonselective opioid receptor antagonist naltrexone

can prevent ethanol-induced DA release in the NAc associated with ethanol self-administration (Gonzales and Weiss, 1998). Furthermore, motor-activating actions of intra-VTA administration of ethanol and DAMGO were completely blocked by prior treatment with naltrexone or the μ -selective antagonist β -fulnaltrexamine (Sanchez-Catalan *et al.*, 2009).

In addition to these μ -opioid mechanisms, it has been recently reported that intra-VTA injection of a δ -selective “agonist” decreases ethanol consumption, potentially via presynaptic inhibition of GABA release onto VTA DA neurons (Margolis *et al.*, 2008a). Furthermore, a number of studies have demonstrated that stimulation of κ -opioid receptors suppresses ethanol consumption and preference (Lindholm *et al.*, 2001; Logrip *et al.*, 2009; Nestby *et al.*, 1999); however, the exact site(s) of action have not been addressed in these studies.

8. Cannabinoids—A single-unit recording study in anesthetized rats has assessed the involvement of cannabinoids in ethanol stimulation of VTA DA neuron firing (Perra *et al.*, 2005). In this study, NAc-projecting VTA DA neurons were identified by antidromic stimulation of the NAc; likewise, NAc neurons receiving inputs from the BLA were identified by their response to orthodromic stimulation of the BLA. Systemic administration of a relatively low dose of ethanol (0.5 g/kg, i.v.) enhanced VTA DA neuron firing and inhibited BLA-stimulated NAc neuron firing. Prior administration of a CB1 antagonist completely blocked the actions of ethanol in both brain areas. Furthermore, treatment with the CB1 antagonist 4 min after ethanol administration also completely reversed the ethanol enhancement of DA neuron firing back to pre-ethanol baseline levels. This post-treatment paradigm may be the only example in the literature showing reversal of ethanol stimulation of DA neuron firing by pharmacological manipulation of any neurotransmitter system. However, it remains to be determined how ethanol stimulates the endocannabinoid system in the VTA and other brain areas.

V. Plastic Changes in DA Neuron Activity after Previous Ethanol Exposure

A number of studies have demonstrated that there is a marked decrease in tonic DA levels in the NAc in animals withdrawn from repeated/chronic ethanol exposure (Diana *et al.*, 1993; Rossetti *et al.*, 1992; Weiss *et al.*, 1996). This hypodopaminergic state in the NAc after withdrawal from repeated/chronic exposure appears to be a common feature that is also observed among other drugs of abuse, such as opiates (Acquas *et al.*, 1991; Crippens and Robinson, 1994; Rossetti *et al.*, 1992) and psychostimulants (Imperato *et al.*, 1992, 1996; Robertson *et al.*, 1991; Rossetti *et al.*, 1992; but see Crippens and Robinson, 1994; Kalivas and Duffy, 1993; Weiss *et al.*, 1992b). Furthermore, human imaging studies have found reduced DA release in the striatum of alcoholics (Martinez *et al.*, 2005; Volkow *et al.*, 2007) as well as in the striatum of addicts to other drugs of abuse (Volkow *et al.*, 2004). It has been hypothesized that animals may compensate for the DA deficit by increasing the amount of drug intake (Melis *et al.*, 2005; Weiss *et al.*, 1992a), which seems to be congruent with the idea that there may be a mechanism to titrate DA levels in the NAc (see Section III.A.). Indeed, ethanol-withdrawn rats self-administer ethanol until NAc DA concentrations are restored to the pre-withdrawal baseline levels (Weiss *et al.*, 1996), indicating that the hypodopaminergic state may be an important component of ethanol dependence. However, it should be noted that other brain systems, such as the one involving CRF, have also been implicated in compulsive drug intake driven by the “negative affective state” during withdrawal (Koob, 2009).

In vivo recording studies have found reduced firing activity of VTA DA neurons after withdrawal from repeated/chronic ethanol exposure, which may account for the decrease in NAc DA levels described above (Diana *et al.*, 2003; Melis *et al.*, 2005). A series of work by Diana and colleagues have demonstrated that both tonic and phasic firing of DA neurons is dramatically decreased in rats withdrawn from 6-day ethanol administration (2–5 g/kg,

intragastric, four times/day) (Diana *et al.*, 1993, 1995). Importantly, not only the overall firing frequency but also the burst frequency, that is, the number of bursts per second, and the number of APs per burst were diminished in ethanol-withdrawn rats (Fig. 4), indicating that the influence of neurotransmitter inputs on DA neuron activity are altered after repeated ethanol exposure. This decrease in VTA DA neuron activity can be observed up to 3 days after the final ethanol administration (Diana *et al.*, 1996). In contrast, Shen *et al.* have reported a decrease in VTA DA neuron population activity (i.e., the proportion of spontaneously active neurons) without any change in the firing frequency or firing pattern of remaining spontaneously active DA neurons in ethanol-withdrawn rats (Shen, 2003; Shen and Chiodo, 1993; Shen *et al.*, 2007). This can be observed 1 day to 6 weeks after withdrawal from 10-day to 8-week ethanol administration (4–5 g/kg, intragastric, twice/day). The reduction in DA neuron population activity has been ascribed to depolarization inactivation (i.e., too much depolarization causing Na⁺ channel inactivation), as evidenced by reversal with apomorphine or amphetamine which hyperpolarize DA neurons via D2 receptor activation. The reason for the discrepancy between these two groups remains to be resolved.

It has been shown that the threshold for brain stimulation reward, assessed with intracranial self-stimulation (ICSS), is elevated after withdrawal from prolonged ethanol vapor exposure (≥ 17 days) (Schulteis *et al.*, 1995). Similar increases in “reward threshold,” that is, decreases in “reward sensitivity,” can be observed during withdrawal from all major drugs of abuse (Koob, 2009), and may reflect reduction in the functional output of the mesolimbic DA system.

In contrast to the studies described above, increases in basal DA levels in the NAc after withdrawal from repeated/chronic ethanol exposure have been reported in a line of rats selectively bred for high alcohol preference, termed alcohol-preferring (P) rats (Engleman *et al.*, 2003, 2000; Smith and Weiss, 1999; Thielen *et al.*, 2004). In addition, it has been shown that these rats become sensitized to the reinforcing effect of ethanol within the VTA after repeated ethanol exposure (Ding *et al.*, 2009a; Rodd *et al.*, 2005a,b). These studies in P rats illustrate genetic differences in ethanol-induced neuroadaptations of the mesolimbic DA system.

A. Plastic Changes in Intrinsic Properties of DA Neurons

No change in baseline pacemaker firing frequency of VTA DA neurons has been found in *in vitro* recording studies using VTA slices prepared from rats or mice that received repeated *in vivo* administration of ethanol (2–3.5 g/kg, i.p., once or twice daily, 5–21 days) (Brodie, 2002; Hopf *et al.*, 2007; Okamoto *et al.*, 2006). However, Bailey *et al.* reported a significant decrease in baseline firing frequency of VTA DA neurons recorded in brain slices 1–6 days after cessation of chronic ethanol consumption (>3 weeks) (Bailey *et al.*, 1998, 2001). The amount of ethanol intake was ~25–30 g/kg/day in these studies, suggesting that prolonged exposure to large amounts of ethanol may lead to suppression of DA neuron pacemaking. The mechanism of this decrease in intrinsic DA neuron activity is yet to be investigated.

Downregulation of I_h density without alteration in voltage dependence of I_h activation has been detected in VTA and SNc DA neurons 1 day after repeated *in vivo* ethanol injection (2 g/kg, i.p., once/day, 5 days) (Okamoto *et al.*, 2006). Reduction in I_h has also been observed in VTA DA neurons after 7 days of withdrawal from repeated ethanol administration (2 g/kg, i.p., twice/day, 5 days) (Hopf *et al.*, 2007). As stated above, these studies failed to detect alterations in baseline pacemaker frequency. However, the contribution of I_h to pacemaker activity appeared to be diminished in ethanol-treated animals, as evidenced by a smaller inhibition of firing frequency by pharmacological blockade of I_h (Okamoto *et al.*, 2006). These observations suggest that changes in other ionic conductances may compensate for I_h reduction to maintain the pacemaker activity of DA neurons (Guzman *et al.*, 2009). In line with this idea, Hopf *et*

al. (2007) detected a reduction in SK-mediated conductance activated by step depolarization in ethanol-treated animals, a change that may counter the effect of I_h downregulation.

The study by Okamoto *et al.* (2006) has found that the magnitude of ethanol stimulation of DA neuron firing is reduced 1 day after 5-day ethanol exposure. This tolerance to ethanol excitation of DA neurons may be a consequence of I_h downregulation, because I_h mediates, at least partially, the excitatory effect of ethanol on pacemaker activity (see Section IV.A.). In contrast, an increase in ethanol stimulation of VTA DA neuron firing has been reported after prolonged ethanol exposure (≥ 21 days) (Brodie, 2002). Therefore, the function/expression of ethanol-sensitive ion channels in DA neurons may undergo differential changes with different ethanol treatment protocols, resulting in tolerance or sensitization to the stimulatory effect of ethanol. In line with this, both tolerance and sensitization to ethanol stimulation of NAc DA release have been reported after repeated ethanol exposure (Ding *et al.*, 2009a; Zapata *et al.*, 2005), although alterations in neurotransmitter inputs to DA neurons may also play a significant role in these studies conducted *in vivo*.

B. Plastic Changes in Neurotransmitter Inputs to DA Neurons

1. Glutamate—No change in NMDA receptor function, assessed with bath perfusion of NMDA onto the slice, has been observed in VTA DA neurons from C57BL/6 mice after a single ethanol exposure (2 g/kg, i.p.) (Wanat *et al.*, 2009), repeated ethanol exposure (2 g/kg, i.p., twice daily for 5 days) (Hopf *et al.*, 2007), or even after more extended ethanol exposure (3.5 g/kg, i.p., twice daily for >21 days) (Brodie, 2002). Nevertheless, NMDA-induced burst firing appears to be enhanced due to downregulation of SK channels (Hopf *et al.*, 2007). Furthermore, prolonged volitional ethanol intake for 35–50 days has been shown to increase AMPA-mediated synaptic transmission onto VTA DA neurons in rats (Stuber *et al.*, 2008), as is well documented after single or repeated exposure to other drugs of abuse (Jones and Bonci, 2005; Kauer and Malenka, 2007). In contrast, a reduction in both AMPA receptor and NMDA receptor functions has been reported after a single ethanol exposure in DBA mice, demonstrating strain specificity of ethanol-induced neuroadaptations (Wanat *et al.*, 2009).

A biochemical study has analyzed the expression levels of the obligatory NMDA receptor subunit, NR1, and AMPA receptor subunits GluR1 and GluR2 in VTA slice homogenates from rats following up to 12 weeks of 5% ethanol liquid diet feeding (Ortiz *et al.*, 1995). Both NR1 and GluR1, but not GluR2, levels were increased by nearly 30% following 12 weeks of ethanol diet, whereas no changes in any of these subunits were detected after a week of ethanol consumption. GluR1 upregulation is consistent with the increase in AMPA-mediated synaptic transmission after prolonged ethanol drinking (Stuber *et al.*, 2008). The functional significance of the increased NR1 levels (e.g., synaptic vs. extrasynaptic) remains to be determined. It should also be cautioned that biochemical assays using tissue homogenates do not discriminate different cell types present in the brain areas being analyzed.

2. GABA—Brodie was the first to directly investigate the potential for neuroadaptive changes in major neurotransmitter systems in the VTA after previous ethanol exposure (Brodie, 2002). In this study, reduced sensitivity to GABA-induced inhibition of VTA DA neuron firing, probably mediated by both GABA_A and GABA_B receptors, was observed in midbrain slices prepared from mice chronically treated with high-dose ethanol (3.5 g/kg, i.p., 21 days). It is not clear if this reduced sensitivity to GABA contributed to the sensitization to ethanol excitation of DA neurons observed in the same study. Conversely, Bonci's group has demonstrated a persistent increase in GABA release onto VTA DA neurons recorded in brain slices 24 h after a single exposure to ethanol (2 g/kg, i.p.) in C57BL/6 and DBA/2 mice (Melis *et al.*, 2002; Wanat *et al.*, 2009). This enhanced GABA release, likely resulting from upregulation of the cAMP–protein kinase A (PKA) signaling cascade at GABAergic terminals,

can be observed even a week after the single ethanol exposure, suggesting that it may have enduring behavioral consequences.

A potential increase in GABAergic inhibition of DA neurons has also been suggested in an *in vivo* recording study of VTA GABA neurons following chronic ethanol exposure. Utilizing either i.p. injection (2 g/kg, twice daily) or intermittent vapor exposure, VTA GABA neurons in freely moving rats displayed an increase in basal firing rate, together with tolerance to the acute inhibitory effect of ethanol, following 14 days of ethanol exposure (Gallegos *et al.*, 1999). However, recordings of VTA GABA neurons in anesthetized rats fed with a liquid diet containing 5% ethanol for 2–3 weeks revealed no differences in baseline firing rate and in the acute inhibitory effect of ethanol (Ludlow *et al.*, 2009). Therefore, neuroadaptive responses of VTA GABA neurons to chronic ethanol appear to vary with different treatment and recording protocols. The contribution of these alterations in GABAergic transmission to the change in DA neuron activity, for example, reduced tonic and phasic firing (Diana *et al.*, 1993, 1995) (Fig. 4), has yet to be addressed.

A reduction in the levels of the GABA_A receptor $\alpha 1$ subunit has been documented in the VTA; however, the duration of ethanol exposure required to detect this reduction appears to be significantly longer (12 weeks) than that required to observe a similar change in other brain areas (2–4 weeks) (Charlton *et al.*, 1997; Ortiz *et al.*, 1995; Papadeas *et al.*, 2001). It should be noted here that the GABA_A receptor $\alpha 1$ subunit is selectively expressed in GABA neurons, but not in DA neurons, in the VTA (Okada *et al.*, 2004; Tan *et al.*, 2010). Therefore, examination of changes in other GABA_A receptor subunit isoforms (e.g., $\alpha 2$, $\alpha 3$, $\alpha 4$) may be warranted in the future.

3. Dopamine (DA)—Prolonged (12-week), but not short-term (1- or 6-week), ethanol (5%) consumption has been shown to increase levels of tyrosine hydroxylase, the rate-limiting enzyme for DA synthesis, by ~70% in the VTA but not in the SNc (Ortiz *et al.*, 1995). Furthermore, an increase in tyrosine hydroxylase (TH) mRNA levels has also been reported after chronic ethanol consumption (5%, 4 weeks) in the VTA with no change in the SNc (Lee *et al.*, 2005). This increase in TH mRNA was completely blocked by concurrent daily administration of the opioid receptor antagonist naltrexone. Consistent with the change in TH levels, increased levels of DA and the DA metabolite DOPAC have been detected in the VTA, but not in the SNc, following consumption of ethanol (6.6%)-containing lipid diet for 1 month (Pellegrino and Druse, 1992). In contrast, a decrease in VTA DA levels has been documented following a very similar ethanol liquid diet administration (6.6%, 6 weeks) in relatively old rats (5 months) (Woods and Druse, 1996). Thus, the effect of chronic ethanol on DA production/turnover in the VTA may depend on the age of animals.

4. Opioids—It has been shown that κ -opioid receptor mRNA is decreased (to ~15% of baseline levels) in rats following repeated ethanol exposure (2 g/kg, i.p., twice daily, 14 days) (Rosin *et al.*, 1999). This may reflect compensatory downregulation of the receptor in response to increased levels of dynorphin, the endogenous κ -opioid, in the mesolimbic system observed after withdrawal from repeated ethanol exposure (Lindholm *et al.*, 2000; Przewlocka *et al.*, 1997). The increase in dynorphin levels might contribute to the aversive emotional state of ethanol withdrawal (Bals-Kubik *et al.*, 1993; Koob, 2009; Trevisan *et al.*, 1998; Wee and Koob).

VI. Concluding Remarks

We started this chapter by asking: “Why do we drink and why do we keep drinking?” Clearly, regulation of the mesolimbic DA output plays a critical role in alcohol drinking in nondependent individuals and also in driving compulsive alcohol consumption in dependent

individuals (alcoholics). Based on this postulate, a number of potentially important “ethanol targets” have been identified over the past couple of decades, both in VTA DA neurons themselves and in the synaptic inputs impinging on them. These include ion channels, neurotransmitter receptors, intracellular signaling molecules, and machinery controlling neurotransmitter/neuromodulator release, as reviewed in this chapter. One of the challenges is to determine which of these ethanol targets in the VTA contribute to specific ethanol actions in behaving animals? Alternatively, could the effects of ethanol on neurons projecting to DA neurons play more important roles than ethanol targets within the VTA? Recent advances in manipulating specific neurons in the brain, such as optogenetic control of neuronal activity (Zhang *et al.*, 2007) or cell type-specific knockin strategies (Skvorak *et al.*, 2006), might give us fresh approaches to these questions over the next decade. Finally, because alcoholism, like addiction to other drugs of abuse, has a strong learning component (Hyman *et al.*, 2006), it is important to fully understand the influence of ethanol (acute and chronic) on the plasticity mechanisms involved in learning of the behaviors and environments associated with ethanol intake, both in the VTA and DA projection areas.

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Fig. 1.

Tonic and phasic DA neuron firing. (A) *In vivo* single-unit recording of DA neuron firing in an anesthetized rat. A spontaneous burst of APs is shown in the background of low-frequency tonic firing. There is a transient pause of activity before tonic firing recommences. Adapted from Overton and Clark (1997). (B) Loose-patch extracellular recording of DA neuron firing in a rat midbrain slice. Iontophoretic application of aspartate was made at the time indicated by the arrow (50 ms). (C) Traces illustrating the effects of the NMDA receptor antagonist AP-5 and the mGluR antagonist MCPG on the phasic burst-pause type of activity evoked by repetitive stimulation of glutamatergic afferents (eight stimuli at 66 Hz; left traces) or aspartate iontophoresis (200 ms; right traces). Also note the tonic pacemaker-like firing before and after the burst-pause. Adapted from Morikawa *et al.* (2003).

Fig. 2.

Spontaneous activity of DA neurons. (A) Intracellular recording of spontaneous DA neuron firing in a brain slice. (B) The Na⁺ channel blocker tetrodotoxin (TTX) abolished APs and revealed the spontaneous membrane potential oscillation, which was completely eliminated by the L-type Ca²⁺ channel antagonist nifedipine. Dotted lines indicate the AP threshold (−40 mV). Adapted from Nedergaard *et al.* (1993).

Fig. 3. Ethanol stimulates DA neuron firing. (A) Example traces of single-unit recording of DA neuron firing before (top) and 5 min after i.v. injection of ethanol (bottom) in an awake rat. Adapted from Mereu *et al.* (1984). (B) Representative traces of DA neuron firing recorded with a perforated-patch configuration in a brain slice. Note the effect of ethanol on the membrane potential trajectory during the interspike interval. Adapted from Okamoto *et al.* (2006).

Fig. 4. Schematic diagram illustrating the effects of acute and repeated ethanol exposure on DA neuron firing. Vertical lines indicate individual spikes. Phasic bursts are marked by arrowheads.