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It's not “either/or”: activation and desensitization of nicotinic acetylcholine receptors both contribute to behaviors related to nicotine addiction and mood

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Abstract

Nicotine can both activate and desensitize/inactivate nicotinic acetylcholine receptors (nAChRs). An ongoing controversy in the field is to what extent the behavioral effects of nicotine result from activation of nAChRs, and to what extent receptor desensitization is involved in these behavioral processes. Recent electrophysiological studies have shown that both nAChR activation and desensitization contribute to the effects of nicotine in the brain, and these experiments have provided cellular mechanisms that could underlie the contribution of both of these processes to nicotine-mediated behaviors. For instance, desensitization of nAChRs may contribute to the salience of environmental cues associated with smoking behavior and activation and desensitization of nAChRs may contribute to both primary and conditioned drug reward. Similarly, studies of the antidepressant-like effects of nicotinic agents have revealed a balance between activation and desensitization of nAChRs. This review will examine the evidence for the contribution of these two very different consequences of nicotine administration to behaviors related to nicotine addiction, including processes related to drug reinforcement and affective modulation. We conclude that there are effects of nAChR activation and desensitization on drug reinforcement and affective behavior, and that both processes are important in the behavioral consequences of nicotine in tobacco smoking.

Keywords

Nicotinic acetylcholine receptors; desensitization; animal models; knockout mice; depression; mood

1. Activation and Desensitization of Nicotinic Acetylcholine Receptors

1.1 Introduction

Whereas it is intuitive that activation of nicotinic acetylcholine receptors (nAChRs) by nicotine can result in behavioral consequences, it is also the case that desensitization of nAChRs, by interrupting the transmission through endogenous acetylcholine, can also alter neuronal function and result in behavioral changes. Emerging evidence for electrophysiological consequences of both nAChR activation and desensitization has provided mechanisms that are consistent with the contribution of both of these processes to nicotine-mediated behaviors. This review will examine the evidence for the contribution of these two very different consequences

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of nicotine administration to behaviors related to nicotine addiction, including processes related to drug reinforcement and affective modulation.

1.2 Functional properties of nAChRs

Nicotine that enters the central nervous system acts at nAChRs that are located throughout the brain on various neuronal populations and are found at the soma as well as on pre- and post-synaptic terminals (McGehee et al., 1995; Wonnacott, 1997). These receptors function as pentameric structures composed of either five alpha subunits (homomeric receptors) or a combination of alpha and beta subunits (heteromeric receptors) (Cooper et al., 1991; Sargent, 1993). The identified receptor subunits in the brain include $\alpha 2$ through $\alpha 7$ subunits as well as $\beta 2$, $\beta 3$ and $\beta 4$ subunits (Boulter et al., 1987; Couturier et al., 1990a; Couturier et al., 1990b; Duvoisin et al., 1989; Sargent, 1993; Wada et al., 1988). The two nAChR subtypes expressed most widely in the brain are the $\alpha 7^*$ nAChRs (where * denotes other nAChR subunits that may not have been identified) and the $\alpha 4/\beta 2^*$ nAChRs (Picciotto et al., 1998). The $\beta 2^*$ nAChRs are thought to have the highest affinity for nicotine and other agonists (Lippiello et al., 1987; McGehee and Role, 1995; Picciotto et al., 1995) while $\alpha 7^*$ nAChRs are thought to have a lower affinity for nicotine *in vitro* (Papke and Thinschmidt, 1998; Wonnacott, 1986).

Activation of nAChRs occurs when acetylcholine or nicotine binds at the interface between an alpha and a neighboring alpha or beta subunit, causing a conformational change and opening of the channel pore (Galzi and Changeux, 1995; Miyazawa et al., 2003) to allow influx of sodium and calcium (Léna and Changeux, 1997; Letz et al., 1997; Rogers et al., 1997). A fundamental property of nAChRs is their susceptibility to desensitization and inactivation following, and indeed in some cases independent of, channel opening (Giniatullin et al., 2005). The equilibrium between channel opening, desensitization and inactivation was originally described by Changeux and colleagues who proposed an allosteric model of nAChR states, and has been refined for different nAChR subtypes using experimental measurements of the rate constants for the transitions between these states (Changeux and Edelstein, 2005; Changeux et al., 1984; Quick and Lester, 2002).

1.3 Desensitization of nAChRs

Studies of nAChR subunits expressed in *Xenopus* oocytes, as well as native currents in brain slices, originally suggested that $\alpha 7^*$ nAChRs were particularly susceptible to inactivation, making $\alpha 7$ -dependent currents difficult to measure unless agonists were delivered very rapidly, whereas $\beta 2^*$ and $\beta 4^*$ nAChRs exhibited desensitization in response to nicotine exposure, but also recovered after several minutes of washout in these *in vitro* preparations (Couturier et al., 1990a; Giniatullin et al., 2005; Pidoplichko et al., 1997). More recent studies have shown that presynaptic effects of lower concentrations of nicotine that are blocked by nicotinic agents that are thought to be selective for $\alpha 7^*$ nAChRs persist in the continued presence of nicotine in rat (Mansvelder et al., 2002) and mouse (Wooltorton et al., 2003) brain slices, whereas presynaptic effects that are blocked with agents thought to be selective for $\beta 2^*$ nAChRs desensitize during continued nicotine application in the brain slice (Mansvelder et al., 2002; Mansvelder and McGehee, 2000). One question that has not yet been answered is whether the pre- and post-synaptic nAChR subtypes differ in their susceptibility to desensitization, and whether nAChRs in different neuronal populations are differentially sensitive to desensitization. One possibility is that accessory proteins strongly influence the likelihood that nAChRs will undergo desensitization. For example, the Lynx1 protein can accelerate the desensitization of various nAChR subtypes, whereas knockout of Lynx1 in mice results in increased nAChR activation that ultimately causes neuronal degeneration (Miwa et al., 2006; Miwa et al., 1999). Thus, it would be interesting to determine the relative susceptibility of $\alpha 7^*$ and $\beta 2^*$ nAChRs to desensitization in pre- and post-synaptic responses of nicotine in mice lacking Lynx1.

Despite the decreased response of AChRs to agonists in the desensitized state, desensitized nAChRs have a higher affinity for ACh and other ligands than those in the “activatable” state (Heidmann et al., 1983; Quick and Lester, 2002); thus equilibrium binding experiments, particularly those using concentrations of nicotine near the K_d for the desensitized state, are likely to measure nAChRs in the high affinity (desensitized) state predominantly. Increased nicotine binding in the brain after chronic nicotine exposure measured in living human subjects using very low concentrations of radiolabeled nicotinic compounds (Staley et al., 2006) may therefore reflect, in part, an increased pool of nAChRs in the desensitized state. Furthermore, as will be discussed throughout this review, the balance between nicotine-mediated activation and desensitization of specific subtypes of nAChRs can influence the functional and behavioral responses to nicotine exposure. The likelihood that chronic nicotine exposure results in both peaks of activation and long-term desensitization of nAChRs suggests that functional responses associated with chronic nicotine exposure due to smoking are due, at least in part, to nAChR desensitization. For instance, it has been suggested that maintained nAChR desensitization may be important for relieving nicotine withdrawal in human smokers (Brody et al., 2006) as is discussed below.

In human subjects, profound effects that are likely due to nAChR activation and desensitization have been observed after just one cigarette. Nicotine levels in the blood, which reach the brain within 8 to 10 seconds (Matta et al., 2006), typically peak shortly after cigarette smoking, ~ 50 ng/ml (300 nM) 5 minutes after 1 cigarette (Henningfield et al., 1993), and then show a steady decline in the subsequent 20 minutes after smoking (Benowitz et al., 1989). Nicotine levels in chronic smokers measured during the day, however, revealed blood nicotine concentrations ranging from 10 to 50 ng/ml (60 to 300 nM) (Benowitz et al., 1989); thus, the sustained elevated nicotine levels experienced by chronic smokers throughout the day could result in cycles of both nAChR activation and desensitization. The desensitization, in particular, may be related to receptor occupancy given the higher affinity of desensitized nAChRs. Furthermore, recent reports suggest that nAChR occupancy is nearly complete after 1 cigarette (Brody et al., 2006). In a recent positron emission tomography (PET) imaging study, $\beta 2^*$ occupancy was $\sim 88\%$ percent after just 1 cigarette, with corresponding plasma levels that have been shown to lead to 50% desensitization of $\alpha 4\beta 2$ nAChRs (Brody et al., 2006). Since venous plasma levels in chronic smokers after multiple cigarettes are higher than those obtained with a single cigarette, the authors further suggest that smoking in these individuals may lead to nearly complete high affinity nAChR occupancy and desensitization and that smoking maintains this desensitization (Brody et al., 2006). It should be noted, however, that this PET ligand may only reveal the highest affinity subclass of nAChRs, which are likely to be in the desensitized state (Changeux et al., 1984). Thus, nAChRs in the activatable state may not be measured using this technique.

1.4 Upregulation of nAChRs following chronic nicotine exposure

While chronic nicotine exposure leads to receptor desensitization, it also leads to compensatory changes such as up-regulation of nicotine binding sites. Chronic nicotine exposure has been shown to lead to robust nAChR up-regulation in both human smokers and in animal models. For instance, in post-mortem brains, increased high affinity nAChR binding has been observed in several regions including the cortex and hippocampus (Benwell et al., 1988; Perry et al., 1999). In addition to these post-mortem observations, up-regulation of $\beta 2^*$ nAChRs in the cortex and striatum have been observed using SPECT imaging of human smokers even after the 7 days of smoking abstinence required to clear nicotine from the brain (Staley et al., 2006). Thus, it appears that both chronic nicotine administration and early withdrawal are sufficient to drive nAChR up-regulation. This observation is supported by animal studies, which also show nAChR up-regulation after chronic nicotine administration. Both $\alpha 7^*$ and $\beta 2^*$ nAChR up-regulation have been observed in various brain regions after chronic nicotine

administration ranging from 8 to 21 days of exposure, although $\beta 2^*$ nAChRs appear to be more consistently upregulated across multiple studies (Marks et al., 1983; Schwartz and Kellar, 1985). Indeed, $\beta 2$ nAChR subunit knockout mice do not show up-regulation of nicotine binding sites (McCallum *et al.*, 2006a). While it is clear that $\alpha 4/\beta 2^*$ nAChRs are upregulated following chronic nicotine exposure, data on upregulation of $\alpha 6/\beta 2^*$ nAChRs has been more variable. Studies of $\alpha 6/\beta 2^*$ nAChRs after nicotine exposure have reported upregulation (Parker *et al.*, 2004), downregulation (Lai *et al.*, 2005) and no change (McCallum *et al.*, 2006b) in this nAChR subtype. Further studies will be required to clarify this issue, but it seems likely that $\alpha 4/\beta 2^*$ nAChRs are more reliably upregulated by nicotine exposure than $\alpha 6/\beta 2^*$ nAChRs.

A number of recent studies have examined the mechanisms underlying nAChR upregulation, and a number of different pathways have been implicated in this process. For example, nicotine can potentiate nAChR assembly in the endoplasmic reticulum followed by transport of the receptor to the cell surface (Corringer et al., 2006; Darsow et al., 2005; Sallette et al., 2005; Vallejo et al., 2005). One question that is still a point of controversy is whether the increase in nicotine binding sites following chronic nicotine exposure results in any increase in functional, activatable nAChRs, or whether increased binding simply reflects an increase in desensitized nAChRs. It appears that the increased surface expression of nAChRs following chronic nicotine can indeed result in increased activation of nAChRs (Vallejo et al., 2005; Wonnacott, 1990), although the discrepancy between studies suggests that there is a dynamic equilibrium between activation and desensitization of nAChRs following chronic nicotine exposure. For instance, nicotine-mediated neurotransmitter release can occur after acute nicotine exposure that activates nAChRs and after chronic nicotine exposure that desensitizes nAChRs (Benwell and Balfour, 1992; Iyaniwura et al., 2001). Furthermore, as will be described in the next section, nicotine exposure can lead to simultaneous activation and desensitization of different nAChR subtypes.

1.5 Continued activation as well as desensitization of nAChRs following chronic exposure to nicotine

Despite compelling evidence that chronic nicotine exposure, for example in human smokers, results in profound nAChR desensitization, examination of the different rates of activation and desensitization between $\alpha 7$ and $\beta 2^*$ nAChRs suggests that not all nAChRs are desensitized under these conditions. In the ventral tegmental area (VTA), $\beta 2^*$ nAChRs are found on the soma of DA neurons (Klink et al., 2001) as well as on GABAergic terminals (Mansvelder et al., 2002), whereas $\alpha 7^*$ nAChRs can be found at lower levels on DA neurons, but are also expressed on glutamatergic terminals (Mansvelder et al., 2002; Mansvelder and McGehee, 2000). Activation of $\beta 2^*$ nAChRs on the soma of DA neurons stimulates the firing of these neurons, as well as acute DA release (Picciotto et al., 1998). As mentioned earlier, at nicotine concentrations of 500 nM or higher, electrophysiological studies in combination with pharmacological agents to isolate individual nAChR-mediated currents have shown that $\alpha 7^*$ nAChR activation is characterized by fast currents that desensitize on a millisecond timescale while $\beta 2^*$ nAChRs desensitize more slowly on a second timescale (Couturier et al., 1990a; Giniatullin et al., 2005; Pidoplichko et al., 1997). However, at lower nicotine concentrations, which may be more similar to those observed at steady state in a smoker's brain (250 nM), $\beta 2^*$ nAChRs also desensitize rapidly (Mansvelder et al., 2002; Mansvelder and McGehee, 2000). Furthermore, when even lower nicotine concentrations (20-80 nM) were examined in an attempt to model the low levels experienced by chronic smokers over long time periods, robust $\beta 2^*$ desensitization was observed without any $\alpha 7$ desensitization (Wooltorton et al., 2003). This $\beta 2^*$ desensitization may be due to a process known as high-affinity desensitization that is observed with low concentration of the agonist, whereby desensitization can occur without activation of the receptor (Giniatullin et al., 2005). This type of desensitization without movement through the open state is predicted by the allosteric model of transitions between

closed, activated and desensitized states, and is based on stabilization of the highest affinity, desensitized state of the receptors by continued presence of low levels of the ligand (Changeux et al., 1984). Thus, these subtype specific effects illustrate how nAChR activation and desensitization can occur simultaneously in response to nicotine administration.

Simultaneous activation and desensitization of specific nAChR subtypes can influence functional responses to nicotine in brain slices. In the VTA, for instance, $\beta 2^*$ nAChRs are located in part on GABAergic terminals (Mansvelder et al., 2002) and cell bodies (Klink et al., 2001) while $\alpha 7^*$ nAChRs are located on glutamatergic terminals (Mansvelder et al., 2002; Mansvelder and McGehee, 2000). The low level nicotine concentrations experienced by smokers over long periods are suggested to preferentially desensitize $\alpha 4\beta 2$ nAChRs on GABAergic terminals and not $\alpha 7$ nAChRs on glutamatergic terminals in the VTA, thus shifting the presynaptic effects of nicotine from a mixed inhibition and excitation of DA neurons, toward the glutamatergic response to nicotine (Mansvelder et al., 2002; Mansvelder and McGehee, 2000). This shift in the functional output in the VTA also has implications for nicotine-mediated behaviors, as will be further discussed throughout this review.

Another functional output that is influenced by rates of nAChR activation and desensitization is nicotine-mediated neurotransmitter release. As mentioned earlier, repeated nicotine administration leads to increased release of dopamine that persists, and indeed shows a sensitized response (increased dopamine (DA) release in response to challenge with the same nicotine dose), despite any nAChR desensitization that is likely to have occurred (Benwell and Balfour, 1992; Iyaniwura et al., 2001). This appears to be due in part to adaptations in the glutamatergic system downstream of nAChRs (Ferrari et al., 2002). Thus, even when nicotine results in desensitization of nAChRs, increased downstream responses may result in increased behavioral responses to nicotine challenge (Fig 1). Recent reports have further demonstrated that sustained nicotine-mediated dopamine responses, particularly after nAChR desensitization, are more likely to occur if the dopaminergic neurons projecting from the VTA to the nucleus accumbens (NAc) are stimulated and firing in burst mode (Rice and Cragg, 2004; Zhang and Sulzer, 2004). Cyclic voltammetry studies have shown that application of both nicotine and an antagonist of $\beta 2^*$ nAChRs to dopaminergic terminals decreases DA release when the DA neurons are firing tonically, but permits ongoing DA release when DA neurons are in a phasic state (Rice and Cragg, 2004; Zhang and Sulzer, 2004). These data suggest that desensitization of nAChRs on DA terminals increases the response to phasic firing of DA neurons. Since sustained nicotine exposure shifts the balance toward glutamate-mediated excitation of DA neurons (Mansvelder et al., 2002; Wooltorton et al., 2003) the combination of increased drive on the cell bodies in the VTA and increased filtering in the striatum/nucleus accumbens suggests that chronic nicotine exposure would greatly increase response to environmental stimuli that increase DA neuron burst firing, and would greatly decrease DA responses under conditions when these neurons were firing tonically (Rice and Cragg, 2004; Zhang and Sulzer, 2004).

Studies in systems involving neurotransmitters other than DA have also demonstrated instances where nicotinic agonists and antagonists have resulted in the same neurochemical effect. For instance, while nicotine increases serotonin (5-HT) release in hippocampal slices, the nAChR antagonist mecamylamine also increases 5-HT levels similar to nicotine (Kenny et al., 2000). This could result from similar physiological mechanisms to those described above that have been studied in the VTA. A number of studies in knockout (KO) mice lacking the $\beta 2$ subunit of the nAChR have shown that both functional deletion in KO mice and nicotine administration in wild-type mice can lead to the same cellular or behavioral effects, suggesting that nicotine administration may normally desensitize $\beta 2^*$ nAChRs in these paradigms and that desensitization then contributes to these cellular or behavioral responses (Brunzell et al., 2006; Cohen et al., 2005; Mechawar et al., 2004; Picciotto et al., 1995). As will be discussed

in the next section, the behavioral consequences of nicotine exposure related to nicotine reinforcement may thus result from a combination of nAChR activation and desensitization.

Finally, while there is much less data available, it is important to note that a number of nAChR subtypes are expressed on non-neuronal cell types in brain (Gahring *et al.*, 2004a; Gahring *et al.*, 2004b) that could potentially modulate the response to nicotine. For instance, nicotine can increase intracellular free calcium and promote calcium waves in astrocytes through activation of nAChRs (Oikawa *et al.*, 2005; Sharma and Vijayaraghavan, 2001). More data are required to determine the effects that these nAChRs could have on nicotine-induced behavioral and neuronal modulation related to reward and mood and to determine whether there is equivalent desensitization of nAChRs in non-neuronal cells following nicotine exposure to what has been seen in neurons.

2. Nicotine Reinforcement

2.1 Introduction

Tobacco dependence is a complex behavioral phenomenon. Although initiation of tobacco use is likely to involve the primary reinforcing effects of nicotine, those initiating smoking, and particularly habitual smokers, also derive pleasure from the sensory cues associated with smoking (Perkins *et al.*, 2001; Rose, 2006; Rose *et al.*, 1985). External cues greatly enhance nicotine self-administration in rodents (Caggiula *et al.*, 2002) and, like a rewarding dose of *i.v.* nicotine (Stein *et al.*, 1998), smoking-associated cues activate the VTA and DA projection areas including the nucleus accumbens, cingulate cortex and amygdala (Due *et al.*, 2002). As noted in Section 1, it is clear that acute activation of nAChRs by nicotine can stimulate the firing of DA neurons (Grenhoff *et al.*, 1986; Klink *et al.*, 2001; Picciotto *et al.*, 1998; Svensson *et al.*, 1990). Studies in striatal synaptosomes, however, show that lower doses of nicotine, consistent with those in the blood of human smokers, result in desensitization rather than activation of nAChRs as measured by nicotine-dependent dopamine release (Grady *et al.*, 1992; Grady *et al.*, 1994; Rowell and Duggan, 1998; Rowell and Hillebrand, 1994). In addition, electrophysiological studies show that steady states of nicotine that would be achieved in the bloodstream of a regular smoker can desensitize midbrain nAChRs with a time course of recovery that requires several minutes (Pidoplichko *et al.*, 1997; Woollorton *et al.*, 2003). This may explain why the first cigarette of the day is often reported as the most pleasurable (Russell, 1989), but it does not explain why people continue to smoke throughout the day. This section will review the evidence suggesting that nAChRs in both the activatable and the desensitized state might drive both primary and conditioned reward associated with tobacco smoking.

2.2 nAChR subtypes that contribute to nicotine reward

The primary rewarding effects of nicotine are likely regulated by a combination of events that include the activation and desensitization of various nAChR subtypes. An accumulation of data suggests that both the $\beta 2^*$ and $\alpha 7$ nAChR subtypes contribute to nicotine-associated increases in DA release and associated nicotine-dependent behaviors (Corrigall *et al.*, 1994; Epping-Jordan *et al.*, 1999; Laviolette and van der Kooy, 2003; Mansvelder *et al.*, 2002; Picciotto *et al.*, 1998; Pidoplichko *et al.*, 2004; Shoaib and Stolerman, 1994). Pretreatment with methyllycaconitine (MLA), an $\alpha 7$ nAChR antagonist, can attenuate nicotine self-administration suggesting a potential role for VTA $\alpha 7^*$ receptors in nicotine reward (Markou and Paterson, 2001); but at doses necessary to penetrate the blood brain barrier, MLA has significant affinity for $\alpha 6/\beta 2^*$ nAChRs (Mogg *et al.*, 2002) suggesting a potential role of these receptor subtypes in nicotine self-administration. At the DA terminals, however, $\beta 2^*$ nAChRs ($\alpha 4/\beta 2$, $\alpha 6/\beta 3/\beta 2$, $\alpha 4/\alpha 6/\beta 3/\beta 2$, $\alpha 4/\alpha 5/\beta 2$) and not $\alpha 7^*$ nAChRs, support nicotine-stimulated DA release (Salminen *et al.*, 2004; Champiaux, 2004 #144).

Activation of $\beta 2^*$ nAChRs in the VTA appears to be critical for the primary reinforcing effects of nicotine. Local and systemic administration of a selective $\beta 2^*$ nAChR antagonist, dihydro-beta-erythroidine (DH β E), blocks nicotine self-administration in rodents (Corrigall et al., 1992; Grottick et al., 2000). Studies in $\beta 2^*$ nAChR null mutant mice further show that $\beta 2^*$ nAChRs are necessary for nicotine self-administration, DA-dependent locomotor activation, nicotine-associated enhancement of NAc DA release, and nicotine-associated enhancement of conditioned reinforcement (Brunzell et al., 2006; King et al., 2004; Marubio et al., 2003; Picciotto et al., 1998). While several subtypes of $\beta 2^*$ nAChRs are found in the VTA, a number of pieces of evidence suggest that $\alpha 4/\beta 2^*$ nAChRs are critical mediators of nicotine reward. Mice with a genetic knockout of the $\alpha 4$ nAChR subunit fail to show nicotine-dependent enhancements of DA release (Marubio et al., 2003), and a single nucleotide mutation that renders the $\alpha 4^*$ nAChRs hypersensitive to nicotine stimulation promotes conditioned place preference at otherwise sub-optimal doses of nicotine (Tapper et al., 2004). Interestingly, $\alpha 4^*$ nAChR knockout mice show an increase in basal DA release in the NAc (Marubio et al., 2003), suggesting that inactivation of $\alpha 4\beta 2^*$ nAChRs may enhance baseline dopaminergic tone. In addition to $\alpha 4/\beta 2^*$ nAChRs, $\alpha 6/\beta 2^*$ nAChRs are highly expressed in DA neurons. Unfortunately, studies using knockout mice have not yet elucidated the role of these nAChRs in nicotine-related phenotypes, likely because the $\alpha 4$ subunit can substitute for the $\alpha 6$ subunit in animals lacking the subunit throughout development (Champtiaux *et al.*, 2002). Thus, pharmacological or conditional knockdown approaches will be necessary to identify the distinct roles of $\alpha 6\beta 2^*$ nAChRs in nicotine reinforcement.

2.3 Desensitization of nAChRs may contribute to salience of environmental cues

Electrochemical cyclic voltammetry studies suggest that nAChRs integrate the activity state of DA neurons to adjust dopaminergic tone (Rice and Cragg, 2004; Zhang and Sulzer, 2004). As noted in Section 1, nicotine and nicotinic antagonists result in similar effects on this tuning process, suggesting that desensitization of nAChRs regulate this integrative process (Rice and Cragg, 2004; Zhang and Sulzer, 2004) (Fig. 1). Both nicotine and DH β E decrease DA release when the DA neurons are firing tonically, but enhance DA release when DA neurons are in a phasic state (Rice and Cragg, 2004), as one would expect during the presentation of a reward (Schultz, 2002). As environmental cues gain more control over behavior following repeated presentation of cues with a primary reinforcer, there is a transition from phasic activity of DA neurons in response to the primary reinforcer, to phasic activity in response to the conditioned stimulus (cue) (Schultz, 2002). Electrochemical cyclic voltammetry studies show that DA release also shifts contingency from the primary reinforcer to the cue after Pavlovian pairing of primary and conditioned stimuli (Day et al., 2007).

Paradoxically, although nicotine administration enhances conditioned reinforcement in wild type mice, knockout of the $\beta 2$ subunit also elevates conditioned reinforcement at baseline (Brunzell et al., 2006). DA release in the NAc shell is necessary for conditioned reward (in (Robbins and Everitt, 2002)). Thus, one potential mechanism for the elevated conditioned reinforcement at baseline in $\beta 2^*$ nAChR knockout mice could be elevated basal DA levels, as has been seen in $\alpha 4$ KO mice (Marubio et al., 2003). Desensitization of $\beta 2^*$ nAChRs on GABA terminals in the VTA following nicotine exposure results in increased activity of DA neurons (Mansvelder et al., 2002; Wooltorton et al., 2003), and may therefore regulate conditioned reinforcement by decreasing an inhibitory effect of the endogenous neurotransmitter acetylcholine on dopaminergic tone. Together, these data suggest that desensitization of high affinity $\beta 2^*$ nAChRs could potentially enhance the response to environmental cues paired with smoking and make them more salient.

2.4 nAChR activation and desensitization contribute to primary and conditioned reward

As has been suggested for other drugs of abuse, sensitization of the DA system might regulate nicotine-associated conditioned reward (Robbins and Everitt, 2002; Robinson and Berridge, 1993; Taylor and Robbins, 1984). Acute, chronic, and prior chronic nicotine exposure all enhance conditioned reinforcement (Brunzell et al., 2006; Olausson et al., 2003; 2004a; b). Unlike conditioned reinforcement at baseline, which seems to be enhanced by nAChR inactivation, nicotine-associated enhancement of conditioned reward appears to require activation of nAChRs. The non-selective nAChR antagonist mecamylamine blocks the ability of nicotine to enhance conditioned reinforcement (Olausson et al., 2004a), and $\beta 2^*$ nAChR null mutant mice fail to show nicotine-associated enhancement of conditioned reinforcement (Brunzell et al., 2006). These data suggest that activation of $\beta 2^*$ nAChRs on DA soma, and potentially activation of $\alpha 7^*$ nAChRs, may contribute to nicotine-associated elevations in cue responsivity.

As noted in Section 1, in human smokers, studies using a PET ligand recognizing $\beta 2^*$ nAChRs, have shown that very low levels of nicotine are sufficient to displace the majority of nAChR binding in human brain (Brody et al., 2006) and one smoking episode is sufficient to occupy most of the brain's high affinity $\beta 2^*$ nAChRs for up to 5 hours after a period of abstinence (Staley et al., 2006). The increased availability of nAChRs following overnight abstinence ought to activate a surplus of high affinity nAChRs, making the first cigarette highly reinforcing to smokers. In support of this theory, smokers show elevated nicotine-associated DA release compared to non-smokers (Brody et al., 2004). The subsequent desensitization of the high affinity $\beta 2^*$ nAChRs that occurs prior to finishing the first cigarette could also enhance the conditioned reinforcing properties of cues associated with smoking as discussed earlier in this section, by increasing DA release from neurons firing phasically. Throughout the remainder of the day, cigarette-associated cues and pleasurable events such as eating would be expected to shift dopamine neurons into a phasic state, during which time smoking a cigarette might enhance DA release via desensitization of $\beta 2^*$ nAChRs. Hence, it is possible that smokers ingest nicotine to both activate and desensitize nAChRs in the brain.

3. Antidepressant Action of Nicotinic Agents

3.1 Introduction

A number of studies have suggested that at least a subset of smokers continue smoking to manage mood symptoms. Thus, in addition to the primary and secondary reinforcing effects of nicotine, the ability of nicotine to alter affective states is also likely to be important for ongoing symptoms. Interestingly, antidepressants can also aid in smoking cessation. For instance, high doses of fluoxetine or other classes of antidepressants can increase quit rates, and these effects are reversed once the treatment is stopped (Hall et al., 1998; Hughes et al., 2000; Kotlyar et al., 2001). The most striking connection between smoking and depression is the norepinephrine-dopamine reuptake inhibitor bupropion, which is prescribed as both an antidepressant and as an aid to smoking cessation (Dwoskin et al., 2006; Hayford et al., 1999). This suggests that depression and nicotine dependence may share some common neuronal substrates. This section will therefore discuss the data on activation and desensitization of nAChRs in behaviors related to mood and depression.

3.2 The cholinergic hypothesis of depression

The involvement of the cholinergic system in the etiology of depression, while not as prominent in the literature as the monoamine hypothesis, was put forward several decades ago. The cholinergic-adrenergic theory of depression postulates that a fine balance exists between cholinergic and noradrenergic systems, and that over-activation of the cholinergic component would lead to depression (Janowsky et al., 1972). This hypothesis stems from clinical

observations showing that physostigmine, a potent inhibitor of acetylcholinesterase that increases ACh concentration, could exacerbate mood disorders. Following treatment with physostigmine, non-depressed subjects had heightened anxiety, aggression and irritability, whereas depressed patients exhibited prolonged and more severe symptoms (Janowsky et al., 1974) and a majority of bipolar patients exhibited signs of depression (Oppenheim et al., 1979). Cholinergic sensitivity was also suggested as a marker of genetic predisposition for depression (Janowsky et al., 1994). In addition, alteration of central acetylcholine turnover has been shown in response to prolonged stress-exposure (Gilad et al., 1987), a key factor in the development of depressive behavior (Nemeroff, 2004; Penza et al., 2003). Finally, imaging studies have demonstrated that depressed patients have increased central concentrations of choline (the limiting precursor of ACh) and this is reversed after recovery (Charles et al., 1994). Another study found elevated levels of choline in the orbitofrontal cortex of adolescents with mood disorders (Steingard et al., 2000). One concern is that Alzheimer's disease patients treated with acetylcholinesterase inhibitors might develop greater symptoms of depression; however, it is difficult to make conclusions about the role of ACh in mood symptoms in these subjects, since the mood of Alzheimer's patients is likely to be affected by numerous factors unrelated to the direct effects of the cholinesterase blockade.

Initially, it was believed that the ability of elevated ACh to result in depression-like symptoms was mediated primarily through muscarinic ACh receptors, and it was not clear whether the effects of heightened cholinergic tone occurred through peripheral or central mechanisms (Janowsky et al., 1972). However, studies have since demonstrated that nAChRs were key in the response to cholinergic stimulation triggered by cholinergic agents including physostigmine (Rhodes et al., 2001). Taken together, these data strongly suggest that heightened cholinergic activity and/or sensitivity can contribute to the development and the exacerbation of symptoms of depression. By extension, it also suggests that over-activation of nAChRs by endogenous ACh could potentially be part of this complex mechanism. While it is clear that the monoaminergic systems are critical for the treatment of depression, a role for the cholinergic system, perhaps through modulation of monoaminergic signaling, is also likely.

3.3 Smoking and depression

Nicotine dependence shows strong co-morbidity with mood disorders including depression. Clinical studies have demonstrated a connection between smoking behavior and genetic susceptibility to depression (Cinciripini et al., 2004; Lerman et al., 1998) and numerous observations suggest that smoking and nicotine can regulate mood in humans and animals. Depressed patients are twice as likely to smoke than the general population (Diwan et al., 1998; Glassman et al., 1990). Further, nicotine patch can alleviate symptoms of depression in non-smokers (Salin-Pascual et al., 1995) and it is believed that some depressed patients initiate smoking as an attempt to self medicate depressive symptoms with nicotine (Markou et al., 1998; Patton et al., 1998). Cigarettes might therefore be used as an efficient method to self-administer nicotine to a desired level. Similarly, it is believed that schizophrenic subjects, who have a very high rate of smoking, may be medicating an attentional deficit with the nicotine in cigarette smoke. An interesting set of studies has suggested that these patients are more likely to smoke because they attribute greater benefits to smoking than a control population (Spring *et al.*, 2003). Indeed, nicotine can reverse some of the neurocognitive impairments associated with schizophrenia (George *et al.*, 2006; George *et al.*, 2002; Sacco *et al.*, 2005).

It should be noted, however, that the self medication hypothesis for smoking alone cannot explain nicotine addiction in depressed subjects, since a large proportion of depressed patients do not smoke, and craving for cigarettes is strongly influenced by other factors including environmental cues associated with smoking (see Section 2) and developmental exposure to

nicotine through maternal smoking (Ernst et al., 2001) or during adolescence (Patton et al., 1998).

Smoking cessation can also exacerbate symptoms of depression (Glassman et al., 1990), although the effects of acute nicotine withdrawal on mood may be the result of distinct mechanisms from the ability of nicotine in tobacco to affect mood during ongoing smoking. Similarly, while depression and smoking show strong correlation and potential common pathways, genetic studies also emphasized that both can also have similar genetic susceptibility but that no causal connections exist between these two phenotypes (Kendler et al., 1993; McCaffery et al., 2003).

3.4 Paradoxical effects of nicotinic agents in depression: balance between activation and desensitization

Animal studies have also shown that chronic nicotine administration can elicit antidepressant-like effects in rats both in the learned helplessness (Semba et al., 1998) and the forced swim (Djuric et al., 1999; Tizabi et al., 1999) paradigms. Further, the nicotinic partial agonist cytisine results in antidepressant-like effects in several behavioral paradigms in mice (Mineur et al., 2007).

Because nicotine is a nicotinic receptors agonist, it seems paradoxical that nicotine administration is antidepressant. If nicotine can relieve depression symptoms, then why does physostigmine, which increases acetylcholine concentration, increase depressive symptoms? Further, several classes of nicotinic agonists have antidepressant actions in both human studies and animal models (Ferguson et al., 2000; Gatto et al., 2004) but several nicotinic antagonists have shown to have potent antidepressant effects (Ferguson et al., 2000; Shytle et al., 2002). These data can be reconciled if the ability of nicotine to desensitize nAChRs is primarily responsible for its ability to alleviate depressive symptoms. Thus, nicotine and other nicotinic agonists and partial agonists may be antidepressant because they limit the ability of endogenous ACh to signal through nAChRs. Similarly, nicotinic antagonists would exert the same effect on depressive symptoms by decreasing cholinergic tone on nAChRs.

Data in human subjects support the idea that blockade rather than activation of nAChRs results in antidepressant-like effects. For example, the non-competitive, non-selective nAChR antagonist mecamylamine as well as the nicotine patch, decrease symptoms of depression in depressed non-smoking patients and patients with Tourette's syndrome (Dursun and Kutcher, 1999; Mihailescu and Drucker-Colin, 2000; Salin-Pascual et al., 1995; Salin-Pascual et al., 1996). Mecamylamine and the competitive nicotinic antagonist Dh β E also have antidepressant-like properties in mice (Caldarone et al., 2004; Mineur et al., 2007; Rabenstein et al., 2006).

A number of studies have shown that chronic administration of nicotinic agonists (including nicotine through regular smoking or as delivered through the nicotine patch) can desensitize rather than activate nAChRs (Reitstetter et al., 1999), leading to functional antagonism (Gentry and Lukas, 2002; Quick and Lester, 2002). Such an effect would be expected to be antidepressant (Djuric et al., 1999; Kinnunen et al., 1996; Tizabi et al., 1999). Indeed, this hypothesis suggests that the increased depressive symptoms observed in some patients following acute cessation from smoking might be explained by the fact that the clearance of nicotine following smoking cessation coupled with persistent nAChR upregulation (Staley et al., 2006) results in increased ability of ACh to activate these upregulated nAChRs.

3.5 Classical antidepressants can block nAChRs

Interestingly, a significant body of evidence is accumulating suggesting that many of the commonly used antidepressants can also block nAChRs in a number of *in vitro* and *in vivo*

assays (see (Shytle *et al.*, 2002) for a detailed review). The doses at which these compounds are able to antagonize nAChRs are in many cases within the low micro-molar range reached when antidepressants are administered chronically. Thus, while the primary targets of classical antidepressants are on monoamine systems, it is possible that nicotinic antagonism could be one component that plays a role in antidepressant response.

Animal studies support the idea that nAChRs may contribute to the antidepressant effects of classical antidepressants. For example, the tricyclic antidepressant amitriptyline has no effect in neurochemical and behavioral assays of antidepressant efficacy in KO mice lacking the $\beta 2$ nAChR subunit, strongly suggesting that $\beta 2^*$ nAChRs are involved in this antidepressant response (Caldarone *et al.*, 2004). Further, a subthreshold dose of the nicotinic antagonist mecamylamine combined with the tricyclic antidepressant amitriptyline results in antidepressant-like effects in mice, reinforcing the idea that blockade of nAChRs yields antidepressant-like effects (Caldarone *et al.*, 2004); however, it is not yet clear whether this additive effect suggests that nicotinic compounds and classical antidepressant act on similar pathways.

3.6 Activation/desensitization of nAChR subtypes in depression-like behavior

Pharmacological studies have demonstrated that non-selective antagonism of nAChRs with mecamylamine can be antidepressant, but further studies have tried to focus on the role of specific receptor subtypes in this effect. For instance, the $\alpha 4/\beta 2^*$ subclass of nAChRs is necessary for the antidepressant-like effects of nicotinic antagonists as demonstrated in KO mice and using selective pharmacological agents (Gatto *et al.*, 2004; Mineur *et al.*, 2007; Rabenstein *et al.*, 2006). In addition, at baseline, KO mice lacking the $\beta 2^*$ subunit show decreased depression-like behavior in the forced swim and tail suspension tests at baseline, supporting the possibility that the $\beta 2$ KO mimics the effect of nAChR blockade (Caldarone *et al.*, 2004; Rabenstein *et al.*, 2006). However, other subtypes of nAChR may also be involved in the effects of nicotinic agents. For example, like mice lacking the $\beta 2$ nAChR subunit, KO mice lacking the $\alpha 7$ subunit also do not respond to the broad spectrum nAChR antagonist mecamylamine in mouse models of antidepressant response (Rabenstein *et al.*, 2006). Similarly, cytisine, a nicotinic partial agonist that blocks $\alpha 4/\beta 2^*$ nAChRs but activates $\alpha 3/\beta 4^*$ and $\alpha 7^*$ nAChRs (Papke and Porter Papke, 2002; Picciotto *et al.*, 1995), is also effective in animal models of antidepressant efficacy (Mineur *et al.*, 2007). Thus, it is not yet clear whether nAChR antagonism is the only mechanism underlying the antidepressant effects of nicotine, or whether antagonism of $\alpha 4/\beta 2^*$ nAChRs coupled with agonism of other nAChR subtype(s) may also contribute.

3.7 Anatomical loci underlying nicotinic effects in depression

The effect of nAChR antagonism appears to be centrally mediated since unlike the permeant antagonist mecamylamine, hexamethonium, a nicotinic antagonist that does not cross the blood-brain barrier, did not result in a significant antidepressant-like response in mice (Rabenstein *et al.*, 2006). The hippocampus has been a major focus of interest in mood disorders, essentially because numerous depressed patients show a shrinkage of this brain region (Sheline, 2000), and also due to the neurotrophin hypothesis of depression (Duman *et al.*, 1999; Malberg *et al.*, 2000). Both $\beta 2^*$ and $\alpha 7$ nAChRs are found in the hippocampus (Gotti *et al.*, 2006) and KO mice lacking the $\beta 2$ nAChR subunit do not show an antidepressant-induced increase in hippocampal neurogenesis, consistent with the lack of a behavioral response to amitriptyline treatment (Caldarone *et al.*, 2004). This further suggests that $\beta 2^*$ nAChRs are necessary for the ability of amitriptyline to increase hippocampal neurogenesis, an effect that has been suggested to underlie the response to chronic antidepressant treatment (Malberg *et al.*, 2000; Santarelli *et al.*, 2003).

In addition to the hippocampus, the amygdala is known to mediate affective behavior and to be the target for classical antidepressants (Clark et al., 2006; Drevets, 2001). Both mecamylamine and cytisine, in agreement with their antidepressant-like properties, decreased c-fos immunoreactivity in the basolateral amygdala, indicating a reduction of neuronal activity in this region (Mineur et al., 2007). This mode of action would be consistent with observations in patients showing an increased activity of this structure during depression episodes and stress (Drevets, 2001), reversed by specific antidepressant treatments (Clark et al., 2006). Because of the correlative nature of the association between nicotinic effects in the amygdala and antidepressant-like effects, further studies will be necessary to establish (or refute) a causal link between decreased activity in basolateral amygdala and the antidepressant-like properties of nicotinic agents. In addition, despite the strong comorbidity that exists between mood disorders and smoking, the overlapping mechanism/neural substrates that could explain the link between these two phenotypes have not yet been identified.

While there are many hypotheses, there is as yet no solid consensus about the neural substrates and brain loci that are critical for mood disorders. Similarly, while nAChR antagonism can be antidepressant, it is not yet known whether the nAChRs involved are pre- or post-synaptic, the cell type(s) involved and whether nicotinic antagonism leads to a net reduction of neuronal activity (through direct inhibition, for instance) or to heightened activity of specific neurons through disinhibition of GABAergic neurons or other inhibitory systems. The cell types on which nAChRs are located (and activated/desensitized) are likely to be critical in the antidepressant-like response induced by nicotinic agents. Thus, nicotinic receptors located on monoaminergic neurons represent a particularly relevant target of interest. Future experiments will be needed to tease out the anatomical substrates underlying the antidepressant effects of nicotinic agents.

An important caveat to the idea that inactivation of nAChRs is antidepressant is that the nicotinic antagonist mecamylamine can block the ability of nicotine to be effective in some models of antidepressant efficacy in selectively bred rats (Tizabi et al., 2000). This suggests that nAChR activation can have antidepressant-like effects, and may reflect the fact that a similar balance of desensitization of $\alpha 4/\beta 2^*$ nAChRs and activation of $\alpha 7^*$ nAChRs that has been shown in the VTA can also modulate monoamine systems related to mood.

4. Conclusions

The studies reviewed here show that there is no simple relationship between smoking and nAChR activation or desensitization. Rather, coordinated activation and desensitization of a number of different nAChRs on different neuronal subtypes likely occurs in response to nicotine administration through smoking. Neither desensitization alone nor activation alone is sufficient to explain the behavioral consequences of nicotine intake, just as nicotine reward alone is not sufficient to explain smoking behavior. Instead, activation and desensitization of nAChRs contribute to an ensemble of behavioral effects, including nicotine reward, conditioned reinforcement and modulation of mood, that promote ongoing smoking behavior.

The fact that desensitization contributes significantly to some of the effects of chronic nicotine intake suggests that some of the effects of nicotine are due to disruption of endogenous ACh signaling. While this is easiest to describe with respect to the cholinergic hypothesis of depression, it is also the case that nicotine reward may be the result of disruption of normal ACh signaling in the VTA and other brain regions. This is supported by studies showing that nicotinic antagonists modulate the rewarding effects of drugs of abuse such as cocaine (Levin et al., 2000; Reid et al., 1999; Zachariou et al., 2001; Zanetti et al., 2006), suggesting a role for ACh in reward circuits more generally than just in nicotine reward.

These studies also suggest that there is a role for tonic ACh signaling in behaviors related to reward and affect. Microdialysis studies using the no-net-flux method have shown that the baseline level of extracellular ACh is in the range of 4.5 nM at rest in the mouse hippocampus (Laplanche et al., 2004). One possibility is that nAChRs act as sensors of tonic ACh levels. The volume transmission hypothesis suggests that basal levels of neurotransmitters can coordinate the excitability of ensembles of neurons across a broad distance (Zoli et al., 1999). The tonic activation and desensitization of nAChRs in the DA system, the hippocampus or the amygdala could regulate reward and affect by setting an overall tone for neuronal activity in these circuits. Thus, the ability of nAChR antagonists to decrease c-fos activity in the amygdala and other brain areas (Mineur et al., 2007) reflects disruption of cholinergic tone in those brain regions that is likely to contribute to the behavioral effects of these nicotinic agents.

The balance between activation and desensitization of nAChRs has been elegantly explored in a number of studies of the DA system (Fig. 1). In this system, the temporal sequence of activation of $\beta 2^*$ nAChRs on DA neurons in the VTA, followed by their desensitization, appears to result in a shift from a brief, direct drive of DA neuronal firing toward presynaptic, $\alpha 7$ -mediated activation of glutamate release onto DA neurons (Mansvelder et al., 2002; Wooltorton et al., 2003). Combined with desensitization of $\beta 2^*$ nAChRs on DA terminals in the NAc leading to decreased transmission of tonic DA neuronal firing, but maintained release of DA in response to phasic DA neuronal firing (Rice and Cragg, 2004; Zhang and Sulzer, 2004), this pattern of nAChR activity could result in increased salience of environmental cues that were paired with nicotine intake. Together, this provides a mechanism for a network level effect of nAChR activation and desensitization that could occur more generally throughout the brain. Future studies may reveal a similar network level of nicotinic regulation in other systems, such as the hippocampus and amygdala. Future studies using agonists and antagonists selective for particular nAChR subtypes could clarify the effects of activation and desensitization particular nAChR populations on nicotine-related behaviors.

It is interesting that several of the current therapeutics for smoking cessation have both activating and desensitizing/inhibiting effects on nAChR function. For example, the nicotine patch does deliver nicotine, but it does so with pharmacokinetics which favor desensitization of nAChRs (Gries et al., 1998). Varenicline is a partial agonist of $\alpha 4/\beta 2^*$ nAChRs (Coe et al., 2005), but also activates $\alpha 7$ -type nAChRs (Mihalak et al., 2006). While there are common behavioral effects observed between smoked nicotine, nicotine patch and varenicline, neither patch (Jorenby et al., 1995) nor varenicline are reinforcing (Rollema et al., 2007). The observation that nicotine patch or mecamylamine can significantly improve mood in patients affected by Tourette's syndrome (Silver et al., 2001) supports the idea that this effect of the patch is likely to be a result of nAChR desensitization. Finally, bupropion, while having effects on monoamine transporters, has also been shown to be a non-competitive antagonist of nAChRs (Dwoskin et al., 2006). Thus, it appears that partial agonism or blockade of nAChRs may be particularly useful for aiding smoking cessation. One potential reason for this possibility is that interference with cholinergic transmission ameliorates negative mood symptoms. It would therefore be predicted that a subset of patients taking varenicline may report that their mood symptoms are decreased by the drug. Further, partial agonism or blockade of nAChRs may be helpful in decreasing cue-induced craving by decreasing the firing rate of VTA neurons, while maintaining the filtering effect on DA terminals that allows other salient reward signals to induce DA release in the NAc.

In summary, both activation and desensitization appear to contribute to the primary rewarding properties of nicotine and to secondary conditioned reinforcement. Further, it appears that blockade of nAChRs can be antidepressant-like in animals and human subjects, through either direct blockade of nAChRs or functional antagonism through desensitization. Thus, agents, like nicotine itself, and partial agonists of nAChRs, have the unique ability to regulate network

properties of ensembles of neurons, through differential activation and desensitization of nAChRs on excitatory and inhibitory neuronal cell bodies and terminals. This may be a widespread mechanism underlying the effects of nAChRs on a number of different brain systems.

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Abbreviation List

nAChR	nicotinic acetylcholine receptor
ACh	acetylcholine
$\alpha 7^*$, $\beta 2^*$, etc	* denotes other subunits that may not have been identified
PET	positron emission tomography
VTA	ventral tegmental area
NAc	nucleus accumbens
DA	dopamine
5HT	serotonin

KO	knockout mouse
MLA	methyllaconitine
DHβE	dihydro-beta-erythroidine
Glu	glutamate
GABA	gamma-aminobutyric acid

A. Baseline **B. Extended nicotine exposure**

Figure 1. Activation and desensitization contribute to physiological effects of nicotine in the mesolimbic circuitry

A. In the absence of nicotine, the endogenous neurotransmitter acetylcholine (ACh) is released in the ventral tegmental area (VTA) from terminals of cholinergic brainstem nuclei including the pedunculopontine tegmental area (Lança et al., 2000). The mixed cholinergic stimulation of glutamatergic (Glu) and GABAergic (GABA) terminals in the VTA supports both tonic and phasic activity of dopaminergic (DA) neurons (Grenhoff and Svensson, 1992). In addition, cholinergic interneurons release ACh onto the terminals of dopaminergic neurons (DA) in the nucleus accumbens (NAc) (Zhou et al., 2001). At baseline, ACh in the striatum allows tonic firing of DA neurons to result in significant DA release (Rice and Cragg, 2004; Zhang and Sulzer, 2004). **B.** The effect of nicotine in the VTA is initially to increase the firing rate of DA neurons (Grenhoff et al., 1986; Picciotto et al., 1998). The increase in firing rate of VTA neurons involves glutamate release (Grillner and Svensson, 2000; Schilström et al., 1998), and the nicotine-mediated increase in glutamate release does not desensitize during continuous administration of nicotine (Mansvelder et al., 2002; Wooltorton et al., 2003). By contrast, the ability of nicotine to increase GABA release in the VTA undergoes rapid desensitization (Mansvelder et al., 2002; Wooltorton et al., 2003). In the NAc, nicotine initially increases release of DA from terminals, but these nAChRs undergo rapid desensitization (Grady et al., 1994; Rowell and Hillebrand, 1994). Desensitization of these nAChRs decreases the ability of tonic firing to release DA in the NAc, but maintains the effect of phasic firing on DA release, thereby acting as a high pass filter for events increasing phasic firing (Rice and Cragg, 2004; Zhang and Sulzer, 2004). Thus, nicotine acts in the VTA to increase phasic firing, and in the NAc to increase the salience of that increase in phasic firing.