THE DOPAMINE HYPOTHESIS OF DRUG ADDICTION: HYPODOPAMINERGIC STATE

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Drug addiction is a brain disorder caused by the repetitive use of various chemicals which alter normal functioning of the central nervous system with consequent behavioral abnormalities. In the search to understand which neurotransmitter systems play upon this behavioral pathology, dopamine has long been thought to play a *prima donna* role. However, its primary role is commonly and erroneously attributed to the increase in activity after acute administration of addicting drugs. On the contrary, the mesolimbic dopamine transmission appears to be drastically *reduced* in its tonic activity when measured in animal models, which mimic the human condition of drug addiction, and in the available human studies conducted in addicted subjects. This paper is a systematic review of the pertinent literature which strongly supports this concept. Various

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experimental approaches such as electrophysiological, biochemical, behavioral, biomolecular and even anatomical, show that dopamine neurons work insufficiently in the crucial phases of the entire drug addiction cycle such as withdrawal from chronic treatment. This hypodopaminergic state is viewed as one of the main causes that triggers drug-seeking and taking, even after prolonged drug-free periods, perpetuating the vicious cycle. In addition, albeit reduced in its activity, the system remains hyperresponsive to abused drugs conferring long-lasting vulnerability to the system. We propose that decreased dopamine function in addicted subjects results in a decreased interest to non drug-related stimuli and increased sensitivity to the drug of choice. Targeting the dopamine system with pharmacological agents, not necessarily classic receptor-oriented drugs, aimed at restoring dopamine transmission may reveal useful new avenues in the treatment of this socially debilitating brain pathology.

I. Drug Addiction as a Brain Disease

Although the phenomenon of drug abuse has been typically perceived as a "moral" (Musto, 1997; O'Brien and Fishman, 2002) defect (and still is by some) and/or character weakness, the persuasive nature of data emerging from rigorous scientific investigation renders this view obsolete and no longer tenable. It is widely and increasingly recognized, nowadays, as a brain disease. This holds true for the scientific community and its ample recognition leans on support from a number of institutions that provide means to investigate its pathophysiological basis. Indeed, not different from traditional diseases, drug addiction bears with it a number of biological abnormalities that have been documented by employing behavioral, electrophysiological, biochemical, and morphological methods, all of which point at an altered brain physiology, which justifies the label *disease*. Although repetitive use of drugs affects different organs (i.e., alcohol affects the liver), the primary target appears to be the brain—thus, *brain disease*.

The conceptualization of drug addiction as a brain pathology has profound social reflections because it implies a total absence of moral connotation, and thus, a drug abuser is not a "criminal" but simply a "patient" who needs treatment irrespective of the causes that triggered the drug-taking behavior. Once accepted, the disease concept prompts further questions: What has occurred in the brain of an addicted individual? A simple attempt to provide an answer will spur such an enormous amount of data that it would be impossible to cover in a chapter; however, a neurotransmitter system (i.e., the mesolimbic dopamine [DA] system) appears to be modified in its functioning more than others and appears to fluctuate differently and predictably, depending on acute drug

challenge, chronic drug treatments, and withdrawal conditions, irrespective of the chemical abused. This is not to say that other systems are not involved or important in the pathophysiology of addiction. It simply suggests that the DA system participates in the most harmful consequences of repetitive drug use and is a major determinant of craving and relapse even after drug-free periods. Accordingly, the DA system reduces its activity under circumstances that mimic "urge" (craving) for the drug that drives behavior toward seeking and ultimately obtaining (drug taking) the desired molecule, thus perpetuating the cycle. In brief, the "dopamine hypothesis" contends that a hypodopaminergic state characterizes animal models of drug addiction and addicted human brains, and the frequently cited increase in activity after acute drug challenge plays only a minor initial role in the context of the disease and its development over time.

Neurobiological mechanisms thought to be at the basis of the disease have been reviewed extensively. In 1978, in an elegant series of studies (Fouriezos *et al.*, 1978; Wise, 1978), Wise first hypothesized that activation of the reward system was closely associated with an increased activity of DA-containing pathways (Corbett and Wise, 1980), and not noradrenergic (Corbett and Wise, 1979; Yokel and Wise, 1975, 1976) pathways, produced by electrical self-stimulation of ascending DA fibers. In particular, the mesolimbic pathway, which projects from the ventral tegmental area (VTA) to the nucleus accumbens (NAcc) has been hypothesized to mediate reward of pleasant stimuli such as various addictive drugs (Bozarth and Wise, 1981; De Wit and Wise, 1977; Yokel and Wise, 1975; 1976), drinking (Gerber *et al.*, 1981), food (Wise *et al.*, 1978a,b), and even sex (Balfour *et al.*, 2004).

Today the role of the mesolimbic DA system is well established: Intracranial self-stimulation (ICSS) electrodes located in the lateral hypothalamus or in the medial forebrain bundle indirectly stimulate (Yeomans, 1989; Yeomans *et al.*, 1993, 2000) (depolarize) ascending DA-containing fibers whose synaptic terminals release DA, which in turn binds postsynaptic DA receptors, thereby potentiating DA neurotransmission. In addition, chemical lesions of DA fibers (Fibiger, 1978; Fibiger *et al.*, 1976) or administration of DA antagonists (Fibiger, 1978; Fibiger *et al.*, 1976) produces a decreased sensitivity to ICSS. Although the issue of neuroleptic-induced motor performance deficits was initially suspected as the cause of ICSS disruption (Fibiger *et al.*, 1976), additional experiments confirmed the major role played by DA in reward (Wise and Bozarth, 1982). A large amount of experimental studies have been carried out with the purpose of clarifying the link between DA and reward, but a detailed account of the specific literature is beyond the scope of this chapter. The reader is referred to the recent excellent reviews (Di Chiara, 1999; Hyman and Malenka, 2001; Kakade and Dayan, 2002; Robbins and Everitt, 1996; Salamone *et al.*, 1997; Schultz,

1998a,b), which have attempted to disentangle the complex role of DA in animal behavior. In spite of the tremendous amount of data, the role of DA neurons in the physiology of reward is still a matter of intense scientific debate. One influential theory suggests that mesolimbic DA mediates reward through pleasurable effects of addictive drug (Wise and Bozarth, 1982), and other works suggest that DA signals interest in reward (Stewart, 1984), the expectation that reward is forthcoming (Schultz, 1998a,b), or wanting reward as opposed to liking it (Berridge and Robinson, 1998; Robinson and Berridge, 1993). Still others argue about the prominent role that DA plays in incentive motivation (Di Chiara, 1999; Di Chiara *et al.*, 1999) or appetitive learning (Cardinal and Everitt, 2004; Robbins and Everitt, 2002).

DA neuronal activity may be part of a continuum in which these cells modify firing rate and/or pattern according to the pleasantness/aversiveness of the stimulus. Indeed, experiments using drugs of abuse as a stimulus, pleasant when acutely administered or unpleasant as during withdrawal, support this conclusion (Pulvirenti and Diana, 2001). In addition, experiments not employing drugs, but various experimental conditions, further support this notion. Schultz *et al.* (1997) have shown that when a monkey receives a reward (apple), DA neurons increase their firing rate but not when the light that signals the reward is turned on. After training, DA neurons increase their activity when the animal sees the light (conditioning stimulus) and not when it receives the actual reward (apple). However, if after the light, the reward is *not* presented, DA neurons "decrease" their firing activity. These experiments indicate that DA neurons are sensitive to both like (reward) and dislike (absence of an "expected" reward). The increase in firing observed after learning upon turning on the light suggests that the "reward value" is now attributed (by the animal) to the light that signals the forthcoming "real" reward. Accordingly, Ungless *et al.* (2004), working with anesthetized rats, have shown that tyrosine hydroxylase–positive (TH^+) units decrease their firing in aversive circumstances, whereas electrophysiologically similar units, probably not dopaminergic and certainly not TH^+, do not. Collectively, these experiments are reminiscent of those employing drugs of abuse as a stimulus (see later discussion) and strongly support the assertion of a direct signaling of DA neurons of pleasant/unpleasant conditions.

In this chapter, we assume that an acute drug challenge is a pleasant stimulus, whereas withdrawal from chronic administration is perceived as an unpleasant or aversive situation.

Controversy and disagreement with respect to the interpretation of data is common in the scientific literature; literature on the involvement of dopaminergic neurons in drug addiction is no exception. Where relevant, we point out some of the current areas of contention and discuss them in light of more recent findings.

II. The Mesolimbic Dopamine System

A. Intrinsic Properties

A first detailed description of the dopaminergic systems in the rat brain revealed three discrete regions containing about 75% of the DA neurons contained in the entire brain (Dahlstrom and Fuxe, 1964). The DA cells located within the VTA project to the limbic subcortical areas (i.e., NAcc, amygdala, and olfactory tubercle) and to the limbic cortices (i.e., medial prefrontal, cingulated, and entorhinal), thereby constituting the mesolimbocortical system (Anden *et al.*, 1966; Bjorklund and Lindvall, 1975; Lindvall and Bjorklund, 1974; Loughlin and Fallon, 1983; Ungerstedt, 1971). This chapter focuses on mesolimbic DA neurons, which have been extensively characterized by means of electrophysiological techniques both *in vivo* (Aghajanian and Bunney, 1977; Bunney *et al.*, 1973; Ungless *et al.*, 2004) and *in vitro* (Grace and Onn, 1989; Johnson and North, 1992b; Lacey *et al.*, 1990).

In vivo, VTA DA neurons display a typical firing pattern that is either single spiking or consisting of bursts of action potentials (Bunney *et al.*, 1973; Grace and Bunney, 1984a,b). The bursting mode has been shown to be more efficient in increasing DA outflow in the terminal regions than the single-spike firing mode (Bean and Roth, 1991; Diana and Tepper, 2002; Gonon, 1988; Gonon and Buda, 1985; Overton and Clark, 1997); therefore, it might mediate synaptic changes and contribute to reward-related learning processes (Gonon, 1988; Reynolds and Wickens, 2002; Reynolds *et al.*, 2001; Schultz *et al.*, 1997; Wightman and Robinson, 2002; Williams and Millar, 1990).

The action potential of a typical midbrain DA neuron has a characteristic triphasic shape of a width greater than 2 ms (Bunney *et al.*, 1973; Diana and Tepper, 2002; Grace and Bunney, 1983a,b, 1984a; Groves *et al.*, 1975), which has been more properly refined to be greater than 1.1 ms when measured from the start of the action potential to the negative trough (Ungless *et al.*, 2004). Interestingly, this latter study showed, for the first time since the first characterization, that *in vivo* the VTA also possesses a third class of cells that are neither dopaminergic nor GABAergic, although they resemble dopaminergic cells based on their electrophysiological properties. Consistently, an *in vitro* study previously showed that the VTA does possess a subset of cells that are nondopaminergic but that do exhibit similar anatomical and electrophysiological features to DA cells in the VTA (Cameron *et al.*, 1997).

In the intact brain (Grace and Bunney, 1983a,b, 1984a,b), it has been difficult to evaluate the intrinsic properties of these cells because of the mutual interactions, multiple inputs, and strong feedback from the target areas and within the VTA. However, intracellular recordings of midbrain DA neurons *in vivo* have

established that DA units display long membrane time constants (5–14 ms), high input resistance (R_{IN}; between 18 and 45 MegaOhms) and resting membrane potentials (RMPs) greater than −50 mV (Grace and Bunney, 1983c, 1984b). In agreement with extracellular recordings of VTA DA cells (Bunney *et al.*, 1973; Ungless *et al.*, 2004), intracellular analysis carried out *in vivo* has shown that DA neurons have long-duration action potentials (2–5 ms) followed by an afterhyperpolarization period (AHP) (3 mV, 1–6 ms) related to activation of a calcium-dependent potassium current (I_{KCa}).

VTA DA cells *in vitro* are mainly characterized by a regular (pacemaker-like), single-spike spontaneous firing (Johnson and North, 1992b; Lacey, 1993) presumably because of the loss of extrinsic afferents impinging on these neurons. The action potential has a long duration (>2 ms), a pronounced AHP, and a sag component, which is mediated by a hyperpolarization-activated, cyclic nucleotide–regulated cation channels (I_h) evoked in response to hyperpolarizing pulses (Grace and Onn, 1989; Johnson and North, 1992b; Mercuri *et al.*, 1995; Richards *et al.*, 1997). Interestingly, Neuhoff *et al.* (2002) have demonstrated that DA midbrain subpopulations significantly diverge from a single electrophysiological phenotype (Kitai *et al.*, 1999) and that differences in I_h, probably corresponding to different densities of functional I_h channels, might be an important mechanism responsible for the functional diversity of DA cells. Particularly, Neuhoff *et al.* (2002) demonstrated that VTA DA cells positively labeled for calbindin (CB^+) and evoking small I_h currents displayed an irregular discharge at higher frequencies with a prolonged AHP. This rebound delayztended to be longer in CB^+ DA neurons whose position was closer to the midline of the midbrain. Because these CB^+ VTA DA cells displayed fast pacemaker frequencies (>5 Hz), a number of authors suggested that they might represent the subpopulation of VTA DA neurons projecting to the prefrontal cortex (Chiodo *et al.*, 1984; Gariano *et al.*, 1989). Conversely, CB^- VTA DA cells localized laterally within the VTA responding with large I_h currents are more likely to form the mesolimbic pathway (Carr and Sesack, 2000; Oades and Halliday, 1987). I_h currents are not essential for setting the R_{IN} of DA neurons (Mercuri *et al.*, 1995), although they play a key role at more hyperpolarized potentials (around −100 mV) (Amini *et al.*, 1999). The R_{IN} of DA neurons *in vitro* is significantly higher (up to 300 MegaOhms) than that observed *in vivo,* which might be the result of deafferentation of the slice preparation (Grace and Bunney, 1983a,c).

It is widely accepted that a Na^+-dependent current mainly contributes to the slow oscillatory potentials (SOPs) (Grace and Onn, 1989; Nedergaard *et al.*, 1993; Ping and Shepard, 1996). Additionally, Amini *et al.* (1999) provided elegant evidence for the Ca^{2+}-dependent mechanisms underlying SOP. They demonstrated that SOPs are due to activation of L-type Ca^{2+} currents ($I_{Ca,L}$), leading to

depolarization of the membrane and increased levels of intracellular Ca^{2+}. The Ca^{2+} increase, in turn, activates small-conductance $I_{K,Ca}$ ($I_{K,Ca,SK}$), hyperpolarizes the cell membrane, and depresses $I_{Ca,L}$ and intracellular Ca^{2+} levels (Amini *et al.*, 1999). This spontaneous pacemaker activity can be blocked by dihydropyridines (Mercuri *et al.*, 1995) because the Ca^{2+} conductance mediating the SOP is dihydropyridine sensitive (Ping and Shepard, 1999). The regular firing pattern is dependent on activation of 4-aminopyridine (4-AP)–sensitive currents (I_A), which slow the recovery of membrane potential (V_M) (Silva *et al.*, 1990).

In vitro VTA DA cells can also display burst activity when *N*-methyl-D-aspartate (NMDA) and the small-conductance Ca^{2+}-dependent K^+ channel (SK) blocker, apamin, are applied (Seutin *et al.*, 1993). Otherwise, DA cells can switch from the pacemaker-like firing to a bursting mode when group I mGluRs are activated and SK reduced (Mercuri *et al.*, 1993; Prisco *et al.*, 2002; Zheng and Johnson, 2002). Both types might bear relevance in information coding and ultimately result in the translation of the glutamatergic signal into the dopaminergic one onto their target neurons in the forebrain.

B. Afferent Regulation

The striking differences between the characteristics of VTA DA cells recorded *in vivo* and *in vitro* reveal the weight of the inputs on the control of both the spontaneous activity of these neurons (Johnson and North, 1992b) and the somatodendritic DA release (Chen and Rice, 2002), which contributes to the regulation of the burst firing through a network feedback mechanism (Paladini *et al.*, 2003). VTA DA cells possess an additional self-regulatory mechanism that involves the endocannabinoid system, a novel class of retrograde messengers (Piomelli, 2003). In fact, VTA DA cells release endocannabinoids in an activity-dependent manner, which depresses glutamatergic afferents on mesolimbic DA cells (Melis *et al.*, 2004a) and ultimately their own firing activity and pattern (Melis *et al.*, 2004b).

The afferent inputs to VTA DA neurons comprise glutamatergic (Alheid *et al.*, 1998; Carr and Sesack, 2000; Charara *et al.*, 1996; Christie *et al.*, 1985; Sesack and Pickel, 1992; Smith *et al.*, 1996; Taber *et al.*, 1995; Thierry *et al.*, 1983), GABAergic (Spanagel and Weiss, 1999; Waddington and Cross, 1978), cholinergic (Garzon *et al.*, 1999; Oakman *et al.*, 1995; Semba and Fibiger, 1992; Woolf, 1991), serotonergic (Herve *et al.*, 1987), and noradrenergic fibers (Bayer and Pickel, 1990).

1. *Glutamatergic*

Main sources of glutamatergic inputs to the VTA arise from the prefrontal cortex (Carr and Sesack, 2000; Christie *et al.*, 1985; Sesack and Pickel, 1992; Smith *et al.*, 1996; Taber *et al.*, 1995; Thierry *et al.*, 1983), the pedunculopontine

region (Charara *et al.*, 1996; Kelland *et al.*, 1993), and the bed nucleus of the stria terminalis (Alheid *et al.*, 1998; Georges and Aston-Jones, 2001, 2002). Through activation of ionotropic (Chergui *et al.*, 1993; Wang and French, 1993a,b, 1995) and metabotropic receptors (Mercuri *et al.*, 1993; Shen and Johnson, 1997), glutamate is thought to regulate the spontaneous activity of VTA DA cells *in vivo* (Charlety *et al.*, 1991; Grenhoff *et al.*, 1988; Svensson and Tung, 1989). Consistently, *in vitro,* electrical stimulation of the afferents elicits postsynaptic currents mediated by activation of ionotropic (Johnson and North, 1992b; Mercuri *et al.*, 1992b; Seutin *et al.*, 1990) and/or metabotropic receptors (Mercuri *et al.*, 1992b; Shen and Johnson, 1997; Zheng and Johnson, 2002). Activation of ionotropic receptors causes depolarization of VTA DA neurons, typically accompanied by an increased cell firing rate or bursting activity (Meltzer *et al.*, 1997; Mercuri *et al.*, 1996; Mereu *et al.*, 1991; Paladini *et al.*, 1999; Seutin *et al.*, 1990; Wang and French, 1993b). Particular emphasis has been placed on the study of the role played by glutamatergic inputs onto VTA DA neurons, given the growing body of evidence suggesting that this transmission in the VTA plays an important role in the actions of many drugs of abuse and addiction (Kalivas and Stewart, 1991; Kauer, 2004; Pulvirenti and Diana, 2001).

2. *GABAergic*

VTA dopaminergic neurons receive GABAergic inputs from two major sources: the medium spiny neurons of the NAcc and the interneurons of the VTA (Diana and Tepper, 2002; Spanagel and Weiss, 1999; Waddington and Cross, 1978). Thus far, it appears that the primary inhibitory regulation of DA cells comes from collaterals of GABAergic projection neurons within the VTA (Churchill *et al.*, 1992; Steffensen *et al.*, 1998) in a similar way to the substantia nigra pars compacta (Tepper *et al.*, 1995). Interestingly, besides having local connections onto DA cells (Johnson and North, 1992b), a subset of VTA GABA cells project to other areas, such as the NAcc (Van Bockstaele and Pickel, 1995), thus providing an output different from the dopaminergic one (Steffensen *et al.*, 1998). GABA hyperpolarizes VTA DA cells through activation of either $GABA_A$ or $GABA_B$ receptors (Diana and Tepper, 2002; Johnson and North, 1992b; Erhardt *et al.*, 2002) presumably originating from distinct sets of presynaptic fibers and regions (Sugita *et al.*, 1992). GABA-mediated hyperpolarization of VTA DA neurons results from the opening of Cl^- and K^+ channels following activation of $GABA_A$ and $GABA_B$ receptors, respectively (Johnson and North, 1992b; Lacey *et al.*, 1988).

3. *Cholinergic*

The cholinergic input to VTA DA cells arises in the pedunculopontine and laterodorsal tegmental nuclei (Garzon *et al.*, 1999; Oakman *et al.*, 1995; Semba and Fibiger, 1992; Woolf, 1991). Acetylcholine, through activation of muscarinic

and nicotinic receptors expressed on DA neurons (Clarke and Pert, 1985; Cortes and Palacios, 1986; Schwartz, 1986; Wada *et al.*, 1989), depolarizes DA neurons *in vitro* (Calabresi *et al.*, 1989a; Grillner and Mercuri, 2002; Lacey *et al.*, 1990a) and excites them *in vivo* (Grenhoff *et al.*, 1986; Mereu *et al.*, 1987), although this effect desensitizes rapidly (Pidoplichko *et al.*, 1997; Yin and French, 2000). The initial fast response seems to be mediated by nicotinic receptors, whereas muscarinic receptors are responsible for a prolonged response (Yeomans *et al.*, 2001). Also, muscarinic and nicotinic receptors may mediate different types of reward, such as eating and tobacco smoking, respectively (Rada *et al.*, 2000; Watkins *et al.*, 2000).

4. *Serotonergic*

Dense serotonergic afferents from the raphe nuclei to VTA DA neurons are reported to be inhibitory (Herve *et al.*, 1987) through activation of 5-HT_{2C} receptors (Di Mascio *et al.*, 1998; Gobert *et al.*, 2000). *In vitro* studies, however, have reported heterogeneous responses of VTA DA neurons to bath application of serotonin (5-HT): In fact, DA cells can be either depolarized, hyperpolarized, or even not affected (Pessia *et al.*, 1994). 5-HT, through activation of 5-HT_{2C} receptors, depolarizes (Pessia *et al.*, 1994) DA cells, while by acting on 5-HT_{1A} receptors hyperpolarizes the tertiary cells within the VTA (Cameron *et al.*, 1997). Thus far, the actions of 5-HT on VTA DA cells appear rather complex given its effects on synaptic transmission (Cameron and Williams, 1994; Johnson *et al.*, 1992; Jones and Kauer, 1999). In fact, early work has reported 5-HT to inhibit $GABA_B$-mediated synaptic currents (Johnson *et al.*, 1992) via activation of 5-HT_{1B} located on a subset of GABAergic presynaptic terminals (Sugita *et al.*, 1992), thus disinhibiting DA cells (Cameron and Williams, 1994). More recently, activation of presynaptic 5-HT receptors has been demonstrated to depress excitatory synaptic transmission onto VTA DA cells (Jones and Kauer, 1999), providing an alternative explanation for the observed amphetamine-induced reduction of burst firing of VTA DA cells.

5. *Noradrenergic*

The noradrenergic afferents to the VTA arise from the locus coeruleus (Bayer and Pickel, 1990) and have long been thought to make the firing pattern more regular without affecting the firing rate (Grenhoff and Svensson, 1989; Grenhoff *et al.*, 1993, 1995). Noradrenaline, *in vitro*, induces an inward current (depolarization) through activation of α_1 receptors (Grenhoff *et al.*, 1995). However, Paladini and Williams (2004) demonstrated that brief activation of α_1 receptors increased an SK (calcium-dependent potassium conductance) and mediated an outward current (hyperpolarization) that completely desensitizes during application of noradrenaline. Thus, either depolarization or hyperpolarization of VTA DA cells would appear to depend on duration of activation of noradrenergic receptors (Paladini and Williams, 2004).

C. Response to Acute Drugs

By altering the amount of excitatory and inhibitory inputs onto the DA neuron, a drug influences neuronal excitability and, therefore, the behavioral actions of DA itself. The positive/rewarding effects of nearly all drugs of abuse, as well as natural rewards, are associated with activation of the mesolimbic DA system (Koob, 1992b,c). This effect is extremely relevant given that the abuse liability of a drug is enhanced by rapidity of onset. As a result, effects occurring soon after drug administration are associated with it and are more likely to trigger a chain of events leading to compulsive drug taking.

In vivo, acute effects of drugs of abuse are not homogeneous. Most substances of abuse (such as ethanol, morphine, nicotine, and cannabinoids) increase the spontaneous activity, in terms of firing rate and busting activity, of VTA DA neurons (Diana *et al.*, 1998a; French, 1997; Gessa *et al.*, 1985, 1998; Grenhoff *et al.*, 1986; Gysling and Wang, 1983; Matthews and German, 1984; Melis *et al.*, 2000; Mereu *et al.*, 1987). Interestingly, acetaldehyde, which is the principal metabolite of ethanol, has also been reported to increase VTA DA neuronal activity (Foddai *et al.*, 2004). Because, traditionally, acetaldehyde was considered simply an aversive metabolite of ethanol, this study suggests that it might actually contribute to the positive motivational properties of ethanol itself (Quertemont, 2004; Rodd-Henricks *et al.*, 2002, 2003). On the contrary, psychostimulants, such as cocaine and amphetamine, decrease VTA DA neuronal activity by blocking DA reuptake, thereby increasing DA release and activating feedback mechanisms (Bunney *et al.*, 1973; Einhorn *et al.*, 1988; Groves *et al.*, 1975). Amphetamine has been shown to produce opposing effects on DA neuronal activity: a DA-mediated feedback inhibition, a 5-HT–mediated suppression of excitatory inputs (Jones and Kauer, 1999), and an α_1-mediated excitation (Shi *et al.*, 2000). However, the overall effects mediated by other psychostimulants (e.g., cocaine, methamphetamine, and methylphenidate) lead to inhibition of DA cells, given that they mimic amphetamine effects (α_1-mediated excitation) on these neurons only in the presence of D_2 receptor antagonists (Shi *et al.*, 2000).

In vitro studies have helped to understand the underlying mechanisms of action of addictive drugs. For instance, morphine-induced excitation of DA neurons results from a hyperpolarizing effect on VTA GABA interneurons (Johnson and North, 1992a). In particular, activation of μ-opioid receptors located on GABA, but not DA, cells accounts for this hyperpolarization. Thus, a reduced GABA-mediated synaptic input to DA cells leads to their depolarization through a disinhibition mechanism. Conversely, ethanol actions on DA neurons seem to be direct, given that it depolarizes mechanically dissociated DA cells (Brodie and Appel, 1998), but does not affect synaptic transmission (Brodie *et al.*, 1990). Specifically, the mechanisms underlying ethanol-induced

excitation of DA cells involve reduction of the amplitude of the AHP (Brodie and Appel, 1998) and of small conductance calcium-activated potassium currents (Brodie *et al.*, 1999). Additionally, ethanol actions on VTA DA cells might be produced by its metabolite acetaldehyde, which directly produces an inward current (Melis and Diana, personal observations, 2004) and, therefore, increases VTA DA neuronal activity (Foddai *et al.*, 2004). In contrast, nicotine exerts direct actions on DA neurons by acting on nicotinic acetylcholine receptors (nAChRs) (Calabresi *et al.*, 1989a). However, the observations that nicotine activates and rapidly desensitizes DA cells (Calabresi *et al.*, 1989a; Picciotto *et al.*, 1998; Pidoplichko *et al.*, 1997) do not provide an explanation for the persistent increased extracellular DA levels detected in the NAcc (Schilstrom *et al.*, 1998). Nonetheless, an elucidation of its long-lasting effects has been offered. In fact, nicotine also binds to and activates presynaptic nAChRs located on glutamatergic afferents, thus enhancing excitatory synaptic transmission to the VTA (Mansvelder and McGehee, 2000). More specifically, activation of presynaptic nAChRs induces long-term potentiation (LTP) of excitatory inputs to VTA DA cells, which outlasts nAChRs desensitization. Additionally, nicotine transiently enhances GABAergic synaptic transmission, which is followed by persistent depression, and shifts DA cell activity toward excitation (Mansvelder *et al.*, 2002).

Despite their high-abuse liability, the actions of cannabinoids on VTA DA cells have long been a matter of debate, and the mechanism of their actions is still poorly understood given that the detection of CB1 receptors in the VTA has long been unsupportive (Herkenham *et al.*, 1991). However, immunohistochemical studies have shown a co-localization of CB1 receptors with TH in the VTA (Marinelli and Mercuri, unpublished observations, 2004; Wenger *et al.*, 2003) and ultimately suggest a functional role for these receptors in the VTA (Marinelli and Mercuri, unpublished observations). In addition, cannabinoids have been shown to activate CB1 receptors in the VTA, inhibit presynaptic GABA release (Szabo *et al.*, 2000), and enhance presynaptic glutamate release in the posterior VTA (Melis, personal observations, 2004), thus providing an explanation for the excitation observed on VTA DA neurons *in vivo* (Cheer *et al.*, 2003; Diana *et al.*, 1998a; French, 1997; French *et al.*, 1997; Gessa *et al.*, 1998).

Regarding the actions produced by psychostimulants, much attention has been focused on the fact that their rewarding and reinforcing properties occur mainly through modulation of DA transmission through an interference with the DA transporter (Ritz *et al.*, 1987; Seiden *et al.*, 1993; Wise, 1996a,b). Amphetamine is preferentially a DA releaser, whereas cocaine is a blocker of DA transporter (Lacey *et al.*, 1990b; Mercuri *et al.*, 1992; Sonders *et al.*, 1997). Amphetamine blocks and reverses the DA transporter, which leads to increased extracellular DA and in turn activates D_2 receptors. Thus, depression of voltage-dependent Ca^{2+} currents downstream activation of D_2 receptors prevents the induction of long-term depression (LTD) at excitatory synapses on DA neurons

(Jones *et al.*, 2000). As a result, a transient blockade of LTD might provide a time window during which the LTP may be facilitated. Like amphetamine, cocaine-induced depression of dopaminergic neuronal activity depends on activation of D_2 receptor (Brodie and Dunwiddie, 1990; Lacey *et al.*, 1990b). However, cocaine has minimal actions on the firing rate of VTA DA cells at low concentrations (Brodie and Dunwiddie, 1990; Bunney *et al.*, 2000). On the contrary, neuronal adaptations resembling LTP take place in the VTA and result from a single *in vivo* exposure to cocaine (Ungless *et al.*, 2001). More specifically, cocaine exposure enhances excitatory, relative to inhibitory, inputs, thus resulting in potentiated excitatory synapses on DA neurons. Consequently, LTP cannot be induced at excitatory synapses as if synapses were already maximally potentiated (Ungless *et al.*, 2001).

D. Response to Chronic Drugs

Investigations examining the acute effects of drugs of abuse provide comprehension of their cellular sites of action but do not give relevant information about the neural changes related to the phenomenon of continuous drug exposure needed to provide realistic experimental models of drug addiction. The path to drug addiction begins with the act of taking drugs, which then becomes chronic, with relapses possible even after long periods of abstinence. Therefore, studying the effects of chronic exposure to drugs of abuse on the mesolimbic DA system is more relevant in the context of drug addiction than studying their acute effects.

Only few investigations have addressed the issue of the effects of chronic exposure to addictive drugs on VTA DA neurons (Brodie, 2002; Diana, 1996, 1998; Diana *et al.*, 1992a, 1993a; Rasmussen and Czachura, 1995; Wu and French, 2000), although several electrophysiological studies were carried out during withdrawal *in vivo* (Diana *et al.*, 1993b, 1995, 1998b, 1999; Lee *et al.*, 1999; Marinelli *et al.*, 2003; Rasmussen and Czachura, 1995; Shen and Chiodo, 1993) and *in vitro* (Bailey *et al.*, 1998, 2001; Bonci and Williams, 1996, 1997; Manzoni and Williams, 1999; Manzoni *et al.*, 1998).

In particular, although chronic morphine and cannabinoids do not alter the spontaneous activity of VTA DA cells (Diana *et al.*, 1995; Wu and French, 2000), a chronic nicotine regimen decreased it (Rasmussen and Czachura, 1995). As for chronic ethanol, conflicting results have been found *in vitro* and *in vivo* (Brodie, 2002; Diana *et al.*, 1992a). More specifically, the spontaneous activity of VTA DA neurons was found to be higher in ethanol-treated rats *in vivo* (Diana *et al.*, 1992a), while no differences were observed in mice *in vitro* (Brodie, 2002). In addition, VTA-DA neurons recorded from ethanol-treated mice showed greater responses to ethanol-induced effects but showed decreased responses to bath-applied GABA, suggesting that a sensitization might occur during chronic ethanol

treatment (Brodie, 2002). Consequently, VTA DA neurons appear to adapt to the presence of the addicting drugs without necessarily changing their own responsiveness to the drug itself. However, once the body has adjusted to the presence of a drug, clear symptoms of withdrawal may result when its use stops. Hence, VTA DA neurons undergo adaptive changes that might be unmasked during withdrawal from addicting drugs. The withdrawal syndrome occurs upon suspension of a chronic regimen, but not from a single exposure to drugs of abuse. This is a particularly relevant issue given that a great number of misinterpretations of the effects of a chronic regimen and/or withdrawal come from investigations carried out some time after acute or subchronic exposures.

E. Activity After Withdrawal from Chronic Administration

The withdrawal syndrome, by definition (DSM-IV, 2000), begins some time (typically within hours) after the drug administration ceases and unmasks a state of physical dependence. The presence of a somatic withdrawal syndrome is among the most concrete evidences of addiction in rodent studies (Deroche-Gamonet *et al.*, 2004; Diana, 1998b; Pulvirenti and Diana, 2001).The signs and symptoms of withdrawal have long been considered rebound effects to the drug that can be unmasked once the drug is withdrawn from the body (O'Brien, 2001). Thus, one might predict that the effects of acute exposure and withdrawal on VTA DA neuronal activity would be opposite. Indeed, the past decade has seen a growing body of evidence indicating that acute withdrawal from addictive drugs results in major changes in the physiology of VTA DA neurons.

Ethanol withdrawal decreases spontaneous activity of rat VTA DA neurons *in vivo* (Diana *et al.*, 1992b, 1993b) and mice *in vitro* (Bailey *et al.*, 1998) with no difference in the number of spontaneously active cells (Diana *et al.*, 1995b; Shen and Chiodo, 1993). This hypoactivity of DA cells correlates well with a reduction of extracellular DA levels in the NAcc (Diana *et al.*, 1993b; Fadda and Rossetti, 1998; Rossetti *et al.*, 1992a) and might represent the neural basis of the dysphoric state observed upon abrupt interruption of chronic ethanol. Interestingly, this hypodopaminergic state outlasts the physical signs of withdrawal (Bailey *et al.*, 2001; Diana *et al.*, 1996) and can be terminated by administration of ethanol itself (Diana *et al.*, 1993b, 1996), suggesting a role for VTA DA neurons in the long-lasting consequences of chronic ethanol ingestion (Pulvirenti and Diana, 2001). Similarly, morphine withdrawal causes a profound decline of firing rate and bursting activity of VTA DA cells (Diana *et al.*, 1995a), which persists long after the behavioral signs of withdrawal have ceased (Diana *et al.*, 1999). The adaptive changes occurring at the synaptic level and underlying the reduction in spontaneous activity of VTA DA cells *in vivo* have been intensively investigated

(Bonci and Williams, 1996, 1997; Manzoni and Williams, 1999). In fact, during acute withdrawal from prolonged morphine administration, an upregulation of the cAMP-dependent cascade produces a long-lasting increased probability of GABA release in the VTA (Bonci and Williams, 1996, 1997). Additionally, an increased sensitivity to presynaptic inhibition by both group 2 mGluRs and $GABA_B$ receptors results in a reduced release of glutamate (Manzoni and Williams, 1999). Thus, withdrawal from chronic morphine modifies both inhibitory and excitatory inputs to VTA DA cells, though in opposite ways. Interestingly, while VTA-DA neurons appear to be back to normal within 2 weeks after acute withdrawal, acute morphine administration produced greater responses in rats with a history of morphine dependence than in controls (Diana *et al.*, 1999). This latter finding suggests an increased sensitivity of VTA DA cells to morphine itself, which may be relevant to the phenomenon of drug craving and relapse (Diana *et al.*, 1999; Pulvirenti and Diana, 2001). These studies strengthen the notion that VTA DA neurons are involved in the mechanisms accounting for the subjective aversive components of withdrawal (dysphoria), rather than the somatic facets of it.

Cannabinoid withdrawal effects on VTA DA neuronal activity (Diana *et al.*, 1998b) are reminiscent of those reported for ethanol and morphine. More interestingly, a reduction in VTA DA neuronal function is also observed when somatic signs of withdrawal are not detectable (Diana *et al.*, 1998b). Furthermore, when a pharmacologically precipitated withdrawal is induced with the specific cannabinoid antagonist SR 141716A, the somatic signs of withdrawal accompany the dampened VTA DA neuronal activity (Diana *et al.*, 1998b).

Similarly, nicotine withdrawal produced a decline of firing rate of VTA DA neurons that rapidly (within 2 days) returned to control levels (Liu and Jin, 2004; Rasmussen and Czachura, 1995). Like the effects of withdrawal from other drugs of abuse (Diana *et al.*, 1993b, 1995, 1998b), the number of spontaneously active DA cells was not altered at any time after nicotine withdrawal. Thus, this study, together with other investigations (Bailey *et al.*, 1998, 2001; Diana *et al.*, 1992, 1993b, 1995a, 1998b), suggests that the hypodopaminergic state accompanying the acute phases of withdrawal is not mediated by depolarization inactivation of DA neurons but most likely reflects alterations of intrinsic properties and extrinsic afferent regulatory mechanisms (Bonci and Williams, 1996, 1997; Diana and Tepper, 2002; Manzoni and Williams, 1999; Pulvirenti and Diana, 2001) modified by a chronic drug regimen and disclosed by withdrawal.

Cocaine withdrawal effects on VTA DA neuronal activity seem to affect the burst firing pattern (Gao *et al.*, 1998). In fact, during the early withdrawal phases a reduced bursting activity of VTA DA cells was observed, which returned to normal within 7 days. In addition, alteration in sensitivity of D_2 receptors (autoreceptors) seems to play an important role in cocaine-induced modifications

of spontaneous activity of VTA DA neurons during the first week of withdrawal (Ackerman and White, 1992; Gao *et al.*, 1998; Lee *et al.*, 1999; Marinelli *et al.*, 2003). Consequently, short-term treatment with D_2 receptor agonists restores the hypodopaminergic neuronal function (Lee *et al.*, 1999; Marinelli *et al.*, 2003), thus representing a potential treatment for intermediate withdrawal phases. The spontaneous activity of VTA DA neurons does not seem to be altered during amphetamine withdrawal (Lee and Ellinwood, 1989), but repeated exposure to amphetamine produces long-lasting changes in the modulation of glutamatergic synaptic transmission by amphetamine in the NAcc (Li and Kauer, 2004).

III. Behavioral Animal Models

A. Self-Administration Studies

Over the past 40 years, experimental psychologists have been developing and refining behavioral models of addiction. Using inventive animal protocols, they have designed behavioral animal models both practical and highly reproducible. Although the human condition of the disease cannot always be reproduced in the finest detail, control over the experimental conditions such as species, environment, nutrition, drug dose, and pattern of administration can be monitored accurately (Bozarth, 1987).

Among behavioral animal models, self-administration has a prominent significance because it reflects an operant (active) behavior phenomenologically identical to the human condition. In the initial work, rats were used as experimental subjects for the intravenous injection of drugs (Weeks, 1962). Subsequently, the method has been refined and adapted to primates (Goldberg, 1973; Thompson and Schuster, 1964) and other mammals (Balster *et al.*, 1976; Bedford *et al.*, 1980; Criswell and Ridings, 1983; Griffiths *et al.*, 1975; Jones and Prada, 1973; Lukas *et al.*, 1982). Basically, the experimental animal presses a lever and receives a bolus of the drug. An intravenous catheter is connected to a pump, which delivers the intravenous fluid injections. The experimental preparation is, therefore, a chronically intravenous catheterized animal, which may be semi-restrained in a chair (e.g., primates) or allowed to freely move within the experimental chamber (e.g., rodents) during the self-administration session. A drug is considered to be self-administered when either the rate of drug responding is greater than the rate of response on a control lever (which results in saline injections), or when the response rate is greater in the subject whose response produces drug injections compared to its yoked control (Davis and Nichols, 1963; Pickens and Thompson, 1975). More specifically, the difference between the

response rate by the animal in the active cage and the animal in the yoked control cage provides an index of the reinforcing properties of the drug itself. Consequently, the drug serves as a reinforcer when a naive animal initiates self-administration of the drug with a rate of lever-pressing that exceeds the control lever or saline responding. The reinforcing properties of the drug can be evaluated by either turning off the injection pump or replacing the drug solution with saline (or vehicle) (Schuster and Thompson, 1969). The animal will then increase the response rate until will stop, eventually. This behavior is termed extinction (Pickens and Harris, 1968; Pickens and Thompson, 1968; Weeks, 1962; Winger and Woods, 1973).

Several schedules of reinforcement are available today (Ator and Griffiths, 2003; Johanson, 1978; Katz, 1989; Spealman and Goldberg, 1978; Young and Woods, 1981) and enable to specify the possibility that an animal is responding to obtain the drug. The most common schedule requires an animal to press the lever for a fixed number of times to obtain an injection (termed *fixed ratio* [FR] schedule). Thus, the self-administration rate within a session varies depending on the effects of the drug and/or the duration of the time out (Ator and Griffiths, 2003). An alternative schedule is the fixed interval (FI), when after a fixed period of time the first response (but not those preceding or following) produces the delivery of the drug. The *progressive ratio* (PR) schedule of reinforcement is the most used paradigm to assess the rank order of potency among different drugs of abuse (Ator and Griffiths, 2003; Gardner, 2000).

Under this schedule, the highest response requirement a drug will sustain represents the so-called "breaking point" (Richardson and Roberts, 1996; Stafford *et al.*, 1998), which can vary according to the determined progression within or across the sessions and typically represents the amount of "work" the subject is willing to perform to obtain a bolus of the drug. Thus, it is possible to build dose–effect curves for a certain drug by comparing the maximum breaking points obtained under the same experimental procedures. Another option to compare reinforcing properties of drugs of abuse is the *choice* procedure, where the response requirement to obtain one of two substances is significantly higher (Ator and Griffiths, 2003). Because of its route of administration, the intravenous self-administration model is almost instantaneous, although the delivery apparatus can have some disadvantages (e.g., viable long-term catheters and solubility of drugs). Though limited (e.g., aversive taste and fluid restriction), oral self-administration has been successfully established and proved useful in the study of ethanol intake (Evans and Levin, 2003; Meisch, 2001). In particular, to overcome the most limiting problem of this procedure (aversive taste of ethanol), the fluid has been sweetened to habituate the animal to the taste while it is exposed to increasing concentrations of ethanol (Meisch and Henningfield, 1977; Meisch and Lemaire, 1991; Turkkan *et al.*, 1989).

Drugs abused by humans have been demonstrated to be readily self-administered by laboratory animals. Indeed, animals will maintain self-administration for psychostimulants such as amphetamine, methamphetamine, cocaine, phencyclidine (Balster *et al.*, 1973, 1976; Bedford *et al.*, 1980; Carroll *et al.*, 1979; Goldberg, 1973; Lukas *et al.*, 1984; Pickens and Harris, 1968; Risner, 1982; Risner and Jones, 1975; Stretch and Gerber, 1970; Thompson and Pickens, 1970; Yokel and Pickens, 1973), opiates such as heroin, morphine, and congeners (Blakesley *et al.*, 1972; Harrigan and Downs, 1978; Lukas *et al.*, 1981), ethanol (Deneau *et al.*, 1969; Smith and Davis, 1974; Weeks, 1962), nicotine (Ator and Griffiths, 1983; Deneau and Inoki, 1967; Goldberg *et al.*, 1981; Lang *et al.*, 1977; Risner and Goldberg, 1983), and THC (Justinova *et al.*, 2003; Tanda *et al.*, 2000). This model allows researchers to extend the knowledge on the neurobiological mechanisms involved in such behaviors and to design and ultimately assess the potential therapeutic value of pharmacological agents. In fact, this behavioral animal model also allows examining the compulsive nature of both drug-seeking and drug-taking behaviors, which cannot be explained on the basis of the acute rewarding properties of the drugs (Bechara *et al.*, 1998; Hutcheson *et al.*, 2001). More specifically, accumulating evidence suggests that the withdrawal phase leads to an increased consummatory behavior of diverse drugs of abuse such as ethanol, opiates, cocaine, and nicotine (Grasing *et al.*, 2003; Hutcheson *et al.*, 2001; Khantzian, 1985; Koob, 1996; Mucha *et al.*, 1986; Valdez *et al.*, 2004; Weiss *et al.*, 1996, 2001). Consequently, the avoidance of the withdrawal syndrome represents a motivational state, in addition to the intrinsic rewarding properties of the drug, which ultimately increases the incentive value of the drug itself. As a result, the aversive signs of withdrawal produce a craving for the drug and increase the self-administration behavior to avert the abstinence phase. This can be explained in light of the compelling evidence (electrophysiological, biochemical, and behavioral) suggesting that neuroadaptive changes occurring within the mesolimbic DA pathway lead to a hypofunctioning DA system during withdrawal (see Chapters 2 and 4 for electrophysiological, biochemical, and anatomical evidence). Accordingly, the decreased mesolimbic dopaminergic transmission occurring during acute withdrawal from ethanol can be restored when the animals increase the self-administration rate (Weiss *et al.*, 1996). Therefore, the observations that ethanol-dependent rats will work more during the acute phase of withdrawal to obtain ethanol, whose consumption reverses the withdrawal-associated decreased DA levels (Weiss *et al.*, 1996), and DA neurons firing (Diana *et al.*, 1993) support the view that the dysphoric state accompanying abrupt interruption of ethanol (and more in general of drug abuse) is causally related to the hypodopaminergic state that outlasts somatic signs of withdrawal (Bailey *et al.*, 2001; Diana *et al.*, 1996) and can be terminated by administration of ethanol itself (Diana *et al.*, 1993b, 1996; Weiss *et al.*, 1996).

B. Intracranial Self-Stimulation

Another behavioral model used to investigate the rewarding/addicting properties of a drug in laboratory animals is the intracranial self-stimulation (ICSS) method (Kornetsky *et al.*, 1979). This procedure is based on the observation that rats will press a lever to pass a small current through electrodes located in various brain areas (Olds and Milner, 1954), including those that give course to the ascending DA-containing axons projecting to the forebrain. This method consists of placing a stimulating electrode in the medial forebrain bundle or other brain areas (such as lateral hypothalamus, VTA, prefrontal cortex, NAcc, etc.) and allowing animals to self-stimulate so neuronal reward circuits are activated. As a result, laboratory animals can directly activate (self-stimulate) those brain circuits that natural and conditioned reinforcers stimulate (Bozarth and Wise, 1981; Goeders and Smith, 1983; Hoebel *et al.*, 1983; Phillips and LePiane, 1980; Phillips *et al.*, 1981).

Once the lever-pressing behavior is established, the difference is made by changing the intensity and/or duration of the ICSS pulse in the presence of a drug. Thus, a relationship between the abuse liability of drugs such as morphine and cocaine and their ability to lower the threshold for ICSS in rat reward brain regions was first found by Kornetsky *et al.* (1979). Subsequently, all addictive drugs, when acutely administered, have been found to lower the threshold of stimulation required to maintain ICSS (Bespalov *et al.*, 1999; Gardner and Vorel, 1998; Gardner *et al.*, 1988; Hayes and Gardner, 2004; Herberg *et al.*, 1993; Williams *et al.*, 1991).

Drugs of abuse such as cocaine (Markou and Koob, 1991), amphetamine (Harrison *et al.*, 2001; Kokkinidis and Zacharko, 1980; Paterson *et al.*, 2000), ethanol (Schulteis *et al.*, 1995), morphine (Schulteis *et al.*, 1994), and nicotine (Epping-Jordan *et al.*, 1998; Harrison *et al.*, 2001) enhance the reinforcing impact of such electrical stimulation.

Conversely, acute withdrawal from diverse drugs of abuse precipitates a deficit in brain reward function, which can be indexed by elevated ICSS reward thresholds. These increases in brain stimulation threshold, to maintain ICSS, have been observed for the major drugs of abuse, such as opiates (Schaefer and Michael, 1986; Schulteis *et al.*, 1994), cocaine (Markou and Koob, 1992), amphetamine (Cryan *et al.*, 2003a; Lin *et al.*, 1999; Wise and Munn, 1995), ethanol (Schulteis *et al.*, 1995), and nicotine (Cryan *et al.*, 2003b; Epping-Jordan *et al.*, 1998; Kenny *et al.*, 2003). In particular, morphine-dependent rats needed an increased threshold current to restore ICSS during acute withdrawal (Schaefer and Michael, 1986). In addition, Kenny *et al.* (2003) provided further evidence for the role of the VTA in ICSS during acute nicotine withdrawal. Indeed, either activation or blockade of group II mGluRs within the VTA elevated or decreased,

respectively, ICSS thresholds in nicotine-dependent rats (Kenny *et al.*, 2003). Therefore, increased ICSS thresholds constitute a good behavioral animal model of the aversive motivational state associated with the negative reinforcement of drug withdrawal in dependent animals and add further evidence to the notion that VTA DA cells and their projections play a key role in perpetuating the addiction cycle (Diana, 1996, 1998; Shippenberg and Koob, 2002). Because accumulating evidence points to neuroadaptive changes induced by long-term abuse of addicting drugs in the mesolimbic regions, it is reasonable to consider that these alterations are involved in the aversive state emerging during withdrawal and motivate the continued use of the drug itself.

C. Place-Conditioning Studies

The place-conditioning paradigm is a pavlovian conditioning procedure in which the animal learns to prefer an environment that is paired with drug effects. This behavioral animal model mimics some aspect of the human condition of addiction. In fact, recovering addicts often return to drug intake when exposed to stimuli and/or environments associated with former use of the drug. Basically, animals are allowed to explore two distinct environments, which are usually different in color and/or pattern and are connected by an open door. The time spent in each compartment is recorded. Subsequently, the door connecting the two compartments is closed and one of the two compartments is paired every other day with a drug (which represents the unconditioned stimulus [UCS]) or vehicle exposure. This procedure is repeated for several days. On test day, the animal is not given any injection but has free access to both compartments, which are again connected by the open door. The time spent in the compartment associated with the drug is considered an index of the reinforcing value of the UCS. The difference between the time spent in the drug- versus vehicle-paired compartment is an indication of the rewarding effects of the drug. The opposite is true for an aversive USC. Thus, this behavioral model provides an animal model of the subjective effects of the drug. Importantly, the context serves as a signal that the drug produces changes at a cellular level. Drugs abused by humans are able to induce, when acutely administered, conditioned place preference in laboratory animals (Tzschentke, 1998) with the exception of THC. In fact, THC can induce either conditioned place preference or aversion depending on the dose and/or the timing of injections (Braida *et al.*, 2001; Lepore *et al.*, 1995; Sanudo-Pena *et al.*, 1997; Valjent and Maldonado, 2000), and opiate receptors (i.e., μ and κ) seem to play opposite roles (Ghozland *et al.*, 2002). Conditioned place aversion is more generally produced by aversive emotional states such as withdrawal from chronic treatment with drugs of abuse (Funada *et al.*, 1993;

Mucha and Herz, 1985). Particularly, this paradigm is one of the most sensitive indices of the motivational (affective) symptoms of drug withdrawal because it can be produced in dependent animals even though they do not show physical signs of withdrawal (Schulteis *et al.*, 1994). In fact, opiate-dependent animals given low doses of antagonist, which would produce motivational but not somatic signs of withdrawal, show aversion to the environment paired with the abstinence (Mucha, 1987; Schulteis *et al.*, 1994; Stinus *et al.*, 1990). Additionally, conditioned place aversion is shown by animals experiencing both ethanol withdrawal and hangover (Morse *et al.*, 2000), as well as cocaine and nicotine withdrawal (Ise *et al.*, 2000; Suzuki *et al.*, 1996). Consequently, drug withdrawal associated with diverse though convergent motivational aversive components plays an important role in maintaining addictive behavior. More specifically, this type of context- and experience-dependent plasticity seems to highlight the VTA as the key structure involved in drug addiction (Kim *et al.*, 2004). Because VTA DA neurons are uniformly inhibited by aversive stimuli (Ungless *et al.*, 2004), the neuroadaptive changes in mesolimbic DA transmission observed during and even long after acute withdrawal (see Chapters 2 and 4 for electrophysiological, biochemical and anatomical evidence) appear to be the most likely perturbations accounting for and contributing to addictive behavior.

IV. Biochemical Studies

A. Microdialysis

Microdialysis is the most widely used technique to monitor extracellular DA levels and is believed to reflect its synaptic concentrations (Imperato and Di Chiara, 1984; Zetterstrom *et al.*, 1983). This procedure allows the monitoring of DA levels in the extracellular space in living tissue and behaving animals (Westerink, 1995). Basically, a dialysis cannula is implanted in the brain region of interest and small molecules such as DA can freely exchange across the membrane of the probe down the concentration gradient. The dialysate is collected and analyzed. It is noteworthy that the exchange of molecules can take place in both directions, so it is possible not only to collect endogenous molecules but also to introduce exogenous compounds. This technique has high sensitivity and specificity for DA, although the probe size is large ($Ø > 200\ \mu m$) and the time resolution is low. In fact, because the samples can be collected only in a minute time scale (usually between 5 and 20 minutes), certain dynamics are undetected, thus resulting in a lack of temporal integration especially with electrophysiological methods. Thus, this technique cannot discriminate between

the spatiotemporal patterns of DA release that lead to diverse behavioral actions of DA and to activation of synaptic versus nonsynaptic DA receptors (Agnati *et al.*, 1995; Zoli and Agnati, 1996). Nonetheless, *in vivo* microdialysis studies have greatly helped the understanding of the neuropharmacological basis of normal and abnormal behavior (Hoebel *et al.*, 1992). In fact, by means of the microdialysis procedure, it has consistently been shown that drugs abused by humans, such as psychostimulants, nicotine, opiates, ethanol, and THC, acutely increase extracellular DA levels in the NAcc (Bradberry and Roth, 1989; Carboni *et al.*, 1989, 2001; Chiamulera *et al.*, 2001; Di Chiara and Imperato, 1985, 1988; Hernandez and Hoebel, 1988; Murphy *et al.*, 2001; Zocchi *et al.*, 1998), and preferentially in the shell subregion of this nucleus, as opposed to its core counterpart (Di Chiara, 2002; Hedou *et al.*, 1999a,b; Heidbreder and Feldon, 1998; Pontieri *et al.*, 1995; Tanda *et al.*, 1997; Zocchi *et al.*, 2003). In line with electrophysiological and behavioral studies, the aversive phase of acute withdrawal is accompanied by a reduction of extracellular DA levels in the NAcc as estimated by microdialysis (Rossetti *et al.*, 1992). Importantly, the changes in extracellular DA levels in this brain region have been observed during acute withdrawal from chronic ethanol (Diana *et al.*, 1993b; Rossetti *et al.*, 1992; Weiss *et al.*, 1996), morphine (Acquas *et al.*, 1991; Pothos *et al.*, 1991; Rossetti *et al.*, 1992), cocaine (Parsons *et al.*, 1991; Robertson *et al.*, 1991; Rossetti *et al.*, 1992; Weiss *et al.*, 1992), amphetamine (Rossetti *et al.*, 1992), nicotine (Hildebrand *et al.*, 1998; Rada *et al.*, 2001), and THC (Tanda *et al.*, 1999). In addition, these changes do not occur in other terminal regions such as the prefrontal cortex (Bassareo *et al.*, 1995; Hildebrand *et al.*, 1998) and do not seem to be correlated with somatic signs of abstinence. Notably, these decreased levels of extracellular DA in the NAcc, together with the dramatically reduced spontaneous activity of VTA DA cells during and after acute drug withdrawal (Bailey *et al.*, 1998; Diana *et al.*, 1993b, 1995, 1995a, 1998a; Gao *et al.*, 1998; Liu and Jin, 2004), strengthen the hypothesis that the hypofunction of mesolimbic DA neurotransmission plays a pivotal role in the aversive state of withdrawal and possibly contributes to renewed drug use.

B. Biomolecular Investigations

Biomolecular investigations have offered insightful data on the genetic basis of drug addiction and still open new avenues into perspectives of therapeutically useful drugs. The molecular studies of drug addiction have provided a better understanding of the mechanisms leading to long-term changes in the brain and ultimately behavior of drug addicts by using animal models of this disease (Nestler and Aghajanian, 1997).

One of the most widely used biomolecular techniques is the candidate gene method. This approach provides useful insights into the influence of specific genes in the regulation of behaviors such as drug addiction, because a candidate gene (or protein) is related to the human disease and therefore considered to be a human risk factor (Nestler, 2001a,b). To correlate a gene to a behavior, manipulation (disruption or enhancement) of a gene within an animal is required, as well as the use of transgenic, knockout and knockin mice. Lastly, by selectively breeding animals within a population that has either a very high or a very low level of a specific trait (e.g., ethanol consumption), it is possible to generate *selected lines*. This approach has been particularly useful in the field of alcoholism because lines with different types of sensitivity, preference, or aversion to ethanol have been generated (Phillips, 2002).

By means of these diverse biomolecular approaches, researches have focused their investigations on the changes occurring when DA is released by VTA DA cells in the terminal regions (e.g., limbic forebrain) in behavioral animal models of drug addiction. Because drugs of abuse alter the amount and time of DA released at the synapses, they might in turn affect the second-messenger cascades downstream activation of DA receptors, such as the cAMP pathway, and intracellular signaling proteins, as well as transcription factors (Bohn *et al.*, 2000; Carlezon *et al.*, 1998; Kelz *et al.*, 1999; Nestler, 2000, 2001a,b). In fact, compelling evidence suggests that upregulation and/or saturation of the cAMP–PKA dependent pathway (Bonci and Williams, 1996, 1997; Melis *et al.*, 2002; Self *et al.*, 1998; Terwilliger *et al.*, 1991) and activation of cAMP–response element-binding protein (CREB) take place in the mesolimbic system after exposure to diverse drugs of abuse such as ethanol, opiates, cocaine, and amphetamine (Asher *et al.*, 2002; Carlezon *et al.*, 1998; Lu *et al.*, 2003; McClung and Nestler, 2003; Shaw-Lutchman *et al.*, 2002, 2003).

Interestingly, some of these drugs (cocaine, amphetamine, and opiates) share the ability to alter the expression of immediate early genes, which, therefore, might represent one of the key elements in the molecular changes underlying drug addiction (Altman, 1996; Conneally and Sparkes, 1998; Hope *et al.*, 1992; Mackler and Eberwine, 1991). Additionally, a specific molecular change seems to exclusively occur after chronic exposure to diverse drugs of abuse (e.g., opiates, cocaine, and nicotine) and to persist long after drug intake ceases. This change involves induction of ΔFosB (a truncated form of the FosB gene) in the NAcc and the striatum, which appears as a hallmark of long-lasting adaptations associated with addiction (Chen *et al.*, 1995; Hope *et al.*, 1994; Kelz *et al.*, 1999; McClung and Nestler, 2003; Moratalla *et al.*, 1996; Nestler *et al.*, 2001a, b; Nye and Nestler, 1996; Pich *et al.*, 1997). By using another biomolecular technique (DNA microarray) (Geschwind, 2001), it has also been possible to establish a putative target for ΔFosB and implicate its signaling pathway in the long-term adaptive changes

of NAcc neurons to cocaine (Ang *et al.*, 2001; Nestler *et al.*, 2001). Additionally, induction of ΔFosB is particularly significant because it mediates stimulation of cyclin-dependent kinase-5 (Cdk5), which appears to be involved in the increased density of dendritic spines on NAcc neurons after chronic cocaine exposure (Bibb *et al.*, 2001; Norrholm *et al.*, 2003).

Taken together, these studies suggest that altered regulation of neuronal gene expression in the mesolimbic DA system, ultimately leading to morphological (see next paragraph) and functional (see previous paragraphs) changes of the system itself, might also explain the need for a continued drug intake in the development of addiction and in the maintenance of such behavior long after drug withdrawal.

C. Microanatomical Studies

Functional alterations accompanying persistent drug intake may represent the phenotype of structural changes occurring at the level of synaptic connections (such as abnormal density and morphology of dendritic spines, synapse loss, and ultimately abnormal synaptic plasticity) and other morphological changes of mesolimbic DA cells.

Dendritic spines "decorate" plasma membranes of several types of "spiny" units in the CNS and appear like knots (they can be thin, stubby, or mushroom shaped) lining on the dendritic shafts and represent independent brain units (Shepherd, 1996). They increase dendritic surface area and modify the electrical signals from synaptic inputs and appear as dynamic structures that can be formed, modified in shape, or eliminated depending on the synaptic activity (Shepherd, 1996). Indeed, a number of studies have suggested that stimulation-induced changes in dendritic spines are related to increased long-term synaptic changes and information processing (Comery *et al.*, 1996; Cox *et al.*, 2003; Daw *et al.*, 1993; Geinisman, 2000; Geinisman *et al.*, 1989; Hayashi *et al.*, 2004; Lynch *et al.*, 1988; Schiller *et al.*, 1998). Thus, size, shape, and number of dendritic spines proportionally affect synaptic plasticity; larger spines have larger heads and constricted necks and support stronger synaptic transmission (El-Husseini *et al.*, 2000; Murthy *et al.*, 2001; Shepherd, 1996).

Because VTA DA neurons (Fig. 1) synapse with dendritic spine necks of medium spiny neurons (Freund *et al.*, 1984) of the NAcc (Fig. 2), drug-induced changes in the structural and functional properties of neurons within the mesolimbic DA system might be relevant in the molecular and cellular basis of long-term behavioral changes observed during drug addiction (Nestler, 1996, 2001b, 2004). In particular, medium spiny neurons of the NAcc receive both excitatory and dopaminergic afferents on the heads and necks of the dendritic spines, respectively, whose integrated actions result in fine-tuning the spontaneous

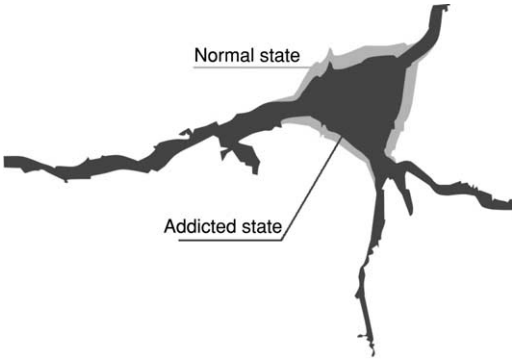

Fig. 1. Schematic drawing of ventral tegmental area (VTA) dopamine (DA) cell morphology during normal and addicted states. VTA DA neurons from a rat of the control group (normal state) are depicted in gray, whereas a VTA DA cell from a rat undergoing acute withdrawal from morphine (addicted state) is overlapping in black. Note that cell size (e.g., area, perimeter) is smaller during the addicted state. Drawing is proportional to actual measures observed experimentally.

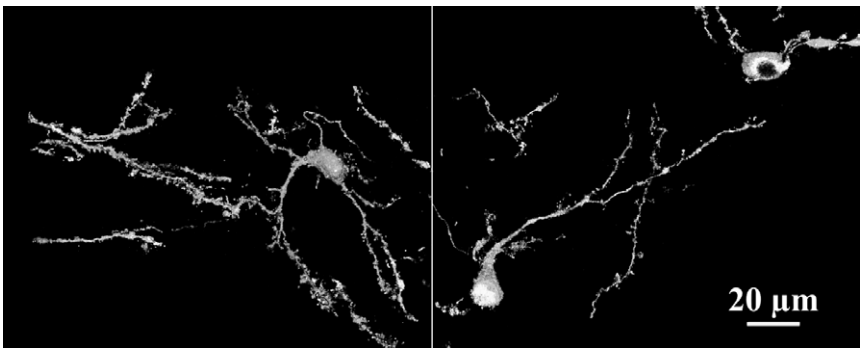

Fig. 2. Confocal images of the nucleus accumbens (NAcc) during normal and addicted states. Each is the projection of a three-dimensional reconstruction of medium spiny neurons within the shell of the NAcc. (Left panel) Medium spiny neuron from a rat of the control group (normal state). Note that dendrites are branched and enriched with spines. (Right panel) Medium spiny neuron from a rat undergoing acute withdrawal from morphine (addicted state). Note that dendrites are less branched and possess fewer spines.

neuronal activity (Pickel and Sesack, 1997). For instance, by using conventional fluorescent microscopy, it has been shown that morphine-dependent animals undergoing acute withdrawal have a dramatic reduction (about 25%) in the size of VTA DA cells (Sklair-Tavron *et al.*, 1996), although changes were ascribed to

chronic morphine (Spiga *et al.*, 2003a) as well as in the density of dendritic spines selectively in secondary dendrites (Diana *et al.*, 2003b) of medium spiny neurons of the NAcc (Robinson and Kolb, 1999a). Similarly, by using confocal laser scanning microscopy, it has been demonstrated that during acute withdrawal from chronic morphine the morphological features (e.g., circularity, area, and perimeter) of VTA DA neurons are profoundly reduced (Spiga *et al.*, 2003). These changes might reflect intracellular alterations occurring in these neurons, such as decreased neurofilament proteins (Beitner-Johnson *et al.*, 1992) and impaired functions such as reduced axonal transport from the VTA to the NAcc (Beitner-Johnson and Nestler, 1993). This reduction in area, perimeter, and circularity, in line with the "size principle" (Henneman *et al.*, 1965a,b; Shepherd, 1994; Somjen *et al.*, 1965) might ultimately represent an additional plastic change that renders the neurons more excitable, to overcome the hypodopaminergic state observed electrophysiologically and biochemically (see Chapters 2 and 4 for electrophysiological and biochemical evidence). Likewise, VTA DA cells are reduced in their size during acute withdrawal from chronic ethanol (Diana *et al.*, 2003a). Additionally, the observation that VTA DA cells have reduced size during acute withdrawal from THC (Spiga *et al.*, 2003b) further suggests that irrespective of the abused substance, VTA DA neurons are reduced in size upon withdrawal. On the other hand, repeated treatment with psychostimulants, such as cocaine and amphetamine, changes the morphology of medium spiny neurons in the NAcc and increases the density of dendritic spines and the number of branched spines on these neurons, thus resulting in augmented arborization that persists long after the last drug exposure (Robinson and Kolb, 1997, 1999b). Similarly, rats self-administering cocaine show increased density and arborization of dendritic spines on the medium spiny neurons of the shell of the NAcc and the pyramidal neurons of the neocortex (Robinson *et al.*, 2001). In a similar fashion, repeated nicotine administration dramatically enhances dendritic length and spine density of NAcc medium spiny neurons (Brown and Kolb, 2001).

The changes in spine density on medium spiny neurons of the NAcc and VTA DA cells' morphology are of particular interest because of their functional role in synaptic transmission and plasticity (Blanpied and Ehlers, 2004; El-Husseini *et al.*, 2000; Murthy *et al.*, 2001; Shepherd, 1996). In particular, augmented density of dendritic spines seems to be a consequence of a change in the number of synaptic inputs onto dendrites (Peters and Feldman, 1976; Wilson *et al.*, 1983) and results in increased synaptic efficacy (Luscher *et al.*, 2000; Malinow *et al.*, 2000; Scannevin and Huganir, 2000). These morphological abnormalities strengthen the view that an abnormal (hypofunctioning) mesolimbic dopaminergic system represents a key substrate involved in and contributing to drug addiction.

V. Primate Studies

A. Nonhuman Primates

Nonhuman primates share with humans 95% of their genetic makeup (Britten, 2002). Studies carried out in nonhuman primates are critical to the understanding of drugs' effects on the body and the brain, as well as the extent to which these changes can be reversed by pharmacological treatments. Electrophysiological recordings of midbrain DA neurons in nonhuman primates have shown them to be stimulated by novel unpredictable rewards and reward-associated stimuli (Schultz, 1998a,b). Interestingly, these neurons are capable of distinguishing between reward and nonreward objects (Romo and Schultz, 1990) and to respond to aversive stimuli with a decreased spontaneous activity (Schultz *et al.*, 1997). Thus, midbrain DA cells are activated when rewards occur without being predicted, are depressed when the predicted reward is omitted, and do not respond when the reward is delivered when predicted (Schultz, 1998a, 1999; Schultz *et al.*, 1997). Particularly, with repeated pairing (stimulus + reward = reward-predicting stimulus), the reward becomes predicted by the conditioned stimulus and the DA neurons cease to respond. However, if the reward fails to occur, DA cells respond with decreased activity at the time the reward is expected (Schultz *et al.*, 1997). Thus, DA neurons play a role in the behavioral adaptation to new situations and salient stimuli in the environment (Schultz, 1998b).

When drugs of abuse such as cocaine (Bradberry, 2000, 2002; Bradberry *et al.*, 2000) and ethanol (Bradberry, 2002) are acutely administered to nonhuman primates, increased extracellular DA levels are detected. This might explain the reinforcing properties of these drugs in nonhuman primates (Spealman *et al.*, 1989). Indeed, increased dopaminergic transmission might facilitate the consolidation of memory of drug experience and help elucidate why nonhuman primates will maintain self-administration for drugs of abuse such as ethanol (Meisch and Stewart, 1994), cocaine (Bradberry *et al.*, 2000), heroin (Mello *et al.*, 1995), and THC (Tanda *et al.*, 2000).

The mesolimbic DA system of nonhuman primates also undergoes major changes during chronic drug exposure, particularly after chronic psychostimulants. In fact, increased activity of TH (Vrana *et al.*, 1993), density of the DA transporter (Farfel *et al.*, 1992; Howell and Wilcox, 2001; Letchworth *et al.*, 2001), and downregulation of D_1 (Moore *et al.*, 1998a) and D_2 receptors have been observed (Farfel *et al.*, 1992; Ginovart *et al.*, 1999; Moore *et al.*, 1998b).

Although the development of physical dependence on drugs of abuse such as opiates (Krystal and Redmond, 1983; Redmond and Huang, 1982) and ethanol (Pieper, 1975) has been explored in nonhuman primates, very little is

known about the neuronal adaptations affecting and occurring in the mesolimbic dopaminergic system following continuous drug intake and unmasked by drug withdrawal. A behavioral study examining withdrawal from cocaine self-administration in monkeys and reporting disruption of the schedule-controlled behavior (Woolverton and Kleven, 1988) suggested that this disruption might cause abrupt changes in DA transmission leading to reinstatement of drug seeking and self-administration. However, whether this disruption reflects a tonically reduced DA receptor stimulation or the absence of phasic DA release due to a decreased activity of DA cells remains to be established. Nonetheless, because DA neuronal responses to aversive stimuli are similar across species (Chiodo *et al.*, 1980; Guarraci and Kapp, 1999; Mirenowicz and Schultz, 1994; Romo and Schultz, 1987; Ungless *et al.*, 2004), we can assume that reduced phasic DA release associated with a dampened spontaneous activity of DA units might play a major role during acute withdrawal. Additionally, indirect evidence supports the idea of a dysfunctional mesolimbic DA system in monkeys following chronic drug intake; indeed, Jentsch *et al.* (2000) showed that repeated exposure to psychostimulants such as phencyclidine induced increased impulsive behavior, possibly resulting from dysfunction of corticolimbic circuits associated with reward and behavioral inhibition (Jentsch *et al.*, 1999). Accordingly, the "impulsivity" shown by monkeys in response to reward-related stimuli (Jentsch *et al.*, 1999) is supported by evidence of deficits in frontal-cortical cognitive function, such as the loss of inhibitory control, resembling those observed in drug abusers (McKetin and Mattick, 1998; Rogers *et al.*, 1999).

B. Humans

Brain imaging techniques have been applied to the study of neurobiological mechanisms of addiction with results unobtainable from virtually any other method. These studies provide insights of great scientific value in understanding the pathophysiology of addiction (Daglish and Nutt, 2003). These relatively new methods enable experimenters to evaluate neural activity or activation via blood flow with positron emission tomography (PET) using radiolabeled water or via glucose metabolism. With single-photon emission computed tomography (SPECT), blood flow can be estimated using technetium-99, and lastly, functional magnetic resonance imaging (fMRI) methods produce images of the blood oxygen level–dependent (BOLD) response, which, in theory, reflects neuronal activation or inhibition. In addition, by administering a ligand, one can monitor receptor occupancy, thereby obtaining additional information on receptor function and ultimately neurotransmission. In the case of dopaminergic systems, the most frequently employed radioligand is ^{11}C-raclopride, which monitors D_2 receptors and other tools such as [^{11}C] D-threomethylphenidate

are frequently used and believed to reflect dynamics of the DA transporter (Volkow *et al.*, 1996). By using these methodologies, Boileau *et al.* (2003) has shown that ethanol administration in healthy volunteers results in a decreased ^{11}C-raclopride in the ventral striatum leading to increased dopaminergic transmission. Similarly, cocaine and methylphenidate produce significant reductions in D_2 receptors, which are associated with decreased metabolism in the cingulate gyrus and orbitofrontal cortex (Volkow *et al.*, 1999a,b), and these changes correlate well with subjective feelings of "high" after the psychostimulants (Volkow *et al.*, 1997). In addition, the level of receptor occupancy appears to predict the degree of drug-induced pleasantness (Fowler *et al.*, 1999; Volkow *et al.*, 1999c). All these studies demonstrate that the potentiation of the dopaminergic response after various addicting compounds observed in laboratory animals can also be detected in humans.

However, as previously stated, the altered brain physiology of the addicted brain limits the significance of these informative experiments in the context of drug addiction. Evaluations after chronic drug intake, and in some cases withdrawal, are nonetheless available and are discussed below.

D_2 receptors have been measured in opiate-dependent subjects and after naloxone-precipitated withdrawal (Wang *et al.*, 1997). Although a reduction of D_2 binding was documented as compared with controls, naloxone did not induce any appreciable change in the striatum of opiate-addicted humans. The reduced D_2 binding could be seen in contrast to the hypodopaminergic state theorized here, because the classic denervation theory would predict the opposite. However, it should be recalled that D_2 binding simply indicates number of receptors, and in animal models, a reduction of dendritic spines (preferential location of DA receptors) has been observed in medium spiny neurons of the NAcc (Diana *et al.*, 2003b; Robinson and Kolb, 1999a), which represent the main dopaminoceptice cell type in the forebrain. Thus, whereas the D_2 binding may, in some cases, reflect dopaminergic transmission, other structural changes produced by chronic morphine and withdrawal should be taken into account and in any event support the hypodopaminergic state outlined in this chapter. On the other hand, the lack of detection of naloxone-induced changes could simply be due to the dorsal-striatal level of investigation, instead of the ventral part.

D_2 receptor availability is also known to be lower in abstinent alcohol-dependent patients (Hietala *et al.*, 1994), whereas the DA transporter seems to be unaffected (Volkow *et al.*, 1996). Similarly, cocaine abusers show reduced ^{11}C-raclopride binding at rest (as compared to healthy controls) and an increased response to cocaine challenge (Schlaepfer *et al.*, 1997; Volkow *et al.*, 1999a).

Though limited in number, human studies in addicted populations, using various drugs, support the concept that dopaminergic transmission is reduced in the brain of dependent subjects, and the response to drug challenge is higher than controls (Lingford-Hughes and Nutt, 2003; Lingford-Hughes *et al.*, 2003; Volkow

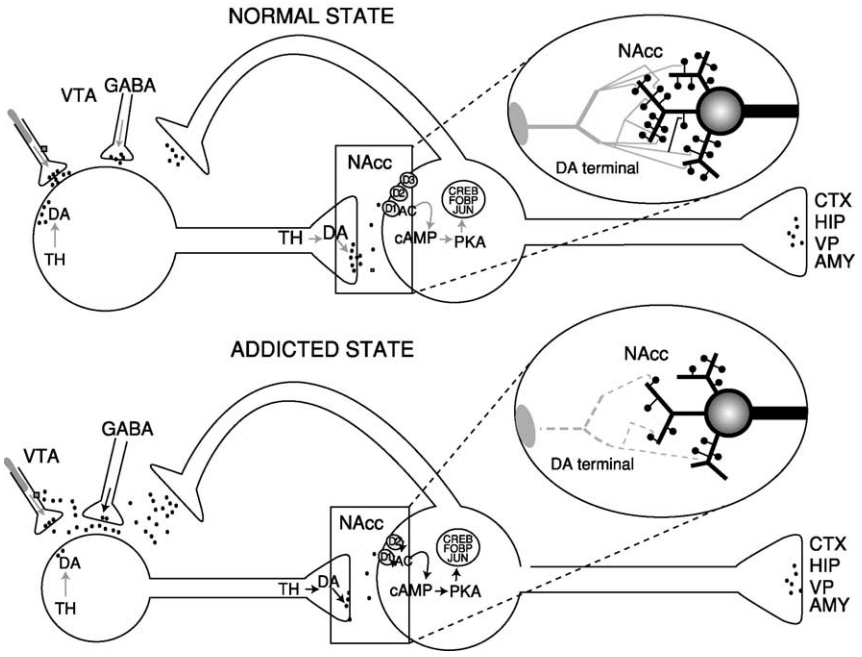

FIG. 3. Schematic summary of changes occurring in the mesolimbic dopaminergic system during the addicted state. (Top) Normal state depicts a control ventral tegmental area (VTA) dopamine (DA) neuron projecting to the nucleus accumbens (NAcc). Shown in the VTA are VTA DA cell and presynaptic glutamatergic ($GABA_B$ receptor is represented as a orange square) and GABAergic terminals. Shown in the VTA DA cell are tyrosine hydroxylase (TH) and DA vesicles. Shown in the NAcc are, in addition to TH and DA, DA receptors (D_1DA and D_2DA), components of the intracellular cyclic adenosine monophosphate (cAMP) system (AC, adenylate cyclase; cAMP; protein kinase A [PKA], cAMP-dependent protein kinase) and possible substrates such as CREB (cAMP-response element-binding protein) ΔfosB (a truncated form of the FosB gene) and jun (*jun* gene), as well as major outputs of this region (CTX, prefrontal cortex; HIP, hippocampus; VP, ventral pallidum; AMY, amygdala). Note the axodendritic synapse between the dopaminergic terminal and the spine necks of NAcc medium spiny neuron in the inset. Also, note that gray lines indicate a normal condition, whereas thick black lines increased activity, and dashed gray lines decreased activity. (Bottom) Addicted state depicts a smaller VTA DA neuron projecting to the NAcc after withdrawal from chronic drug exposure. TH levels are decreased (*dashed gray arrow*) in the VTA DA cells body and increased in the terminal region of the NAcc (*thick black arrow*). In the NAcc medium spiny neuron AC, cAMP, and PKA activities are increased; changes that could account for the D_1DA receptor supersensitivity (*thick black arrow*) and reduced number of D_2DA receptors. It should be noted that alterations in dopaminergic transmission influence spine density and number within the medium spiny neuron of the NAcc.

et al., 2004). These two facts are in line with the preclinical literature described in preceding chapters and support the idea of a hypodopaminergic state that characterizes the addicted brain.

VI. Conclusions

The last few decades have produced a wealth of experimental data on the neurobiological basis of drug addiction both in laboratory animals and in clinical settings with human subjects, drastically improving our knowledge of the disease. The present analysis of the literature suggests that the mesolimbic DA system (Fig. 3) displays a reduced spontaneous activity after chronic drug intake and withdrawal—crucial phases of addiction. In experimental animal models, when direct measures of DA neurons' functioning (such as electrophysiological models to monitor cells firing and pattern, and biochemical models such as microdialysis to monitor DA release) are employed, a reduction in spontaneous activity is reported irrespective of the addicting chemical. When methods such as place-conditioning studies, self-administration, and ICSS are applied, aversion, increased self-administration, and higher current thresholds (BP) respectively, are observed in similar conditions. Studies of human addicts coherently report a reduction of D_2 receptors and a reduction in DA release (Volkow *et al.*, 2004). Collectively, these data support the concept of a hypodopaminergic state at both presynaptic and postsynaptic level. The reduction in spine density observed in rats correlates well with the reduction in postsynaptic D_2 receptors in humans and would further impoverish an already defective DA transmission, thereby "weakening" the entire system. Targeting these abnormalities therapeutically will make it possible to develop pharmacological tools more efficacious than those available.

The strong consonance between experimental animal data and human findings renders drug addiction one of the human pathologies in which the term "evidence-based medicine" appears to be adequately applied.

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