Historical and current perspective on tobacco use and nicotine addiction

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Although the addictive influence of tobacco was recognized very early, the modern concepts of nicotine addiction have relied on knowledge of cholinergic neurotransmission and nicotinic acetylcholine receptors (nAChRs). The discovery of the ‘receptive substance’ by Langley, that would turn out to be nAChRs, and ‘Vagusstoff’ (acetylcholine) by Loewi, coincided with an exciting time when the concept of chemical synaptic transmission was being formulated. More recently, the application of more powerful techniques and the study of animal models that replicate key features of nicotine dependence have led to important advancements in our understanding of molecular, cellular and systems mechanisms of nicotine addiction. In this review, we present a historical perspective and overview of the research that has led to our present understanding of nicotine addiction.

Introduction

Tobacco use spread around the world from its origins in the Americas (Box 1). Early anecdotal accounts of tobacco’s captivating influence and of associated health consequences inspired research into its addictive properties. However, our present molecular and cellular concepts of nicotine addiction (see Glossary) arising from tobacco use are relatively recent. The specific investigation of nicotine addiction arose from a long history of basic cholinergic research. It is valuable, captivating and, for some of us, even romantic to look back at the early work that laid the foundation for our present understanding of nicotine addiction. The early discovery of autonomic cholinergic transmission and nicotinic acetylcholine receptors (nAChRs) spurred physiological characterization of the neuromuscular junction (NMJ). Those studies served as guideposts, or they may more appropriately be called points-of-reference, for the more recent investigations that revealed surprisingly different and diverse mechanisms of nicotinic cholinergic transmission in the brain. Most commonly, humans self-administer nicotine using an exquisite dosing device, the cigarette. Acting directly and mainly through nAChRs, nicotine impinges upon neural circuitry that shapes short-term and long-term behavior. This review provides a compact summary of tobacco use and the scientific advances that led to the modern research into nicotine addiction. We hope this brief article and the referenced review publications and books will inspire both novices and experts to explore these fascinating historical events further.

Brief history of nicotinic cholinergic signaling

As tobacco use spread around the world (Box 1), interest in the active ingredients and their effects stimulated scientific investigation. Early in the 20th century, nicotine had been synthesized and the classic studies of drug action by John Newport Langley and his colleagues had begun [1]. These initial efforts with nicotine culminated in the 1905 study in which Langley refers to the ‘receptive substance’ that would eventually turn out to be nAChRs [2].

As the concepts of specific receptors, drug pharmacology and synaptic transmission were being formulated and advanced (Figure 1), Otto Loewi published the results of his most famous experiment, indicating the importance of neurotransmitters in chemical neural communication [3] (Figure 2). Two frog hearts were separately bathed in saline solutions, and the vagus nerve was stimulated to slow the beat of one frog heart. When solution was taken from that bath and applied to the second bath, it slowed the beat of the second frog heart. This ‘Vagusstoff’, as Loewi termed it, was identified by Henry Dale and his colleagues.
**Box 1. Brief history of tobacco use**

As we know, tobacco originated in temperate climates of America. The native populations chewed and smoked the leaves, and they spread the plant throughout the Americas [44,46]. By the time of Columbus’s landing on San Salvador in 1492, tobacco had reached across the continent and nearby islands, and the leaves had become a form of barter. During the 1500s, tobacco use began to spread as Spanish, Portuguese and later English sailors introduced its use at ports [Borio, G., 2007, The Tobacco Timeline; http://www.tobacco.org/resources/history/Tobacco_History.html]. Early on, court physicians studied and fostered the plant. Jean Nicot de Villemain, the French ambassador to Lisbon, learned about the ‘medicinal’ properties of tobacco, and introduced the plant to the French court. Eventually his name was used for the plant that has become the most widely cultivated tobacco, *Nicotiana tabacum* (Figure 1), and for the addictive alkaloid nicotine [44].

In 1612, John Rolfe began cultivating tobacco in Jamestown, the earliest successful English settlement in present day USA. Within a few years, he married Pocahontas, the daughter of a Native American chief, and their relationship was later romanticized into American folklore. Very rapidly, tobacco became the primary cash crop and even a currency of the colonies and the early United States. In 1880, James Bonsack invented the cigarette-rolling machine, and commercial cigarettes became widespread. By the beginning of the 1900s, billions of cigarettes and cigars were sold yearly.

Throughout the early 20th century, cigarette use grew, scientific advances accelerated and resistance to smoking began to grow. In 1950, epidemiological studies linked smoking to lung cancer and other diseases, and thereby extensively publicized the hazards of tobacco [45,46]. Shortly after, Ernst Wynder and colleagues induced tumors in mice by painting cigarette tar onto their exposed skin [151]. Those epidemiological and experimental studies spurred other research that led to the general scientific and government acceptance of a causal link between cigarette smoking and ill health. In 1965, when the US Congress passed the Cigarette Labeling and Advertising Act requiring a warning label on every pack of cigarettes, 42% of adults in the United States smoked [152]. Smoking rates have generally fallen in developed countries, and in 2006 about 21% of adults in the USA smoked [Rock, V.J. et al., 2006, Cigarette Smoking Among Adults - United States; http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5644a2.htm]. Demographic estimates for the early 2000s indicated, however, that there were more than 1.2 billion smokers worldwide, and 80% were in less developed countries. Presently, 5.4 million deaths worldwide are attributed to tobacco use, accounting for about 9% of deaths globally [153,154].

The mechanistic and quantitative analysis of nicotinic synaptic transmission began at the NMJ and was catalyzed forward by the works of John Eccles, Bernard Katz and Stephen Kuffler [5]. As the concepts of nicotinic cholinergic quantal release and postsynaptic response were being delineated [6,7], biochemical and structural characterization of the nAChR was aided by the extremely dense expression of muscle-like receptors in the Torpedo electric organ and by the high affinity binding of the snake toxin, α-bungarotoxin [8]. The kinetics of the muscle nAChRs were finally examined in detail using single-channel electrophysiological approaches [9,10]. The sequences of the Torpedo and the muscle nAChRs were revealed as the subunits were cloned [11]. The structure of nAChRs was progressively delineated [8,12] (Figure 3), leading to an understanding of ACh binding and gating that was critically advanced owing to images from two-dimensional nAChR crystals [13,14] and from the crystal structure of the molluscan ACh binding protein [15].

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**Figure 1.** An historical pathway and representative contributors to our present use and understanding of tobacco. 1490s: At their first encounter with Native Americans in 1492, Christopher Columbus and his crew of Spanish sailors were given merchandise, including dried tobacco leaves that they discarded because they had no use for them. Later, members of Columbus’s crew were again given the highly prized leaves, and they were shown the process of smoking. 1560s: As France’s ambassador to Portugal, Jean Nicot sent tobacco products to the French court as a potential medicinal treatment in 1561. The tobacco plant, *Nicotiana*, and the addictive substance, nicotine, derived their names from him. Early 1900s: In the early 1900s, Henry Dale isolated acetylcholine and demonstrated that it was the parasympathetic neurotransmitter that Otto Loewi had called ‘Vagusstoff’. Mid-1900s: In the 1950s, Ernst Wynder contributed to an epidemiological study linking smoking to cancer [45]. Wynder and colleagues also painted cigarette tar onto the skin of mice during the first study using tobacco to induce cancer in a laboratory setting [151]. Later 1900s: Bridging from the 1960s to the present, Jean-Pierre Changeux has made important contributions to our understanding of the molecular and cellular properties and roles of nicotinic acetylcholine receptors (nAChRs). Using genetically engineered mouse models, he and his colleagues also contributed detailed information about the roles of specific nAChR subtypes in the mesolimbic dopamine system during the nicotine addiction process.

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**European’s first encounter with tobacco**

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**Figure credit:** iStockphoto.com.
Although behavioral effects of nicotine had been recognized and described hundreds of years earlier, the physiological roles and importance of neuronal nAChRs in the brain were not widely appreciated until as recently as the early 1980s. Cloning of neuronal nAChR subunits [16] and their identification throughout the central nervous system (CNS) by in situ hybridization [17] generated more attention to nAChRs within the brain. Neuronal nAChRs were found to be much more diverse than the several kinds of muscle nAChRs. Neuronal subunits formed heteropentameric nAChRs in \(a\beta\) combinations of \(\alpha(2–6)\) and \(\beta(2–4)\). Some subunits \((\alpha7–9)\) were also found to form homomeric nAChRs, and \(\alpha10\) formed a heteromeric channel with \(\alpha9\) subunits [8,18,19] (Figure 3). Consequently, the number and diversity of neuronal nAChRs were remarkably large.

The functional progress began by characterizing nAChR currents from neuronal cells mainly cultured in vitro and later using in vitro brain slices [8,18,20–25]. The modality of nicotinic synaptic transmission in the brain differs significantly from that in the peripheral and the central nervous systems. Muscle and ganglionic nAChRs commonly mediate fast direct nicotinic synaptic transmission at the NMJ and autonomic ganglia (Figure 4a). In the mammalian brain, however, sparse diffuse cholinergic innervation more commonly provides a slower signaling that arises as ACh diffuses away from the immediate release site [25,26]. This type of neurotransmitter signaling is often called volume transmission as a convenient short hand.

Nicotinic receptors operate in the brain using a wide variety of mechanisms. The most well studied process is modulation of neurotransmitter release by presynaptic nAChRs [22,23,25,27–29] (Figure 4b). The activity of presynaptic nAChRs initiates a direct and indirect effect.

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**Figure 2.** Didactic representation of Otto Loewi’s most famous experiment, which was published in 1921 [3]. The idea for the experiment came to him in a dream. Two beating frog hearts were isolated in containers of Ringer’s solution. The vagus nerve was stimulated in one heart (a), slowing the heart rate A. Fluid moved from container (a) and applied to the heart in container (b) slowed heart rate B. This demonstrated that a soluble substance released by the vagus nerve modulated the heart rate. He called the unknown substance ‘Vagusstoff’, and Henry Dale’s lab later identified the chemical to be acetylcholine (ACh).

**Figure 3.** Schematic representation of a generalized nicotinic acetylcholine receptor (nAChR). (a) The arrangement of a single nAChR polypeptide subunit within the plasma membrane. The four transmembrane segments (M1–M4) of a nAChR subunit are oriented within the membrane, with both the amino and carboxyl ends being located extracellularly. (b) Many nAChRs are constructed from \(\alpha\) and \(\beta\) subunits, with the most common nAChR subtype being the \(\alpha4\beta2\) nAChR in the mammalian brain. The \(\alpha7\) subunit forms the most common homomeric nAChR in the mammalian brain. More complex subunit combinations are possible, with more than one \(\alpha\) and/or more than one \(\beta\) subunit combining to form other nAChR subtypes. Looking down on the receptor from above, the central water-filled cation-conducting pore is represented by the white circle in the center of the subunits. (c) A side cut-away view of the nAChR showing the subunits arranged around the central pore that passes through the membrane. There are three main conformational states of the nAChR: closed pore at rest, open pore with two acetylcholine (ACh) molecules bound to the agonist binding sites, and closed pore in the desensitized state with two ACh molecules bound.
intracellular calcium signal that enhances neurotransmitter release [22,30–36]. Properly localized calcium signals mediated by nAChRs initiate intracellular signaling that is known to modify synaptic transmission [37,38]. Furthermore, properly timed presynaptic nAChR activity, arriving just before electrical stimulation of glutamatergic afferents, boosts the release of glutamate and enhances the induction of long-term synaptic potentiation [39,40].

Nicotinic receptors also have different patterns and levels of expression at preterminal, axonal, dendritic and somatic locations where they modulate excitability and neurotransmitter release [18,24,25,41,42]. Preterminal and axonal nAChRs located before the presynaptic terminal indirectly affect neurotransmitter release by activating voltage-gated channels and initiating local action potentials [41,43] (Figure 4c). Somatodendritic receptors initiate or modulate synaptic inputs to the soma, and thereby modulate plasticity and information flow. Postsynaptic nAChRs also contribute to the depolarization and intracellular calcium signal normally assigned to glutamatergic synapses [39,40]. Because ACh spreads from synaptic and possibly non-synaptic release sites [26], nAChRs at non-synaptic locations influence not only the excitability of neurons but also their overall electrical and chemical responsiveness [25].

**Brief history and scientific advances of nicotine as an addictive drug**

Although the modern concepts of tobacco or nicotine addiction are relatively recent, the captivating influence of tobacco was known by Native Americans and was quickly documented by Europeans [44]. For example, Francis Bacon, an English philosopher, statesman and Renaissance scientist, observed the spread of tobacco and wrote:

‘In our time the use of tobacco is growing greatly and conquers men with a certain secret pleasure, so that those who have once become accustomed thereto can later hardly be restrained therefrom.’ (Francis Bacon, *Historia vitae et mortis* (1623), quoted by others [44]).

Scientific progress indicating that tobacco smoke is addictive post-dates the first evidence in the early 1950s that smoking was associated with a substantially increased incidence of lung cancer [45,46]. Initially, tobacco smoking was seen as a habit, but by 1971 researchers were beginning to recognize that many smokers were addicted to nicotine present in tobacco smoke [47,48]. Much of the work focused on the evidence that chronic or repeated exposure to nicotine results in a withdrawal effect if the drug is precipitously withheld. By the 1980s, the validity of the nicotine dependence hypothesis was becoming more widely accepted, and the concept of using nicotine replacement therapy to aid successful cessation attempts had moved to the fore [49–51]. This period also saw the development of tests that could measure the degree of tobacco dependence. The best known is the Fagerstrom Test of Nicotine Tolerance and Dependence [52,53], which remains the standard measure of tobacco dependence. This test allowed researchers to evaluate anti-smoking treatments and, then, to relate the cessation outcomes to the ways in which people smoked and their level of dependence.

**Intracranial self-stimulation and nicotine self-administration**

The idea that nicotine caused dependence also influenced work with animal models. Although some early animal studies on nicotine pre-dated the wide acceptance of nicotine dependence in humans [54,55], the results with humans largely spurred research with animal models. Dependence upon nicotine is characterized by compulsive drug-seeking and drug-taking behavior. The psychological and neurobiological factors that underlie drug taking and...
Nicotine self-administration was difficult to examined experimentally using animals trained to self-administer small intravenous doses of nicotine. However, the seminal studies of Corrigall and colleagues [60] were the first to describe a reliable methodology for studying nicotine intravenous self-administration in rats. This methodology was a pivotal development because it allowed researchers to investigate both the psychological and neurobiological substrates that influence nicotine reinforcement. Subsequent studies showed that nicotine reinforcement in this self-administration model depends upon the stimulation of the mesocorticolumbic dopamine (DA) neurons [61,62]. Since the earliest studies of intracranial electrical self-stimulation (Figure 5c), cortical and limbic structures of the brain have been identified as mediating reward. In particular, the mesocorticolumbic DA system plays an important role in intracranial self-stimulation, in drug self-administration and in processing environmental reward.

The landmark studies of the brain reward circuitry predated the initial nicotine self-administration advances, but those fields of reward circuitry and drug addiction later converged [63]. In the 1950s, James Olds and his colleagues showed that particular neural nuclei and fiber tracts supported intracranial electrical self-stimulation [64,65] (Figure 5c). Rats worked to the exclusion of other goals to self-administer intracranial electrical stimulation when delivered to ‘rewarding’ regions of the brain [66–68]. In this same time period, Arvid Carlsson and his colleagues showed that DA was a neurotransmitter [69], and midbrain DA systems had an important role in brain self-stimulation [70–72]. Self-stimulation of the medial forebrain bundle facilitated DA release, and DA receptor antagonists or DA neuron lesions inhibited brain self-stimulation [70,73,74]. The DA efferents originating from the midbrain ventral tegmental area (VTA) and targeting the prefrontal cortex and nucleus accumbens (NAc) of the ventral striatum became recognized as paramount neural structures shaping reward-related behavior [75–78]. These mesocorticolumbic DA pathways became the major focus of research into reward-motivated behavior and drug addiction.

**Mesocorticolumbic dopamine system**

The studies of brain reward circuitry and mesocorticolumbic DA signaling preceded the demonstration that nicotine self-administration depended upon the stimulation of DA neurons [61,62]. Nicotine injection into the whole animal induced an increase in DA neuron firing (as measured by *in vitro* and *in vivo* electrophysiological recordings) [79,80], and elevated DA concentrations particularly in the NAc (as measured by *in vivo* microdialysis) [81]. The increased DA release in the NAc shell was thought to mediate the reinforcing properties of the drug, and this process drove the acquisition and maintenance of responding for the primary reinforce [82,83]. Subsequent studies suggested that the DA projections to the shell and core subdivisions of the NAc mediated differential and complementary components of nicotine dependence [81–88].

Recent studies showed that long-lasting DA neuron activity was driven by nicotine-induced synaptic potentiation of excitatory glutamatergic afferents onto DA neurons [25,89]. Nicotine has multiple actions upon synaptic events and circuitry within the midbrain DA centers. By activating presynaptic nAChRs (Figure 4b), nicotine boosts glutamate release, and by activating postsynaptic nAChRs at
glutamate synapses, nicotine boosts the postsynaptic depolarization and calcium signal mediated by glutamate receptors. Both of these actions enhance the likelihood of initiating synaptic potentiation that increases DA neuron activity. In addition to its direct effects on midbrain DA neurons, nicotine also alters DA signaling in the NAc itself [90–94]. Taken together, these results implicate the mesocorticolimbic DA system and indicate that nicotine has behavioral and neurobiological properties that are like those of other psychostimulant addictive drugs [95–97].

Withdrawal from nicotine
Much of the early work on nicotine addiction in animals focused on measures of withdrawal to assess the level of dependence. The work of David Malin and his colleagues was seminal in this regard. They were the first to describe a nicotine abstinence syndrome in rats, which they argued models important components of the abstinence syndrome experienced by many smokers when they first quit [98]. More recent experimental studies have employed a withdrawal model in which rats or mice are constantly infused with nicotine for a week or more before the drug is precipitously withdrawn or antagonized by the administration of a nAChR antagonist. The abstinence syndrome evoked in this model is attenuated by the re-administration of nicotine (which is comparable to nicotine replacement therapy in humans). The abstinence syndrome is also attenuated by pharmacotherapies (e.g., the atypical antidepressant, bupropion) that treat tobacco dependence in humans [99]. Some behavioral consequences of nicotine withdrawal in this paradigm are mediated by the periphery, but there also is a CNS component [100–102]. For example, CNS withdrawal effects are accompanied by decreased brain reward function that is estimated by measuring the threshold for intracranial self-stimulation of brain reward pathways [103] (Figure 5c). Stimuli or drugs, such as nicotine, reduce the threshold current for self-stimulation. By contrast, nicotine withdrawal evokes a robust increase in the threshold current, which is thought to reflect anhedonia (a reduced ability to respond to pleasurable stimuli), a core symptom of tobacco withdrawal [103]. These methodologies continue to be used in the present day as a measure of the somatic and central symptoms of nicotine withdrawal [102].

The anhedonia associated with nicotine withdrawal has been associated with reduced extracellular DA in the NAc shell [104]. A number of brain regions that innervate the NAc and VTA have been implicated in the expression of drug withdrawal [105]. Recent studies have begun to focus on the neurons that project to the VTA from the lateral habenula. Those projections can inhibit mesoaccumbens DA neurons, providing a source for negative reward [106,107]. In addition, the medial habenula is particularly rich in α3 and β4 subunits that are sometimes expressed in combination with the less common α5 subunit. In addition, a direct target of the medial habenula, the interpeduncular nucleus, expresses α2 subunits. All of these subunits contribute to nAChRs that have been implicated in the expression of the somatic symptoms of the nicotine withdrawal syndrome [108,109]. These nAChR subtypes are also implicated in the aversive effects (negative reward) experienced at higher doses of nicotine [110]. Their action in the medial habenula and interpeduncular nucleus contribute to the decrease in nicotine self-administration seen at higher nicotine doses. Thus, receptors comprised of α2, α3, α5 and β4 subunits contribute to the falling arm of the inverted U dose-response curve for nicotine self-administration (Figure 5b).

Up-regulation of nAChRs
Another important advance toward understanding the effects of chronic nicotine came in the early to mid 1980s. After prolonged exposure to nicotine there is an ‘up-regulation’ of nicotine binding sites. Of the many drug-induced neuroadaptations caused by chronic nicotine, this up-regulation is the most widely appreciated, but its mechanistic importance is still actively under investigation. The nAChR up-regulation is observed in rodent animal models [111,112] and in postmortem tissue from the brains of smokers as increased radiolabeled nicotine binding [113–115]. The results suggest that prolonged exposure to a smoker’s concentration of nicotine increases the binding and possibly the number of excitable nAChRs. However, the up-regulation is not uniform; there is variation in the up-regulation across locations of the brain and among the nAChR subtypes. For example, the presynaptic regulation of catecholamine release appears to be enhanced by nAChR up-regulation, and this action may enhance the responses to a subsequent challenge with nicotine [116]. It also is hypothesized that nAChR up-regulation contributes to the development of sensitization: as more nAChRs become available to respond to the same nicotine dose, less nicotine is needed to cause the same effect. By contrast, continuous exposure to nicotine caused by smoking throughout the day produces desensitization (Figure 3c) of particular nAChR subtypes, which may contribute to acute forms of tolerance. That is, more nicotine is needed to achieve the same effects because many nAChRs are desensitized and unable to respond, owing to maintained low concentrations of nicotine. During longer periods of smoking abstinence, such as overnight or during attempts to quit, when nicotine is not present, the up-regulated nAChRs recover from desensitization. Then, reactivation of an increased excitable population of nAChRs may play an early role in the expression of the nicotine abstinence syndrome [117–119].

Tobacco-associated environmental cues and learning
Although the addictive properties of tobacco smoke clearly depend upon the presence of nicotine, the powerful addictive properties of tobacco reflect a complex interaction between the drug and the context in which it is delivered. When compared with other addictive drugs, nicotine alone in animal models does not seem to be as powerfully addictive as tobacco experienced by many smokers. Increasingly, researchers have begun to consider the role of the tobacco smoke vehicle in which nicotine is most commonly delivered. An early study showed that the satisfaction experienced by smokers who inhale tobacco smoke is substantially diminished if the upper airways are anaesthetized with a local anesthetic [120]. Rose and colleagues [120] concluded that the sensory components of tobacco
smoke contribute to the satisfaction experienced by the smoker. In this case, the drug-associated sensory cues become conditioned (learned) reinforcers associated with smoking tobacco.

As the addiction process progresses, neural plasticity and neuroadaptations throughout the brain are influenced by the drug experience. Environmental stimuli become conditioned cues because they are paired with the unconditioned rewards arising from the nicotine dose delivered by tobacco use [121–124]. For example, rodent studies have shown that pairing a sensory cue (usually a tone or light) with delivery of the drug in a self-administration paradigm enhances the reinforcing properties of nicotine [125]. By repeated association with nicotine, the cue acquires reinforcing properties in its own right [125,126].

In a similar manner, memories associated with addictive behaviors become internal motivational drives to continue drug use [25,127–129]. Learned associations arise as nicotine acts locally upon memory-related circuits [130] while also inducing DA release from midbrain centers [124]. The cellular mechanisms underlying these system-level effects arise in part from the ability of nicotine to alter local GABAergic inhibition, thereby enhancing synaptic mechanisms that underlie systems-level learning [124,130]. The results indicate that nicotine induces a DA signal that serves to increase the probability and strength of synaptic potentiation that underlies drug-associated learning.

Because nicotine acts throughout the brain and influences synaptic mechanisms that normally mediate the neural plasticity of learning, cues associated with smoking come to elicit neuronal activity in regions linked to attention, memory, emotion and motivation [131]. Consequently, nicotine-associated cues reinstate extinguished nicotine-seeking behavior, which contributes to relapse [132,133]. Inhibition of DA receptors significantly attenuates the magnitude of cue-elicited reinstatement of nicotine-reinforced behavior [134], and that result is consistent with a DA contribution during the associative learning linked to drugs of abuse [124].

In addition, other components of tobacco smoke may influence the addictive properties of nicotine by interacting with the neural responses to the drug. Although not addictive on their own, monoamine oxidase inhibitors, which slow the breakdown of monoamines such as DA, may affect the overall motivational impact of nicotine [135]. Although it has been consistently established that nicotine has the behavioral and neurobiological properties of an addictive drug, environmental associations and components of the tobacco smoke vehicle play a pivotal role in the severity of the dependence developed by some smokers.

Concluding remarks
The decreasing cost of sequencing the human genome has brought human genetic diversity into the forefront of mental health and addiction research. Genome-wide association analysis of single nucleotide polymorphisms (SNPs) has already linked nAChRs to tobacco use and health problems. For example, the CHRNA5-CHRNA3-CHRNB4 gene cluster, which encodes the α5, α3 and β4 nAChR subunits, has been implicated in various aspects of nicotine dependence and cigarette-related health issues [136]. Individuals homozygous for the less common SNP, rs16969968, of the CHRNA5 gene are more likely to be heavy smokers and nicotine dependent [137,138]. Future efforts will expand the genetic links to various aspects of smoking, such as the initiation of use, the difficulty in quitting and the risk for relapse. This research will indicate mechanistic pathways for more detailed animal studies that will ultimately indicate further targets for therapeutic interventions to aid smoking cessation.

The future holds more powerful experimental tools. New approaches are continually being added (Box 2) to the arsenal of methodologies used to study cholinergic mechanisms and addiction. One such advance toward understanding the roles of nAChR subtypes within the addiction process is the creation of genetically-engineered mutant mice lacking specific subunits or containing gain-of-function subunits [8,139–142]. Studies with such mouse models have shown that β2-containing (β2*) nAChRs mediate much of the reinforcing influence of nicotine [143,144], and those receptors potently regulate DA release in the NAc [92,145]. Mutant knockout mouse models also helped to show that nAChRs containing the α5, α3, α2 and β4 subunits are implicated in the somatic manifestations of nicotine withdrawal [108,109,146]. The next level of sophistication is rapidly entering the nicotinic field. Conditional knockout models and re-expression of a subunit on the null background have demonstrated the important nAChR subunit and its neural location for tasks involving reinforcement and cognition [147], passive avoidance [148], locomotion [144] and nicotine dosing [110]. Another significant advance is the ability to control the activity of specific neurons by introducing light-activated molecules (i.e. optogenetics). For example, channelrhodopsin-2, which was introduced into ‘cholinergic’ habenula

Box 2. Rapid progress in nicotine addiction studies

The general field of addiction and the specific field of nicotine addiction arising from tobacco use have seen transformational advances in recent times. Like many areas of scientific research, the progress seems to be accelerating. Behavioral advances are being linked to underlying mechanisms, providing targets for therapeutic and prophylactic developments. Increasingly powerful techniques and experimental approaches enable scientists to address questions that were unapproachable only a decade ago. The excitement, energy and progress seem unique to our time, but our impressions were expressed well by Henry Dale more than 50 years ago. Dale, who identified ACh, when in his eighties, wrote to his friend Thomas R. Elliott, who also had made seminal contributions toward understanding synaptic transmission [4]. An excerpt from Dale’s 1958 letter expresses the dizzying rate of scientific advances he observed when visiting Bernard Katz’s lab while Katz was in his astonishing prime:

‘I feel almost bewildered by the kind of detail which such people are now elucidating with the aid of electron-ultramicroscopy, and also with an electrical recording which they can now achieve of the transmitted excitatory process at the motor end-plate of a single muscle fibre...I find it really exciting to think of the contrast between physiology as we had it from Langley and Gaskell, and what it is becoming today. A great deal indeed has happened since you first suggested a chemical mechanism for the transmission of the excitatory process from a nerve ending; and it goes on happening with a constant acceleration.’ (Letter from Dale to Elliott, 29 June 1958, Royal Society Archives, quoted by Tansey [4].)
neurons, was recently used to show that glutamate was co-released with acetylcholine [149]. Yet another ever-expanding capability is the increased power of in vivo recordings from freely-moving animals [150]. Dozens of neurons can be separately recorded from multiple areas of the brain while an animal performs a behavioral task.

In the future, the convergence of these powerful approaches will provide unprecedented data. Molecular, cellular, systems and behavioral approaches will be combined to study the various animals models created as discussed above, as well as those models based on human genetic association studies. As interesting and exciting as the past has been, the future promises even more innovation and surprises (Box 2).

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