Dopaminergic and cholinergic learning mechanisms in nicotine addiction

Manivannan Subramaniyan and John A. Dani
Department of Neuroscience, Mahoney Institute for Neurosciences, Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania

Address for correspondence: John A. Dani, Department of Neuroscience, 415 Curie Blvd, 211 CRB, University of Pennsylvania, Philadelphia, PA 19104. johndani@upenn.edu

Nicotine addiction drives tobacco use by one billion people worldwide, causing nearly six million deaths a year. Nicotine binds to nicotinic acetylcholine receptors that are normally activated by the endogenous neurotransmitter acetylcholine. The widespread expression of nicotinic receptors throughout the nervous system accounts for the diverse physiological effects triggered by nicotine. A crucial influence of nicotine is on the synaptic mechanisms underlying learning that contribute to the addiction process. Here, we focus on the acquisition phase of smoking addiction and review animal model studies on how nicotine modifies dopaminergic and cholinergic signaling in key nodes of the reinforcement circuitry: ventral tegmental area, nucleus accumbens (NAc), amygdala, and hippocampus. Capitalizing on mechanisms that subserve natural rewards, nicotine activates midbrain dopamine neurons directly and indirectly, and nicotine causes dopamine release in very broad target areas throughout the brain, including the NAc, amygdala, and hippocampus. In addition, nicotine orchestrates local changes within those target structures, alters the release of virtually all major neurotransmitters, and primes the nervous system to the influence of other addictive drugs. Hence, understanding how nicotine affects the circuitry for synaptic plasticity and learning may aid in developing reasoned therapies to treat nicotine addiction.

Keywords: dopamine; acetylcholine; VTA; hippocampus; nucleus accumbens; memory

Introduction

The nervous system has evolved reward- and punishment-based learning to guide individuals to select actions that sustain life and species. Addictive drugs activate and modify these innate learning circuits. Nearly a billion people around the globe smoke tobacco, driven mainly by the addictive plant alkaloid nicotine. Tobacco use, most commonly by smoking, initiates a powerful addictive process, ensuring its persistent use despite willful attempts humans make to break out of the addiction. Consequently, tobacco use is the leading cause of preventable death, causing nearly six million deaths per year worldwide. Why is the human nervous system, in particular, susceptible to such a phenomenon? What are the key neural systems that nicotine influences to initiate the acquisition of this addictive drug use?

Nicotine is the primary addictive substance in tobacco. In addition, environmental stimuli and sensory experiences associated with smoking play an important role in the addiction process. When smoked, nicotine enters the blood stream mainly through the lungs and reaches the brain in tens of seconds. In the bloodstream at pH 7.4, nicotine exists in charged (69%) and uncharged forms (31%). The uncharged form can pass through lipid membranes and directly alter intracellular signaling. It is the charged form that binds to the nicotinic acetylcholine receptors (nAChRs), which are ligand-gated ion channels formed by homo- or heteropentameric subunit combinations. The subunits are grouped into two classes, α and β. In the mammalian brain there are eight α subunits (α2–α7, α9, and α10) and three β subunits (β2–β4). The nAChRs are distributed widely in the...
nervous system, including cortical and limbic regions that contribute to the addiction process, such as the cerebral cortex, hippocampus, amygdala, ventral tegmental area (VTA), and nucleus accumbens (NAc). In individual neurons, nAChRs are located in preterminal, presynaptic, axonal, dendritic, and soma compartments. Hence, the influence of nicotine on brain function is determined, at least in part, by the receptors’ subtype-specific localization, density, and functional properties.

**Associative learning and addiction**

Because mammals function within broad and highly variable environments in the world, they must perform behaviors and voluntary movements in order to achieve success and to avoid danger. It is also advantageous for them to learn the causal relationship between specific actions and outcomes. Behavioral psychologists have made significant progress in understanding the structure of the associations among stimuli (S), actions or responses (R), and outcomes (O). Learning these associations is one of the most fundamental capabilities of the nervous system of animals, ranging from worms to humans. When animals perform rewarded instrumental actions in the presence of a stimulus, they learn all combinations of binary associations among S, R, and O: namely, R–O, S–R, and S–O (Fig. 1). In addition, they learn the higher order association S–(R–O), where the stimulus becomes associated with the R–O relation. This higher order association of instrumental learning can be viewed as a Pavlovian association of S with R–O.

In general, these associations play a significant role in addiction. In particular, the nicotine addiction process resembles instrumental learning in some respects. For example, the smoker performs the instrumental action of lighting the cigarette and smoking it, with the outcome being (in some cases) positive effects such as a sense of well-being, feeling more relaxed, reduced anxiety, enhanced working memory, increased alertness, better attention, increased cognition, relief of withdrawal symptoms, and so on, depending on the smoker and circumstances. In such a case, the smoker’s nervous system likely learns several associations. First, it learns the R–O association between lighting the cigarette and the positive outcomes, motivating the smoker to repeat the action. Second, it learns the Pavlovian S–O and S–(R–O) associations, whereby the location and other environmental input from the smoking episodes gain salience and eventually lead to the initiation of smoking. Although these associations are rather simple, a diverse set of neuronal systems, including reward-related structures, are involved in this process. Because an explicit comparison between natural learning and addiction at the mechanistic level is beyond the scope of this review, we will only briefly touch upon it. Our main focus instead will be on the mechanistic learning processes in the acquisition phase of the addictive process. Topics related to the maintenance and withdrawal phases of nicotine addiction are not considered here, but they have been reviewed elsewhere.

**Learning circuitry modified by nicotine**

In modern neurobiological studies, the two classically evaluated learning paradigms—Pavlovian conditioning and instrumental conditioning—have been brought under a common conceptual framework called reinforcement learning, where rewards and punishments act as positive and negative reinforcers, respectively. When a goal directed action is pursued, such as foraging for food, successful outcomes are marked by reward-related signals. The reward signals are thought to play a crucial role in linking the precedent action sequence and cues with the positive experience of the outcome. The main sources of these reinforcement signals are the midbrain dopaminergic neurons located in the VTA and substantia nigra pars compacta (SNc). Several crucial brain structures in the addiction process that receive dopamine (DA) include the prefrontal cortex, hippocampus, amygdala, and dorsal and ventral striatum (which includes the NAc). The response of dopaminergic neurons has been modeled as a
reward prediction error that is thought to send a teaching signal that initiates plasticity in key learning and memory circuits. When appropriately cued, the learned sequences are repeated in order to receive the reward. This learning is important for survival, as it provides an efficient means of obtaining essential resources based on previous successful outcomes. Unfortunately, it is these learned associations among stimuli, action sequences, and rewards that all drugs of abuse (including nicotine) usurp to achieve their strong undesired effects on behavior. In the following, we will review how nicotine engages some of the key reward-related structures to achieve its addictive influence.

**Influences of nicotine over DA signaling by the VTA**

The VTA consists of heterogeneous group of cells: the parnigral nucleus, the parainterfascicular nucleus, the rostral VTA, and the rostral midline at the floor of the midbrain (Fig. 2). It is an important node in the reward-based learning circuit (Fig. 2, top) and projects to many brain structures including the NAc, PFC, and hippocampus. The VTA dopaminergic neurons spontaneously fire at a tonic average rate of around 4 Hz (2–10 Hz range) with randomly interspersed bursts. During the acquisition phase of reward-based learning, they fire briefly in phasic bursts, signaling and broadcasting the arrival of reward. Once the association between reward predicting cues and rewards is learned, the arrival of reward no longer elicits burst firing. Instead, the conditioned incentive stimuli that predict reward elicit burst firing, and many conditioned behaviors depend on this burst firing. It should be noted that there are similarities and differences between natural rewards and drug reward. For example, in rats that have learned to self-administer cocaine, similar to associative learning in food rewards, the presentation of a cue paired with cocaine elicits phasic DA release in the NAc. However, unlike natural rewards such as food, cocaine acting as a primary reward continues to elicit prolonged DA release even after learning the association between cocaine reward and the cues that predict it. Although such studies do not exist for nicotine addiction, nicotine likely acts in a similar fashion because nicotine also elicits prolonged burst firing in VTA DA neurons and causes prolonged DA release in the NAc. This unnatural non-habituating DA release has been hypothesized to be an important contributor to the excessive motivational power attributed to the cues and contexts associated with drug use.

Although the VTA receives convergent inputs from many areas, prominent input sources, when considering reward-based learning, include the pedunculopontine/lateral dorsal tegmentum (PPTg/LDTg) and the PFC. The PPTg sends glutamatergic/cholinergic projections to the VTA, and activation of PPTg elicits burst firing in VTA DA neurons. The PPTg is, in turn, activated by cortical and limbic inputs, including PFC and extended amygdala, and by sensory inputs from visual, auditory, and somatosensory modalities. This convergence of inputs marks the PPTg as a crucial structure through which various behaviorally relevant inputs control the burst firing of VTA DA neurons. For instance, the PPTg/LDTg has been shown to control the conditioned responses of VTA DA neurons in behaving rats. The LDTg sends glutamatergic/cholinergic/GABAergic projections to the VTA and plays a permissive role in the burst firing. This conclusion is supported by the observation that inactivation of the LDTg leads to severe reductions in burst firing and leads to tonic pacemaker-like activity in DA neurons, similar to that found in brain slices. In addition, NMDA receptors play an essential role in burst firing, as suggested by the finding that the NMDA receptor antagonist AP-5, infused into the VTA, reduces the burst firing markedly, whereas the non-NMDA receptor antagonist CNQX has less influence over bursting. Although the PPTg and LDTg inputs significantly regulate burst firing in VTA neurons, the number of DA neurons that undergo burst firing is likely controlled by the ventral pallidum (VP). The VTA receives tonic inhibition from the VP, leading to hyperpolarization of the DA neurons. This hyperpolarization likely results in Mg block of the NMDA receptors, which in turn diminishes the burst firing. Removing the inhibition from the VP not only leads to an increase in the number of spontaneously active DA neurons but also likely leads to removal of Mg block, thereby, permitting afferent inputs to elicit burst firing.

When nicotine arrives in the brain, among its many actions is to excite the midbrain dopaminergic...
Figure 2. A simplified and incomplete schematic diagram (top panel) that highlights components of the circuitry contributing to reinforcement learning in rodents. Top panel: Sagittal section of a rodent brain didactically depicting strategic connections. Bottom panels: Coronal sections approximately at the anterior–posterior axis levels indicated by the dashed arrows, adapted from Figs. 20 and 74 of Ref. 29. Ac, anterior commissure; Amg, amygdala; LDTg, lateral dorsal tegmentum; NAc, nucleus accumbens; PFC, prefrontal cortex; PPTg, pedunculopontine tegmentum; SNc, substantia nigra pars compacta; VP, ventral pallidum; VTA, ventral tegmental area.

centers of the VTA and SNc. Nicotinic receptors are found on the GABAergic interneurons and DA neurons within the VTA and on the excitatory and inhibitory input axons and/or terminals. Although there are many types of nAChRs located on VTA DA neurons, the β2 subunit in combination with α4 and/or α6 subunits have been shown to be essential for nicotine addiction in animal models. In vivo recording from VTA DA neurons in anesthetized mice lacking the β2 subunit (Chrnb2<sup>−/−</sup>) showed that spontaneous burst firing was absent in all of the recorded neurons. However, re-expression of the β2 subunit selectively in the VTA restored the spontaneous firing patterns. In addition, the re-expression also restored the nicotine-induced increase in burst firing and intracranial self-administration (ICSA) of nicotine into the VTA. Various subtypes of β2 containing nAChRs are located on both dopaminergic and GABAergic interneurons of the VTA. Interestingly, using in vivo recording in anesthetized Chrnb2<sup>−/−</sup> mice, a recent study showed that re-expression of the β2 subunit on DA neurons or GABA interneurons alone did not restore nicotine-induced burst firing in DA neurons, whereas re-expression in both cell types restored nicotine-induced burst firing. In addition, re-expression of the β2 subunit in either cell population alone did not sustain the ICSA behavior whereas re-expression on both cell populations restored ICSA to the wild-type level. These results show that the concerted activation of β2* nAChRs located on both dopaminergic and GABAergic interneurons is involved in nicotine self-administration.
Under natural circumstances, usually processed information such as reward, novelty, and behaviorally salient stimuli gain access to the VTA to enable phasic burst firing. However, nicotine bypasses such preprocessing and directly activates VTA DA neurons to cause burst firing. In vivo tetrode recording from VTA DA neurons in behaving rats showed that an acute nicotine dose induces prolonged burst firing (Fig. 3) that can continue intermittently for more than 30 min, and causes DA release in the NAc (Fig. 4) that lasts for more than an hour. When nicotine binds to nAChRs located on the soma of DA neurons, it causes direct depolarization, resulting in an increased firing rate. However, some nAChRs located on the soma desensitize quickly, raising the question of how nicotine causes prolonged DA release.

Early in vitro studies indicated that both presynaptic mechanisms contribute to nicotine-induced prolonged DA release. The α7-containing nAChRs (α7*) located at presynaptic glutamatergic terminals are highly permeable to calcium and are less prone to desensitization at the nicotine concentrations experienced by smokers. The increased Ca²⁺ entry acting through the vesicle release machinery enhances glutamate release onto DA neurons. Conversely, the high-affinity α4β2 receptors located on the GABAergic neuron somas and terminals desensitize quicker, thereby, reducing the inhibitory drive onto the DA neurons. The net effect was thought to provide strong presynaptic drive to the DA neurons. Accordingly, when brief nicotine application was paired with postsynaptic depolarization, long-term synaptic potentiation (LTP) was induced that lasted for many minutes and

Figure 3. Nicotine increases burst firing in putative DA neurons within the VTA of freely moving rats. (A) Normalized average firing rate before (control, black) and after (nicotine, red) intraperitoneal (i.p.) injection of 0.4–0.5 mg/kg nicotine. Error bars represent SEM. (B) Representative traces from a single putative DA neuron before (black) and after (red) nicotine injection. Reproduced from Ref. 41.

Figure 4. Nicotine (0.6 mg/kg, i.p.) increases DA release in the nucleus accumbens shell of freely moving rats as measured by microdialysis with HPLC. Error bars represent SEM. Modified with permission from Ref. 40.
hours. Both the α7 antagonist methyllycaconitine (MLA) and the NMDA antagonist AP-5 prevented the nicotine-induced LTP in vitro,84 but in vivo, the role of α7 is more subtle.74 These experiments inspired studies in which nicotine was first given to the animals and VTA slices were then cut after various time periods. This approach revealed that nicotine causes its effect on VTA neurons in the intact animal, while still allowing in vitro techniques to measure the outcome. Consistent with the in vitro studies, a single dose of systemic nicotine increased the ratio of synaptic currents mediated by AMPA relative to NMDA receptors (AMPA/NMDA ratio) in VTA DA neurons, indicating increased numbers of AMPA receptors induced by LTP.86–88 This increase lasted up to 3 days (Fig. 5A) with a single nicotine dose and up to 8 days (Fig. 5B) after the cessation of 1 week of a daily nicotine dose.86 Although the early studies pointed out the crucial role of α7* presynaptic receptors in LTP, their roles are more subtle, as α7* gene knockout mice self-administered nicotine to wild-type levels.75

What is the role of this increased AMPA current measured by the AMPA/NMDA ratio in nicotine addiction? An increased AMPA/NMDA ratio does not appear to be sufficient for nicotine addiction, as Chrm2−/− mice, which do not self-administer nicotine, still show an increased AMPA/NMDA ratio.86 This indicates that the nicotine-induced increased AMPA current on DA neurons by itself cannot establish addiction and additional factors are needed. It must also be recalled that β2-containing nAChRs not only play a role in DA neuron activation in the midbrain, they also are vitally important in controlling DA release arising from action potentials reaching the DA neuron target areas.89,90 Moreover, future studies need to establish the causal role of the potentiated AMPA currents (i.e., DA neuron LTP) in nicotine addiction. It is also currently unknown which synapses onto VTA DA neurons contribute to the increased AMPA current.89,90 Although PFC glutamatergic inputs have been the focus of many studies, it is surprising that those inputs actually synapse onto VTA neurons that project mainly back to PFC, not to the NAc.50 On the contrary, DA neurons that receive inputs from PPTg project to the NAc,49 and it has been shown that those glutamatergic projections are modulated by nicotinic mechanisms.40 Hence, it is important for future studies to elucidate which synapses are actually strengthened by nicotine-induced LTP. The role of metabotropic glutamate receptors (mGluRs) in the regulation nicotine-mediated DA release also is important and has been recently reviewed.91

In addition, to understand the long-lasting DA signal induced by nicotine, we must consider that nAChR desensitization to low nicotine concentrations is incomplete. The DA neurons from the posterior VTA that projection to the NAc commonly express the α6 subunit with the α4 and β2 subunits.14 The presence of the α6 subunit, particularly in α6α4β2* nAChRs, slows the rate and degree of desensitization seen with the higher affinity α4β2 nAChRs.92 Thus, the subtype of nAChR commonly expressed in DA neurons (i.e., α6α4β2*) is not as susceptible to desensitization as the higher affinity α4β2-nAChR subtype, which is more common on GABA neurons.
Learning the smoking context through hippocampal processing

The hippocampus is involved in many diverse functions, including spatial navigation, working memory, transitive inference, arbitrary association, and temporal ordering of events.93–95 The hippocampus is anatomically in a strategic position to perform these functions because it receives highly processed information from diverse cortical and subcortical areas. While spatial information is conveyed mainly from the medial entorhinal cortex through the medial perforant path (Fig. 6), object information mainly from the lateral entorhinal cortex reaches the hippocampus through the lateral perforant path.96,97 In addition, the hippocampus receives several neuromodulatory inputs, which include noradrenergic input from the locus coeruleus,98 serotonergic input from the raphe,99,100 cholinergic input from the medial septum,101–103 and dopaminergic input mainly from the VTA.104 In addition, the dorsal (septal) and ventral (temporal) halves of the hippocampus have different input-output configurations.105 Not surprisingly, the hippocampus has been implicated in a multitude of functions ranging from spatial navigation to anxiety. How does nicotine engage the hippocampus with such diverse functions in achieving its addictive influence?

Given that a wide variety of nAChR subtypes have been reported throughout the hippocampus,12 one important likely role played by the hippocampus in addiction is to process the contextual information associated with nicotine use. Nicotine has been shown to induce LTP in the medial perforant path-DG synapses, the mossy fiber CA3 synapses, and the Schaffer collateral CA1 synapses (reviewed in Ref. 106). A large number of behavioral studies also support the idea that nicotine improves learning and memory performance in various tasks (reviewed in Ref. 107). It is interesting to note that in the hippocampus the GABAergic interneurons express much higher densities of nAChRs than the pyramidal cells,108–111 and recent studies112,113 have started examining the crucial role of a special class of inhibitory neurons in hippocampal plasticity. These neurons are called oriens lacunosum moleculare, or OLM interneurons, as their cell bodies reside in the stratum oriens and their axonal projections go up to the stratum lacunosum moleculare (Fig. 6, right). Activation of these GABAergic neurons by nicotine results in facilitating the Schaffer collateral pathway by inhibiting the inhibitory interneurons that synapse onto the pyramidal cell dendrites at the stratum radiatum (Fig. 7). On the other hand, OLM interneurons inhibit the temporoammonic (TA) pathway by directly synapsing at principal neuron dendrites in the stratum lacunosum moleculare. The Schaffer collateral pathway is hypothesized to bring information retrieved from CA3 to CA1, while the temporoammonic pathway brings sensory information directly from the entorhinal cortex.114,115 Accordingly, CA1 is thought to compare these two streams of inputs in order to determine the novelty of the information.116 Controlling the information flow in this way by OLM interneurons using endogenous acetylcholine may...
serve specific functions. For example, selectively blocking the TA pathway may facilitate retrieval of stored representations from CA3. However, future studies need to address how such control of information flow leads to nicotine addiction.

Being mobile organisms, humans experience various events throughout the day under different contexts. Yet, not all these events and contexts are worth committing to memory. How does the hippocampus “choose” what to store in memory? One idea is that the VTA sends DA signals to the hippocampus to “stamp” behaviorally relevant information. This idea would predict that the hippocampus might play a similar role in committing drug-associated contexts to memory if the hippocampus received DA signals when the context was experienced. Accordingly, when nicotine activates VTA DA neurons, DA will be released in the hippocampus and the contextual information will be marked for memory storage. First, in freely moving mice, systemic nicotine injection potentiates LTP (the medial perforant path input to the dentate gyrus) in a hippocampal slice. LTP was induced by weak theta burst stimulation (wTBS) and was quantified by comparing the slopes of field EPSPs in the presence and absence (baseline) of nicotine in the perfusate. Compared to the control condition (open circles), nicotine (filled squares) enhanced LTP in the Chrna2-cre mice. Removing inhibition from OLM cells (filled triangles) abolished the LTP-enhancing effect of nicotine. Error bars represent SEM. Modified with permission from Ref. 112.

Figure 7. Nicotine’s facilitation of LTP in the Schaffer collateral pathway depends on OLM interneurons. The inset shows the stimulating electrode placed in the Schaffer collateral (SC) path and the recording electrode in the stratum radiatum (SR) in a hippocampal slice. LTP was induced by weak theta burst stimulation (wTBS) and was quantified by comparing the slopes of field EPSPs in the presence and absence (baseline) of nicotine in the perfusate. Compared to the control condition (open circles), nicotine (filled squares) enhanced LTP in the Chrna2-cre mice. Removing inhibition from OLM cells (filled triangles) abolished the LTP-enhancing effect of nicotine. Error bars represent SEM. Modified with permission from Ref. 112.

Nucleus accumbens

One of the key targets that receives potent nicotine-induced DA release from the VTA is the NAc. The NAc has been hypothesized to serve an important role in translating motivation to action. It has earned a distinction in addiction because all major addictive drugs, including nicotine, cause DA release into the NAc. The NAc is implicated in a diverse set of functions that include stress response, motor function, sensory-motor gating, and instrumental learning.

The NAc is divided into core and shell subregions based on anatomy and neurochemical makeup. The core region is surrounded on the medial, lateral, and ventral sides by the shell region (Fig. 2). On the dorsal side, the core is directly continuous with the dorsal part of the striatum. In many respects, the core boundary cannot be easily distinguished from the dorsal part of the striatum. The core and shell regions receive distinct but overlapping inputs. Anatomical connections also exist between the core and shell, allowing interactions between them. Given the distinction between the core and the shell, many studies have explored the relative role of these two regions in instrumental learning in general and nicotine addiction in particular. In acquiring simple instrumental learning tasks such as lever pressing for food reward, the core region has been shown to be involved. Furthermore, this learning depended on coincident activation of NMDA and DA D1 receptors in the core region. The consolidation of this learning also depends on protein synthesis in the NAc core.
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Figure 8. Nicotine’s ability to enhance LTP in the medial perforant path-DG synapse depends on DA in freely moving mice. (A) Nicotine enhances LTP in vivo. Top row: field potential traces showing population spike (PS). Black, blue, and red traces indicate evoked responses after saline, 0.1 mg/kg nicotine, and 1 mg/kg nicotine injection (i.p.), respectively. Gray traces show the baseline evoked response. Bottom row: Normalized PS amplitude measured a day prior to nicotine injection (–1 day), on the day of nicotine injection (arrow, 0 day), and 1 (1 day), or 2 days (2 days) after the treatment. (B) Blocking VTA neuronal action potential with tetrodotoxin (TTX) abolishes nicotine-induced LTP (n = 5). (C) Blockade of DA D1 receptors by local hippocampal infusion of SCH23390 also abolishes nicotine-induced LTP (n = 6). Error bars represent SEM. Modified with permission from Ref. 117.

not shell.\textsuperscript{134} If the acquisition of smoking behavior engaged instrumental learning, those results would predict a greater involvement of the core region. Paradoxically, initial intravenously injected nicotine was shown to increase DA release in the shell region, not the core.\textsuperscript{41,123} These microdialysis studies showed that the initial large nicotine-induced DA increase was in the shell. The DA
signal did initially change in the core, but microdialysis does not see changes in DA afferent activity well owing to its slow sampling over a large (averaged) area of the background DA concentration. Experimentation showed that the NAc shell was best able to capitalize on afferent burst firing by DA neurons to increase DA release.41 Repeated noncontingent injections of nicotine, however, lead to a sensitized DA release in the core, whereas the shell, although it showed a significantly enhanced DA release, did not show such sensitization.124,135 This sensitized response in the core was thought to underlie the enhanced locomotor response observed in rodents following repeated nicotine administration.136 However, subsequent studies showed a dissociation between the sensitized DA release in the core and the locomotor sensitization.137,138 It should be noted that in the above nicotine studies the rodents did not perform any instrumental action to receive nicotine. In a nicotine self-administration (SA) study,139 rats were trained to nose poke to receive 0.03 mg/kg intravenous nicotine. It was found that starting from the first week, when the rats were learning to self-administer nicotine, the DA level increased in the NAc shell. Consistently, significant enhancement of DA release in the core region was only observed from the fourth week, well after the animals learned to nose-poke the nicotine-delivering port. The authors also showed that over the entire 6-week period of SA, there was no significant change in the dialysate concentration of DA measured during SA in the core or the shell when the intake of nicotine was taken as a covariate, suggesting very little sensitization in both sub-regions of the NAc. These observations suggest that response-contingent and non-contingent nicotine administrations may lead to different DA release sensitization outcomes. Another study140 that examined nicotine-induced CPP found that infusion of D1 antagonist in the shell region impaired acquisition of CPP; infusion into the core region did not have an effect. The basis for the difference in DA signaling to the NAc shell by nicotine was found to arise from the differences in DA release induced by phasic burst firing when decoded in the NAc shell versus the dorsal striatum (at least during the initial exposures to nicotine).41 These studies indicate that, initially, nicotine preferentially engages the shell region of the NAc to bring about its reinforcing properties.

The core may become important for the later stages in the addiction process, such as cue-induced reinstatement of nicotine seeking.141 However, the role of nicotine-induced DA release in the core region in nicotine addiction is largely unexplored. The studies summarized above and other studies suggest that acquisition of instrumental learning reinforced by food and nicotine may engage different neural circuits.142–144

Nicotine modulates DA levels in the NAc in two ways. First, nicotine activates VTA neurons, which leads to DA release in the NAc. Second, nicotine modulates nicotinic activity in the target areas of VTA, including the NAc.89,90,145–148 Ongoing nAChR activity located at the dopaminergic terminals in the NAc augments DA release.89,90,149,150 While nAChRs containing the β2 subunit are highly important for DA release in the striatum,89,90,145,148 the α7 subunit–containing nAChRs also may have some role since NAc infusions of either α-bungarotoxin or the α7* nAChR antagonist methyllycaconitine reduces the DA level in the NAc.151 In addition, nicotine enhances the contrast between tonic and phasic DA signals in the NAc by suppressing DA release resulting from tonic presynaptic inputs, while enhancing DA release from phasic burst-like presynaptic activity.90 Potential functional roles of nicotine-induced DA release in the NAc have been addressed in a recent review.91

The main projection neurons out of the NAc are the medium spiny neurons (MSNs), which receive convergent inputs from the medial prefrontal cortex (mPFC), ventral subiculum of the hippocampus, and basolateral amygdala (BLA).152–155 The mPFC inputs carry information about executive and motor plans. The hippocampal inputs provide contextual information, while the BLA provides discrete cue information and emotional salience information. These areas express two general types of DA receptors (D1 and D2), and their activation is hypothesized to lead to facilitation of one input over another in the NAc. Both kinds of DA receptors are found pre- and post-synaptically in the NAc.156–158 The DA affinity of D1 receptors is lower than that of D2 receptors.159,160 As a consequence, while activation of D1 receptors requires higher DA levels afforded by phasic release, tonic levels of DA more continuously activate D2 receptors. D1 activation in the NAc has been shown to favor hippocampal inputs. On the other hand, activation of D2
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receptors leads to suppression of PFC inputs. Hence, to overcome the suppression by D2 activation during phasic DA release, stronger PFC inputs are required for them to activate the NAc. Interestingly, there is another condition, which may potentially allow PFC inputs to activate the NAc. When the tonic DA level goes below the baseline level, for example when reward is withheld, the D2 activation level will decrease, allowing PFC inputs to activate NAc neurons. In summary, these mechanisms allow the NAc to engage in different information processing: (1) phasic DA signaling during reward to integrate hippocampal contextual inputs and behaviorally relevant PFC motor plans, and (2) engage PFC inputs to enable behavioral flexibility mediated through the NAc. Since nicotine increases burst firing in VTA neurons, we hypothesize it may then favor hippocampal inputs while suppressing PFC inputs. Since the PFC is implicated in executive function and behavioral flexibility, the shift from the PFC may partly explain the pursuit of tobacco despite its harmful effects. This shift from PFC processing to more limbic, hippocampal processing may also lead to the inflexibility in changing the smoking behavior to better alternatives.

Many observations indicate that the hippocampus and NAc work closely in learning and memory of place-reward associations. First, under anesthetized conditions, the MSNs of the NAc exhibit a relatively depolarized “up” state and a hyperpolarized “down” state. The MSNs fire action potentials more readily in their “up” state. Inactivating the hippocampus completely abolishes the state transition, indicating that the hippocampal inputs are necessary for the bistable MSNs to enter the depolarized “up” state. Second, the NAc exhibits hippocampus-entrained theta rhythm. Place cells, which are most commonly identified within the hippocampus, are also observed in the NAc and show phase precession similar to hippocampal place cells. Finally, reward-related information is reactivated in the NAc during quiet wakefulness and slow wave sleep, similar to the findings in the hippocampus. Although the hippocampus and NAc have been studied separately in nicotine addiction, the interaction between the two has not been well explored. Since both areas express nAChRs, it would be valuable to explore the above-mentioned interactions to understand how drug-associated memories gain abnormal strength and persistence.

The amygdala

The amygdala is traditionally associated with fear and emotion processing and memory. Although studied mainly for fear processing, the amygdala also mediates positive affect hence, its relevance to addiction is a fertile area for exploration. In addition to the context in which smoking is carried out, discrete cues also play a role in reinstating smoking behavior. For example, the click of a cigarette lighter or the smell of smoke can trigger a smoker’s desire for tobacco. The effect of nicotine on the plasticity in the lateral amygdala was recently explored in mice. A 7-day oral intake of nicotine led to enhanced LTP in the entorhinal cortex–lateral amygdala synapses that lasted up to 72 hours. This enhancement was pathway specific since the effect was not present in thalamus–lateral amygdala or lateral BLA synapses. The effect was mediated by α7- and β2-containing nicotinic receptors. Reduction of inhibition was found to contribute partially to the LTP enhancement. It remains to be seen if long term changes in nicotinic receptors also played a role in the enhanced LTP. Since the entorhinal cortex provides sensory information to the lateral amygdala, enhanced neurotransmission in this pathway may enhance processing of discrete cues associated with smoking.

The function of the amygdala is not simply to associate stimulus with reward (or negative reward). Rather, it is involved in associating a stimulus with the value of the reward. This expectation arises from the observations that lesions of the amygdala lead to insensitivity to reward devaluation. While the hippocampus processes contextual information associated with drug use, the amygdala has been implicated in adding and updating the value of cues associated with reward. The amygdala is reciprocally connected with the orbitofrontal cortex (OFC) and works closely with it to assign value to S–O associations. Working together these regions update S–O values in the face of changes in the value of the outcomes. Once the updating is complete, the value of the expected reward outcome is stored in the OFC. Hence the amygdala–OFC axis may be pivotal in the addiction process. In chronic nicotine use, the positive effects of nicotine substantially decrease, changing the value of the outcome of smoking. A question of future exploration is whether nicotine addiction...
or addiction in general involves impairment in the amygdala–OFC-dependent value updating process.

**Nicotine-induced long-term changes in synaptic potentiation**

Smoking continues for years in addicted smokers. This results in long lasting modifications in the nervous system, ranging from changes in receptor subtype composition and expression levels to systems level adaptations.2,13 These changes contribute to the continued smoking. From the perspective of learning, another important issue is which areas of the brain undergo elaborate changes orchestrated by chronic nicotine that lead to smoking addiction. Although many areas of the brain are affected, there is accumulating evidence that chronic use of cocaine leads to habit learning in the dorsal striatum.174 Future studies are needed to address the extent of habit learning driven in the dorsal striatum by nicotine. Finally, many studies have started exploring the role of the insular cortex in nicotine dependence.175–178

Apart from being an addictive substance on its own, nicotine upon continued use primes the nervous system for addiction to other substances of abuse such as cocaine and alcohol.179–181 In support for this gateway role of nicotine, a recent mouse study182 showed that a single low dose of cocaine or a 7-day chronic oral nicotine administration did not enhance LTP. However, the same single dose of cocaine when given after the 7-day chronic nicotine exposure increased LTP substantially as measured using in vitro brain slices. Interestingly, this effect was also dependent on D1/D5 DA receptor activation as seen for nicotine-induced LTP in the hippocampus.117,183 Similar priming effects of nicotine in LTP enhancement by cocaine have been shown for the striatum184 and amygdala.185 Also, pre-exposure to nicotine increased alcohol SA, while alcohol-induced DA responses measured in the NAc shell decreased.186 The blunted DA response was due to increased inhibitory synaptic transmission onto DA neurons. Inhibiting stress hormone receptors prior to nicotine exposure prevented the decreased alcohol-induced DA responses and the increased alcohol SA. Those results indicated that pre-exposure to nicotine recruited neuroendocrine systems to influence neurotransmission and behavior associated with alcohol reinforcement.186 It remains to be seen whether these gateway effects of nicotine are widely generalizable to other drugs of addiction.

**Summary**

Nicotine addiction, still a growing health problem, is the leading cause of preventable death in developed countries.10,14,91,187,188 Many of its addictive influences can be understood within the framework of reinforcement learning. Nicotine orchestrates changes in key learning-related structures such as the VTA, NAc, hippocampus, and PFC. However, there are many psychopharmacological factors that are involved in nicotine addiction. For example, nicotine is known to alter the release of virtually all major neurotransmitters.12,189–194 In addition, due to the widespread expression of nAChRs throughout the nervous system, structures outside the traditional reward learning pathways are likely involved in nicotine addiction. Recent studies point out yet another way nicotine could affect cellular function: in its uncharged form, nicotine can pass through the lipid bilayer without the need to bind to nAChRs, and then alter intracellular signaling of targeted cells.9 The importance of understanding and preventing nicotine addiction is further underscored by nicotine’s ability to act as a gateway drug.

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**Conflicts of interest**

The authors declare no conflicts of interest.

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