Published in final edited form as:

Semin Cell Dev Biol. 2009 June ; 20(4): 418–431. doi:10.1016/j.semcdb.2009.01.001.

Cellular Events in Nicotine Addiction

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Abstract

In the twenty-five years since the observation that chronic exposure to nicotine could regulate the number and function of high affinity nicotine binding sites in the brain there has been a major effort to link alterations in nicotinic acetylcholine receptors (nAChRs) to nicotine-induced behaviors that drive the addiction to tobacco products. Here we review the proposed roles of various nAChR subtypes in the addiction process, with emphasis on how they are regulated by nicotine and the implications for understanding the cellular neurobiology of addiction to this drug.

Keywords

Upregulation; desensitization; tolerance; ion channel; plasticity

1. Introduction

The tobacco plant alkaloid, nicotine, is generally agreed to be the major, if not sole, compound responsible for driving the addiction of more than one billion people ($\approx 20\%$ world population), which, in turn, results in five million deaths worldwide each year [1]. The unaided quitting rate for smokers is 3–5% [2,3] and, despite the availability of several nicotine replacement therapies [4], only about one third of people that would like to stop using tobacco products are permanently successful by the age of 60, usually after multiple failed attempts [reviewed in 5]. After nicotine enters the body, it binds to nicotinic acetylcholine receptors (nAChRs) of the central nervous system (CNS), specifically those in the brain, and initiates drug addiction [6, 7]. The persistent interaction between nicotine and nAChRs must ultimately lead to downstream plasticity at the molecular, cellular and circuit levels that then results in the behavioral desire to continue to intake nicotine.

Transmembrane nAChRs are fast-activating ligand-gated ion channels that produce membrane depolarization and cellular excitation [8]. While much is known about the physiological role of nAChRs in the peripheral nervous system (PNS), the relevance of these receptors in CNS signaling has been somewhat obscure [for a concise historical perspective see 9]. In the PNS, it is now firmly established that the major role of nAChRs present postsynaptically at both the neuromuscular junction (NMJ) and within the autonomic ganglia, is to faithfully detect the presence of the chemical neurotransmitter, acetylcholine (ACh), thereby enabling efficient synaptic signaling. The synapses at the NMJ are designed never to fail and vertebrates would

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no doubt not have survived long if they were less than 100 % reliable [see discussion in 10, 11]. Exceptions to this generalization occur during development and with disease, e.g. myasthenia gravis, and usually result in significant changes in postsynaptic nAChR number [12,13]. Autonomic ganglion synapses may be more pliable than the NMJ, as indicated by their ability to support activity dependent long-term changes in synaptic transmission [14]. Mechanisms underlying the plasticity of nAChRs in the periphery may provide useful clues for understanding the changes in CNS receptors following chronic exposure to nicotine, particularly those in the autonomic ganglia, which share an overlapping neuronal subtype.

In the CNS the situation is more elusive, with only a few clear examples of fast synaptic transmission involving nAChRs, despite the widespread expression of these receptors and innervation by cholinergic fibers [9,15,16]. Coupled with a lack of anatomically defined cholinergic synapses, this has led to the postulation that nAChRs may function in part through more diffuse signaling, perhaps contributing to “volume” transmission –with receptors detecting ambient levels of ACh [17–19]. Although there is a somewhat limited knowledge of the operation of nicotinic synapses in the CNS, it is well established that nAChRs can contribute to long-lasting neuronal plasticity – including changes induced by the exogenous drug nicotine. Importantly, such plasticity likely helps condition the brain to secondary drug-related cues and/or context that make successful withdrawal from drugs like nicotine extremely difficult [20, 21], lending support to the idea that chemical addiction is a form of associative learning [22]. Indeed, it is now appreciated that changes in synaptic efficacy as well as downstream gene regulation may provide a common molecular and cellular basis for both normal learning and addiction [23]. The effects of nicotine on synaptic transmission and plasticity have been discussed elsewhere [24–26] and here we will focus on the mechanisms that lead to nicotine-induced alterations in nAChR number and function, changes in other classes of proteins, and their relationships to long-lasting nicotine dependence.

2. nAChRs in the brain

The diversity of nAChR subtypes, both in terms of their regional and subcellular distribution, implies that specific receptors may be localized to control cellular events that ultimately underlie a variety of discrete behaviors [16,27–29]. Thus, in order to understand how nicotine-induced plasticity contributes to the long-term disruptions of neuronal activity in the CNS that underlie the behavioral adaptations acquired during nicotine exposure and withdrawal, it is necessary to know how these receptors operate and what specific properties, based on their subunit composition, allow them to interact with the low concentrations of nicotine in the cerebrospinal fluid (CSF) that are associated with the use of tobacco products.

2.1. Composition of nAChRs in the CNS

Unlike muscle type nAChRs whose subunit composition is known and fixed, except during development [30], the molecular composition of neuronal nAChRs expressed in the nervous system is not completely defined [28,31–35]. Indeed, even armed with the knowledge that only certain subunits can co-assemble, the question of just how many different native nAChR subtypes exist is difficult to answer. The mere presence of a large number of subunits [and possibly more based on sequence homology in other species, e.g. *C. elegans*;36] has led some to conclude that diversity is the general rule rather than the exception [27,28]. Based to some extent on the non-overlapping cellular distributions of nAChR subunit mRNAs in the CNS [e.g. 37], it is likely that the number of native nAChR subtypes will be restricted by specific patterns of subunit expression. Conversely, because many central neurons express multiple genes, their potential promiscuous assembly may not always limit the number of nAChRs subtypes found on single cells – and hinder determination of receptor composition [38]. However, despite the enormity of the task, the wide range of available technologies, including high signal-to-noise ligands for radiolabeling binding sites, more recently developed selective

pharmacological tools [39,40] and genetically modified mice [28,41], have helped define which subunits are incorporated into receptors, along with their regional and in some cases subcellular distribution. It is now clear that *all* subunits can participate in at least one type of nAChR present in the brain (Table 1). Below we have highlighted the essential characteristics of the major nAChRs and provided brief descriptions of their known or potential roles in the mechanisms of nicotine addiction. Of the receptor subtypes it is the $\alpha 4\beta 2^*$ subtype which has to date served the pivotal role in nicotine addiction [42], perhaps because its relatively high affinity allows it to interact with the low levels of nicotine present in the CSF after tobacco smoke inhalation [see discussion in 43]. However, roles for other subtypes of nAChRs – especially those localized to the central reward pathways – are beginning to emerge. Nomenclature follows international standards, where an asterisk represents the inclusion of possible additional subunits [34].

2.1.1. $\alpha 4\beta 2^*$ nAChRs—Receptors containing $\alpha 4$ and $\beta 2$ subunits represent the main population of central nAChRs, and account for the majority of high affinity binding sites labeled by [^3H]nicotine [44,45]. Indeed, the ligand specificity profiles of receptors formed from heterologously expressed $\alpha 4$ and $\beta 2$ subunits are quite similar to high affinity [^3H]nicotine sites [46]. Moreover, genetic knockout of either the $\alpha 4$ or $\beta 2$ subunits –aside from a small population in the interpeduncular nucleus (IPN) – eliminated all high affinity binding sites in the brain [47,48]. However, where detailed functional comparisons of nAChRs expressed in *Xenopus* oocytes/mammalian cells have been made, no exact matches with native nAChRs were possible, implying that some $\alpha 4\beta 2^*$ receptors may incorporate additional subunits, including $\alpha 5$ [49–51]. This suggestion is echoed in data from single cell RT-PCR studies showing co-expression of $\alpha 4$, $\beta 2$ and $\alpha 5$ in cortical neurons [52]. Although originally described as having a fixed stoichiometry [53], these receptors can exist in two distinct stoichiometries – a higher affinity $(\alpha 4)_2(\beta 2)_3$ and a lower affinity $(\alpha 4)_3(\beta 2)_2$ form – both in expression systems [54] and within the brain [55]. The stoichiometry and precise subunit composition of $\alpha 4\beta 2^*$ nAChRs is likely an important factor governing their upregulation by nicotine (see section 3).

Indeed, it was upregulation of these high affinity [^3H]nicotine binding sites after chronic nicotine that began to define a central role for $\alpha 4\beta 2^*$ nAChRs in addiction [56,57] – a finding that is difficult to reconcile, at least at the level of the receptor, with the accompanying development of tolerance to nicotine [58]. Following on from the initial demonstration that animals would self-administer nicotine [6], it was shown that the $\alpha 4\beta 2^*$ receptor-preferring antagonist, dihydro- β -erythroidine (DH β E), reduced drug-seeking behavior when infused into the ventral tegmental area (VTA), but not after it had been introduced in the nucleus accumbens [59]. Because DH β E is not a pure $\alpha 4\beta 2^*$ nAChR antagonist, it was not until the development of suitable *in vivo* genetic manipulations that the involvement could be confirmed. In the first example, knockout of the $\beta 2$ gene in mice produced parallel deficits in the activation of midbrain dopaminergic neurons and the self-administration of nicotine [42], which could be reversed by a viral rescue of $\beta 2$ expression specifically in the VTA [60]. These results have now been extended to $\alpha 4$ subunits [61]. Moreover, in mice containing a hypersensitive $\alpha 4$ allele knocked-in in place of the wild-type $\alpha 4$ gene, it was possible to show that *activation* of $\alpha 4$ subunit-expressing nAChRs was sufficient to produce conditioned place-preference [62]. Although $\alpha 4$ and $\beta 2$ subunits can contribute to other receptors, e.g. those with $\alpha 6$ (see Table 1; [63]), in sum these experiments reveal a central role for $\alpha 4\beta 2^*$ nAChRs in nicotine addiction. Evidence from brain slice electrophysiological studies is beginning to unmask how this may take place at the level of synaptic integration, including studies that show how acute nicotine-induced desensitization of putative $\alpha 4\beta 2^*$ receptors on midbrain interneurons blocks their endogenous activation by acetylcholine and shifts the balance of inhibitory-excitatory input to the VTA dopaminergic cells in the direction of excitation [64,65]. Although chronic nicotine may have additional regulatory effects on these particular VTA nAChRs [66; see section 3],

these data imply that nAChR desensitization, along with receptor activation, likely contributes to aspects of nicotine addiction [67].

2.1.2. $\alpha 7^*$ nAChRs— $\alpha 7$ subunit-containing receptors provide a molecular explanation for the large number of [125 I] α -bungarotoxin (α BTX) binding sites found in the brain [68,69] and the pharmacological sensitivity of fast activating nAChRs in areas such as the hippocampus [70,71]. Although $\alpha 7$ subunits readily form homomeric receptors in *Xenopus* oocytes [but see 72], it is unclear whether they exist in this form in the brain [73]. Brain receptors purified using α BTX as a probe only contain $\alpha 7$ subunits [74,75], and mice null for the $\alpha 7$ gene have a complete loss of both α BTX binding sites in the CNS and functional α BTX-sensitive nicotinic responses in hippocampal neurons [76]. However, $\alpha 7$ can co-assemble with other α and β subunits [77] resulting in receptors present *in situ* with a lower sensitivity to α BTX [78] conceivably missed in the above α BTX purification experiments. The $\alpha 7^*$ nAChR conductance is unique amongst nAChRs; typified by very fast activation and desensitization kinetics and a high calcium permeability [68,69,71,79]. No generalized role for these receptors in central synaptic transmission can be ascribed; in the CNS they are present and function at postsynaptic [80,81] and presynaptic sites [for review see 82].

As discussed above, $\alpha 4\beta 2^*$ nAChRs have an essential role in nicotine self-administration (section 2.1.1), but recent data show that this is not likely to be sufficient to account for all aspects of addiction to nicotine. Some of the most convincing evidence that other nAChR subtypes are involved comes from further analysis of $\beta 2$ knockout mice [83]. In these animals, nicotine can act through methyllycaconitine (MLA)-sensitive $\alpha 7^*$ nAChRs to normalize aberrant neurochemical and behavioral deficits. Changeux and colleagues conclude that both $\alpha 4\beta 2^*$ and $\alpha 7^*$ receptors are necessary to produce the homeostatic neuroadaptations that develop in the presence of nicotine [83]. However, with respect to a direct role in establishing drug-seeking behavior, it appears that $\alpha 7$ subunit-containing receptors are not required [61]. Using both pharmacological and genetic approaches it was concluded that $\alpha 7^*$ nAChRs do not support conditioned place preference [84], although high doses of MLA were shown to reduce nicotine self-administration [85], implying a possible $\alpha 7^*$ nAChR contribution to the reinforcing properties of nicotine. Precipitation of somatic withdrawal symptoms using the non-specific blocker, mecamylamine, was attenuated in $\alpha 7$ knockout mice [86; but see 85], however, MLA-induced withdrawal was unaffected in the absence of the $\alpha 7$ gene, urging caution and implying that non- $\alpha 7$ MLA-sensitive nAChRs may be also involved in withdrawal from nicotine [86].

At the synaptic level, $\alpha 7^*$ nAChR receptors may contribute to plasticity of glutamate synapses, including those impinging onto dopaminergic neurons in the VTA. Here nicotine triggers release of glutamate, which under the appropriate conditions can increase synapse efficacy for prolonged periods of time, both *in vitro* [87] and *in vivo* [88]. These persistent effects of $\alpha 7^*$ nAChR stimulation may be representative of a more generalized cognitive role for this receptor subtype [89] – especially given the findings of $\alpha 7^*$ receptor-induced synaptic plasticity in the hippocampus [90]. Moreover, its potential role in information processing is highlighted in schizophrenic subjects, who smoke at four times that of the general population, possibly in part to control sensory gating dysfunction caused by an altered $\alpha 7$ gene [91].

2.1.3. $\alpha 3\beta 4^*$ nAChRs—The initial limited expression of $\beta 4$ subunits in the CNS [92] supported the idea that $\beta 4$ subunit-containing nAChRs served a dominant function only in peripheral ganglia [28]. However, closer analysis of $\beta 4$ distribution revealed that it was often associated with the $\alpha 3$ gene [93–95], and analysis in regions that co-expressed these two subunits, e.g., medial habenula (MHb), locus coeruleus, and superior colliculus, revealed receptors with functional properties and a likely minimal molecular composition including $\alpha 3$ and $\beta 4$ subunits [96]. Like $\alpha 4\beta 2^*$ nAChRs, these native receptors may also incorporate $\alpha 5$

[51,97–99], a finding that, if true, makes sense as all three subunits are present within a single gene cluster [100]. The $\beta 3$ subunit may also be included, further increasing the diversity of these nAChRs [101]. The lower affinity of these receptors for nicotine [43] prevents them from being detected using [^3H]nicotine, but the higher affinity, and relatively non-selective agonist, epibatidine, strongly labels these sites. Nicotine displaces radiolabeled [^3H] or [^{125}I]epibatidine with high and low components corresponding to the $\alpha 4\beta 2^*$ and $\alpha 3\beta 4^*$ nAChRs, respectively [102–104]. As expected, these receptors remain in the $\beta 2$ subunit knockout mouse, in which they can be further subdivided into additional classes [103,105]. Unlike its dominant role in ganglionic transmission [106,107], the synaptic function of the $\alpha 3\beta 4^*$ nAChR within the CNS has not been established.

In part due to their scarcity, little attention has been paid to the role of non- $\alpha 4\beta 2^*$ heteromeric nAChR subtypes with respect to nicotine addiction. However, two sets of independent results link the $\beta 4$ subunit to mechanisms of withdrawal from nicotine (and other drugs) and provide circumstantial support that the habenulo-interpeduncular axis may represent an additional component of the “reward center”, especially given its ability to support intracranial self-stimulation [108]. First, somatic signs of withdrawal are reduced in $\beta 4$, but not $\beta 2$, null mice [109]. Second, the natural alkaloid, ibogaine, and a synthetic derivative, 18-methoxycoronaridine, known antagonists of the $\alpha 3\beta 4^*$ receptor subtype [110], when injected into the MHb or IPN can reduce sensitization [111], self-administration [112] and withdrawal symptoms [113] to morphine. That these compounds also attenuate nicotine-conditioned place preference [114] may point to a more generalized role for habenulo-interpeduncular $\alpha 3\beta 4^*$ nAChRs in addiction.

2.1.4. $\alpha 6\beta 2\beta 3^*$ nAChRs—Although both $\beta 3$ and $\alpha 6$ subunits can be incorporated into heteromeric receptors in heterologous expression systems [101,115], the contribution of these subunits to native nAChRs in the CNS was until recently unclear. The function of these two subunits in the brain is perhaps best observed after removal of the majority of high affinity $\alpha 4$ -subunit containing binding sites: [^{125}I]epibatidine binding in the presence of cytosine (to mask high affinity nAChRs) leaves a population of sites with a similar distribution to $\alpha 3$ subunit mRNA, as predicted [103]. Some of these “ $\alpha 3$ ”-receptor sites represent $\alpha 3\beta 4^*$ nAChRs, like those in the MHb [96,105], whereas others may contain different gene members. This idea was confirmed using α -conotoxin MII (α -MII), a nicotinic antagonist toxin with originally described selectivity for $\alpha 3\beta 2$ over $\alpha 3\beta 4$ subunit-containing receptors [116], which displaced some of the remaining [^{125}I]epibatidine binding [103]. Moreover, [^{125}I] α -MII binding is lost in either $\beta 2$ [117] or $\beta 3$ [118] null mice, suggesting initially that this receptor may be comprised of $\alpha 3$, $\beta 2$ and $\beta 3$ subunits [39]. However, not least because many [^{125}I] α -MII sites are found in the $\alpha 6$ subunit-rich striatum [119,120], attention became focused on the contribution of this previously obscure subunit [see 121]. Thus, it is likely that α -MII sensitive presynaptic nAChRs on dopaminergic terminals [117,122] do not contain $\alpha 3$ subunits, but rather express an $\alpha 6\beta 2\beta 3^*$ nAChR subtype [123,124], a view supported by the lack of effect of $\alpha 3$ subunit knockout on [^{125}I] α -MII binding in most areas of the brain [125].

The release of dopamine in the striatum is controlled by presynaptically localized $\alpha 6\beta 2\beta 3^*$ (in addition to $\alpha 4\beta 2^*$) nAChRs and, because of their additionally highly restricted distribution in the reward centers of the brain, these receptors are strongly implicated in mechanisms of nicotine addiction [123,124,126,127]. Under normal conditions, the nAChRs on dopaminergic terminals are activated by endogenously released ACh from intrinsic striatal cholinergic neurons and this activity appears to be critical for the normal maintenance of dopaminergic transmission [128]. Thus, “chronic” nicotine-induced desensitization of these presynaptic nAChRs (although the precise nature of the subtype(s) affected is unknown) can produce a profound decrease in dopamine release [128] and potentially interfere with other known mechanisms of synaptic plasticity in this region [for review see 129]. Recently, it has been

established that $\alpha 6^*$ nAChRs specifically within the VTA are an absolute requirement for nicotine self-administration in mice [61], and coupled with similar data for $\alpha 4$ and $\beta 2$ subunits (see section 2.1.1), permits the suggestion that regulation of dopamine release by presynaptic $\alpha 4\alpha 6\beta 2^*$ nAChRs on VTA neuron terminals [130] may be critically important in establishing addiction to nicotine [61].

2.1.5. Other nAChR subtypes—While the above sections have discussed the most abundant subtypes present in brain it remains an incomplete list. It is worth mentioning other nAChR classes that may be more restricted in their distribution, but none-the-less have important roles. Obvious members of this group are receptors comprised of $\alpha 9$ / $\alpha 10$ subunits, found only in sensory epithelia [131,132], where they mediate transmission of efferent *inhibitory* information via release of ACh onto hair cells in the cochlea – apparent as a postsynaptic hyperpolarization triggered by activation of potassium channels via calcium flux through the nAChR channel [133,134]. Although the only remaining “homomeric” subunit, $\alpha 8$, is absent in mammals, it is likely to have important function in chick retina/brain, possibly in combination with $\alpha 7$ [135–137]. The discovery that most [125 I] α -MII binding sites represent $\alpha 6\beta 2\beta 3^*$ -nAChRs [121] has led to a decreased emphasis on $\alpha 3\beta 2^*$ receptors in the brain. However, within the habenulo-fasciculus retroflexus tract, [125 I] α -MII are almost exclusively dependent on the $\alpha 3$ subunit [125], implying that the $\alpha 3\beta 2$ subunit combination exists either alone or as one of the interfaces of an $\alpha 3\beta 4^*$ nAChR [96,104]. Neurons in the IPN have been known for some time to express a channel functionally distinct from those in the MHb [138]. Recent work suggests that this receptor is a second class of $\beta 4$ subunit-containing receptor that may include the $\alpha 2$ and/or potentially the $\alpha 4$ subunit [35,105]; $\alpha 2$ subunits exist almost exclusively in the IPN [37,139]. Essentially nothing is known about the functional role of this highly restricted receptor subtype. Other mixed interface nAChRs may exist with up to four distinct subunits [140].

2.1.6. nAChR mutations and smoking behavior—As discussed in the preceding sections, pharmacological and genetic manipulations indicate that specific nAChR subtypes/subunits are likely to be involved in various aspects of addiction to nicotine. Thus, it is highly likely that individual differences in nAChR alleles may help explain variability in the predisposition to smoking in the human population. Recent studies have identified single nucleotide polymorphisms (SNPs) within the $\alpha 3$ - $\alpha 5$ - $\beta 4$ gene cluster [100] located on chromosome 15 that correlate with the intake of nicotine [141,142], implying that genetic factors at the level of nAChRs may predispose certain individuals to nicotine addiction. In particular, one SNP in the presumed cytoplasmic domain of the $\alpha 5$ gene produces an amino acid switch and a decrease in the functional expression of heterologously expressed $\alpha 4\beta 2\alpha 5$ nAChRs [142] allowing the authors speculate about cellular mechanisms that may increase the risk of transitioning from casual smoking to addiction. Other researchers have found that, in addition to the number of cigarettes smoked, there is a genetic association of the same gene cluster with certain smoking-related diseases such as lung cancer and peripheral artery disease [143], although it is unclear at present whether this is due to individual patterns of smoking.

In addition to the nAChR gene cluster, there is some evidence that other receptors, including the $\beta 3$ nAChR, may be genetically linked to nicotine addiction [144]. On the other hand there is no apparent correlation between $\alpha 3$ - $\alpha 5$ - $\beta 4$ genes and smoking cessation [145], although $\beta 2$ subunits may be implicated [146]. These latter findings are somewhat puzzling and worthy of further investigation given the known relationship between $\beta 4^*$ receptor subtype(s) and somatic withdrawal symptoms in rats [109] discussed above.

3. Consequences of chronic nicotine on nAChR number and function

Animals are highly capable of adapting to different sets of conditions so that they can continue to perform optimally. Following this rule, chronic exposure to certain drugs will initiate mechanisms that act to restore normal brain function in the constant presence of the chemical. That is, the system has undergone some sort of plastic change, which persists after removal of the drug, and presumably contributes/underlies both drug seeking and a withdrawal state. For certain drugs, e.g., opiates such as morphine, this type of homeostatic mechanism at the level of the drug-receptor interaction has been useful for explaining the molecular and cellular processes that underlie behavioral addiction [147]. However, it is unlikely that this mechanism alone accounts fully for addiction, and other mechanisms including direct and lasting interference with normal reward systems, and the induction of learned associations involving contextual cues are also of central importance [20,22,23]. The behavioral aspects of nicotine addiction have been dealt with effectively elsewhere [148], and a central goal here is to formulate models that can account for the cellular consequences of exposure to nicotine.

Nicotine acts at the ACh binding site of nAChRs, and thus any model of nicotine-induced plasticity must begin with the interaction of nicotine and its receptors. Continuous exposure to nicotine through its interactions with nAChRs will alter the pattern of ongoing endogenous neuronal activity in the brain, which is then restored to normal in the chronic presence of the drug, i.e., homeostatic neuroadaptation [see 149]. In the case of nAChRs, based on data collected over the last twenty-five years, a common starting point invokes that the minimal homeostatic changes occur in the number and/or function of the nAChRs that bind to nicotine. Obviously, the situation becomes complicated with multiple nAChR subtypes, their cellular distribution within neuronal networks, and their downstream consequences, which themselves could drive additional compensation.

3.1. Autoregulation of $\alpha 4\beta 2^*$ nAChRs

One of the most enduring aspects of nAChR plasticity has been the discovery that chronic *in vivo* nicotine treatment (equivalent to persistent tobacco smoke inhalation) causes a marked increase in the number (change in $B_{\max}$ but not K_d) of [3H]nicotine ($\alpha 4\beta 2^*$) binding sites in the CNS [45,56,57,150]. The increase is long-lasting, but not permanent [151], and was also observed in the postmortem human brains of smokers [152]. Because nicotine is an agonist, upregulation of the receptors appears to contradict the simplest of homeostatic mechanisms that predict downregulation as a compensatory measure, leading to the introduction of the desensitization hypothesis [58].

3.1.1. Is a homeostatic model viable?—Nicotine, like most other agonists, including ACh, does not simply activate nAChRs, but ultimately induces desensitized states of the receptor [153]. In practical terms this means that nicotine acts in a different manner from ACh. ACh is rapidly terminated (in milliseconds) by hydrolysis and keeps nAChRs at the synapse in an activatable state [10]. Nicotine has a long half-life ≈ 1 hr [154] essentially keeping it present in the CSF permanently given repetitive intake [155]. The resulting accumulation of desensitized receptors at synapses would effectively depress ongoing transmitter action – homeostasis would then predict receptor upregulation [56,57,156; see Fig. 1]. Assuming that the nAChR can detect and respond to less ACh activation, this simple scenario predicts that antagonists should also increase nAChR number, but the results from such studies have been mixed [156–160]. However, assuming antagonists do not induce desensitized receptor conformations [although see 161], it has been argued that agonist-induced desensitization is an absolute requirement for upregulation, and that receptor blockade *per se* is insufficient [58]. Two lines of evidence support this view. First, in cells isolated from their synaptic input, nicotine treatment still produces upregulation [162], i.e., upregulation is cell-autonomous

[163]. Second, upregulation can, in some cases (e.g., when surface nAChRs are considered), be directly correlated to occupation by nicotine of high-affinity desensitized states [164]. However, while the desensitization model gains extra credibility because the concentrations of nicotine in the CNS resulting from tobacco smoke inhalation are submicromolar [165] and will tend to favor interaction with desensitized receptor conformations [153,166], alternate mechanisms have been proposed in which nicotine can interact with other receptor conformations. Recently, the concept of desensitization-induced upregulation has been challenged using mice expressing hypersensitive $\alpha 4$ subunit-containing nAChRs. In these animals, ultra low concentrations of nicotine, which interact only with the mutant $\alpha 4^*$ receptors, cause functional upregulation and induce drug-seeking behavior [62]. Because the sensitivity of agonist for receptor desensitization is only marginally affected compared to a marked shift in the ability of agonist to open the mutant ion channels [167], it seems reasonable to suggest that activation (not desensitization) of native $\alpha 4\beta 2^*$ nAChRs can drive both the molecular and behavioral aspects of drug addiction. However, this explanation does not satisfactorily account for upregulation observed in the absence of channel opening [see below; 163].

One of the more controversial aspects of upregulation has concerned the source of the additional surface receptors. The factors governing the regulation of receptor assembly, membrane targeting and recycling are not fully understood [168,169], and it is therefore not surprising that the mechanism of nicotine-induced regulation of nAChR function is also unclear. Efforts have also been hampered by artificial expression systems, in which the majority of nAChRs reside in intracellular pools, possibly because factors necessary for surface membrane trafficking and stability are missing from these systems [72]. Nevertheless, cumulative data from experiments involving both native and over-expressed nAChRs have provided potential explanations for upregulation. There is general agreement that nicotine does not induce changes in the levels of $\alpha 4$ and $\beta 2$ mRNA transcripts [158,170], and as such post-transcriptional mechanisms including increased translation [171], assembly [172], and trafficking from an intracellular pool [173], or decreased turnover [158,174] have all been proposed to account for an increased surface expression. One problem with the desensitization theory of upregulation is that the concentration of nicotine required for upregulation is not optimal for a pure interaction with the desensitized state of the receptor [164]. Indeed it is generally found to be intermediate between the measured “affinities” for the main activatable and desensitized states of surface nAChRs [160,164], seemingly implying that nicotine must be interacting with some unknown receptor conformation [160]. Resolving the nature of this state may therefore shed light on the precise mechanism of receptor upregulation. Because nicotine can readily cross membranes [175], it could have access to assembly intermediate forms of the nAChR, which conceivably could have different binding “affinities” for agonists compared to mature surface receptors. Although non-membrane permeable quaternary amines, e.g., tetramethylammonium, are as efficacious as nicotine in producing upregulation [160], implying that surface receptors are the trigger [see 164], it has been suggested that nicotine and quaternary amines, including ACh, act as chaperones to promote assembly and maturation of nAChRs within the endoplasmic reticulum [174,176,177]. Importantly, if these assembly intermediates (dimers and tetramers) have a lower “affinity” for interaction with agonist than the desensitized conformations of mature surface nAChRs, then this may explain why higher concentrations of nicotine are needed for upregulation than predicted directly from desensitization binding measurements.

3.1.2. Functional consequences of upregulation—An essential component of the homeostatic model is that upregulation should represent an increase in function – to compensate for the blocked and/or desensitized receptors (see Fig. 1). Here the story becomes more complicated because initial studies showed that nicotine exposure caused functional downregulation, at least in the case of $\alpha 4\beta 2^*$ nAChRs [158,178], a finding that may partially

be explained in retrospect by the slow release of intracellularly accumulated nicotine and re-desensitization of surface nAChRs [175]. In contrast, several studies have shown that an increase in the function of expressed $\alpha 4\beta 2$ nAChRs accompanies the change in number [e.g., 165,174]. Such parallel changes in number and function are easy to interpret, but other studies imply more complex relationships between function and number of upregulated receptors [179]. Vallejo et al. argue that surface receptor number is unaffected by nicotine treatment, but rather there is an allosteric-induced shift in pre-existing nAChRs to a higher affinity conformational state [180]. Other studies also imply a shift in the affinity of receptors following upregulation, but attribute this change to increased expression of an altered $\alpha 4\beta 2$ stoichiometry [174]. Interestingly, native $\alpha 4\beta 2^*$ nAChRs with a more complex subunit composition containing the $\alpha 5$ subunit do not appear to undergo upregulation *in vivo* [181]. As a whole these experiments support every aspect of the homeostatic upregulation theory, but with critical differences with respect to mechanism. An all-inclusive but less than satisfying interpretation is that multiple modes of nicotine-induced modulation can occur, with exact conditions determining the prevailing form. Nevertheless, Nashmi and Lester have recently offered a thermodynamic solution that reconciles many of the inconsistencies described in the preceding sections [163]. They propose that ligands acting at the binding site of nAChRs (including agonists like nicotine and high affinity competitive antagonists such as DH β E) will stabilize high affinity conformational receptor states (e.g. desensitized) that, due to slow kinetics, will outlast the binding of ligand, i.e., after chronic nicotine exposure a greater fraction of the receptors will be receptive to agonist and as a consequence receptor function will be enhanced. Furthermore, because these ligands bind at the $\alpha\beta$ interface, this argument can be extended to the stabilization of the multimeric nAChR during intracellular assembly [163].

It has been accepted that, despite quantitative differences, there is widespread upregulation of high affinity binding sites across multiple brain regions during chronic nicotine exposure [170,182,183]. New experiments have now re-quantified upregulation specifically for functional $\alpha 4$ subunit-containing receptors by measuring fluorescence from mice expressing an $\alpha 4$ -YFP knock-in construct. While other CNS nuclei were affected, these data highlight particularly significant increases in $\alpha 4^*$ nAChR function in GABAergic neurons of the VTA and substantia nigra (but not in the principle dopaminergic cells), as well as in the perforant path of the hippocampus [66].

3.2. Other receptors

The effects of chronic nicotine on non- $\alpha 4\beta 2^*$ nAChRs are less than clear. Midbrain dopaminergic neurons terminate in the striatum, where the release of dopamine can be directly affected by two types of presynaptic nAChRs, $\alpha 4\beta 2^*$ and $\alpha 6\beta 2\beta 3^*$ [123,124,126,127]. Although *in vivo* chronic nicotine experiments have largely focused on upregulation of $\alpha 4\beta 2^*$ nAChRs [45], work in striatum and other areas of DA axon termination indicated that a different receptor subtype may be functionally upregulated by nicotinic ligands [184,185; but see 186]. Making use of α -MII (see section 2.1.4), it has been shown that $\alpha 6$ subunit-containing receptors are markedly increased in number compared with $\alpha 4\beta 2^*$ receptors following chronic nicotine treatment [187; but see 188], in agreement with a preferential upregulation of $\alpha 4\beta 2^*$ nAChRs on GABAergic cells in this region [66]. In addition mRNAs for $\alpha 6$ and $\beta 3$ subunits are increased in the substantia nigra [189], implying a possible transcriptional regulation, in contrast to $\alpha 4\beta 2^*$ nAChRs [45]. While these data support functional upregulation of striatal $\alpha 6^*$ receptors, other groups have shown that chronic nicotine produces a slight depression of agonist-stimulated release [56,190]. It is possible that treatment paradigms or species differences underlie these disparate results. Indeed, experiments from Walsh et al. have shown that different nAChR subtypes, including those containing $\alpha 6$, when expressed in heterologous cell systems, can undergo upregulation, although the nicotine concentration-and time-dependence varies with receptor type [191].

There also appears to be a concentration-dependence to the nicotine-induced upregulation of various non- $\alpha 4\beta 2^*$ nAChRs observed in other studies. As a general rule, higher concentrations of nicotine are necessary - reflecting the lower affinity of the activatable and desensitized states of these receptors [43]. Some $\alpha 3$ subunit-containing receptors are upregulated depending on the expression system [191,192], but more importantly native $\alpha 3^*$ nAChRs in the habenulo-interpeduncular axis, as characterized by a component of [125 I]epibatidine binding, are not altered by chronic exposure to nicotine *in vivo* [183], possibly because the CSF concentration of nicotine is too low. Likewise, the very low affinity $\alpha 7^*$ nAChRs/ α BTX binding sites are upregulated (but only at elevated doses of nicotine compared to those needed to increase $\alpha 4\beta 2^*$ nAChRs) both *in vitro* [193] and *in vivo* [182].

4. Downstream molecular targets

In addition to the commonly reported changes in nAChRs, chronic *in vivo* nicotine treatment also leads to changes in the expression of non-cholinergic targets within reward-related structures (Table 2). Many of these changes are in opposing directions depending on the structure, but given that the different structures may have unique roles in the addiction process [194], different drug administration paradigms can differentially effect gene expression [195], and alterations of these targets by nicotine can occur in multiple ways (see below), it perhaps should not be surprising that there are varied effects. The effects observed are common to most drugs of abuse and include changes in ligand-gated ion channels, the dopamine system, neuropeptides, transcription factors and growth factors [196]. Additionally, changes in members of the two-pore domain family of potassium channels [197,198] and L-type Ca^{2+} channels [199,200] have been found following nicotine treatment (Table 2), and while changes in the *expression* of K^+ channels and Ca^{2+} channels in response to other drugs of abuse may not have been reported in the literature as of yet, the *function* of voltage-gated channels that control the excitability of neurons within the prefrontal cortex, nucleus accumbens, and hippocampus have been shown to be altered [201–207]. Because nicotine has the ability to alter the induction of synaptic plasticity [24–26] it may influence changes in gene expression that typically occur downstream of plasticity induction leading to changes in certain molecular targets. In addition to influencing gene expression by altering plasticity, nAChRs may directly flux Ca^{2+} [32,208–210], which is the principle mediator of plasticity induced gene expression [211]. Nicotine may also play an even more direct role by accumulating in cells [174–177] and directly interacting with the ubiquitin proteasome system, thereby influencing the degradation of proteins [212].

5. Implications

The data reviewed here show that chronic exposure to nicotine can induce changes in its own receptors in addition to causing altered expression of many other cellular targets. In particular there is general agreement that high-affinity nicotine binding sites can be functionally upregulated and although the precise mechanism by which this occurs is not fully resolved it most likely involves nicotine entry into the cell and interference with the assembly process. Based on this evidence we are forced to conclude that upregulation of $\alpha 4\beta 2^*$ nAChRs does not represent a direct homeostatic response to persistent receptor desensitization, but may in fact be a fortuitous event resulting from the ability of nicotine to cross membranes, accumulate in cells, and bind to nAChR assembly intermediates –effectively all thermodynamics, as discussed by Nashmi and Lester [163]. Do we have to further conclude that the upregulation of nAChRs is an unfortunate epiphenomenon with no causal role in addiction to nicotine? Based on genetic and pharmacological elimination of either and/or both $\beta 2$ and $\alpha 7$ subunit-containing receptors, Besson et al. found it difficult to incorporate the upregulation of high affinity sites into a homeostatic model in which the “opposite” action of nicotine at these two receptor classes offset both neurochemical and behavioral effects [83]. Conversely, other

researchers have incorporated the upregulation of these receptors into models of sensitization and tolerance to nicotine. It has been proposed that the transient upregulation of nAChRs increases the excitation of dopamine neurons in the VTA by permitting long-term potentiation of synaptic transmission. Together these changes result in enhanced dopamine release, which underlies behavioral sensitization [213]. Conversely, focusing on the mechanism of tolerance to nicotine in midbrain neurons, Nashmi et al. argue that selective upregulation of $\alpha 4^*$ nAChRs on GABAergic cells coupled with chronic stimulation by nicotine may lead to a decreased output of dopaminergic neurons [66]. Ultimately it will be important to test the validity of these models and find methods of probing directly whether upregulation of nAChRs is a necessary step in the development of behaviors that lead to nicotine addiction. There will be a similar challenge with respect to determining which of those changes in other nAChR subtypes and other cellular processes are a direct consequence of the presence of nicotine, compensatory secondary adaptations, or driven by reward associated behaviors.

Acknowledgments

Supported by PHS grant NIDA DA11940

Abbreviations

nAChRs	nicotinic acetylcholine receptors
CNS	central nervous system
PNS	peripheral nervous system
NMJ	neuromuscular junction
ACh	acetylcholine
IPN	interpeduncular nucleus
VTA	ventral tegmental area
αBTX	α -bungarotoxin
MLA	methyllycaconitine
MHb	medial habenula
DHβE	dihydro- β -erythroidine
GFP	green fluorescent protein

References

1. WHO Report on the Global Tobacco Epidemic 2008. The MPOWER package. Geneva: World Health Organization; 2008.
2. Hughes J, Keely J, Naud S. Shape of the relapse curve and long-term abstinence among untreated smokers. *Addiction* 2004;99:29–38. [PubMed: 14678060]
3. Nides M. Update on pharmacologic options for smoking cessation treatment. *Am J Med* 2008;121:S20–31. [PubMed: 18342163]
4. Stead LF, Perera R, Bullen C, Mant D, Lancaster T. Nicotine replacement therapy for smoking cessation. *Cochrane Database Systematic Reviews* 2008:CD000146.
5. Russell MA. The nicotine addiction trap: A 40-year sentence for four cigarettes. *Br J Addiction* 1990;85:293–300.
6. Corrigan WA, Coen KM. Nicotine maintains robust self-administration in rats on a limited-access schedule. *Psychopharmacol* 1989;99:473–8.
7. Corrigan WA, Franklin KB, Coen KM, Clarke PB. The mesolimbic dopaminergic system is implicated in the reinforcing effects of nicotine. *Psychopharmacol* 1992;107:285–9.
8. Dani JA, Mayer ML. Structure and function of glutamate and nicotinic acetylcholine receptors. *Curr Opin Neurobiol* 1995;5:310–7. [PubMed: 7580153]
9. Brown, DA. The history of the neuronal nicotinic receptors. In: Clementi, F.; Fornasari, D.; Gotti, C., editors. *Neuronal nicotinic receptors*. Berlin, New York: Springer; 2000. p. 3–11.
10. Colquhoun D, Ogden DC. Activation of ion channels in the frog end-plate by high concentrations of acetylcholine. *J Physiol* 1988;395:131–59. [PubMed: 2457675]
11. Wood SJ, Slater CR. Safety factor at the neuromuscular junction. *Prog Neurobiol* 2001;64:393–429. [PubMed: 11275359]
12. Sanes JR, Lichtman JW. Induction, assembly, maturation and maintenance of a postsynaptic apparatus. *Nat Rev Neurosci* 2001;2:791–805. [PubMed: 11715056]
13. Lindstrom J. Autoimmune diseases involving nicotinic receptors. *J Neurobiol* 2002;53:656–65. [PubMed: 12436428]
14. Briggs CA, McAfee DA. Long-term potentiation at nicotinic synapses in the rat superior cervical ganglion. *J Physiol* 1988;404:129–44. [PubMed: 2855347]
15. Role LW, Berg DK. Nicotinic receptors in the development and modulation of CNS synapses. *Neuron* 1996;16:1077–85. [PubMed: 8663984]
16. Jones S, Sudweeks S, Yakel JL. Nicotinic receptors in the brain: Correlating physiology with function. *Trends Neurosci* 1999;22:555–61. [PubMed: 10542436]
17. Descarries L, Gisé V, Steriade M. Diffuse transmission by acetylcholine in the CNS. *Prog Neurobiol* 1997;53:603–25. [PubMed: 9421837]
18. Zoli M, Jansson A, Syková E, Agnati LF, Fuxe K. Volume transmission in the CNS and its relevance for neuropsychopharmacology. *Trends Pharmacol Sci* 1999;20:142–50. [PubMed: 10322499]
19. Lendvai B, Vizi ES. Nonsynaptic chemical transmission through nicotinic acetylcholine receptors. *Physiol Rev* 2008;88:333–49. [PubMed: 18391166]
20. Di Chiara G, North RA. Neurobiology of opiate abuse. *Trends Pharmacol Sci* 1992;13:185–93. [PubMed: 1604711]
21. Caggiula AR, Donny EC, White AR, Chaudhri N, Booth S, Gharib MA, et al. Cue dependency of nicotine self-administration and smoking. *Pharmacol Biochem Behav* 2001;70:515–30. [PubMed: 11796151]
22. Kelley AE. Memory and addiction: Shared neural circuitry and molecular mechanisms. *Neuron* 2004;44:161–79. [PubMed: 15450168]
23. Hyman S, Malenka R, Nestler E. Neural mechanisms of addiction: The role of reward-related learning and memory. *Annu Rev Neurosci* 2006;29:565–98. [PubMed: 16776597]
24. Dani JA, Ji D, Zhou FM. Synaptic plasticity and nicotine addiction. *Neuron* 2001;31:349–52. [PubMed: 11516393]
25. McKay BE, Placzek AN, Dani J. Regulation of synaptic transmission and plasticity by neuronal nicotinic acetylcholine receptors. *Biochem Pharmacol* 2007;74:1120–33. [PubMed: 17689497]

26. Role LW, Mansvelder HD. Electrophysiology and synaptic plasticity in nicotine addiction. *Seminars Cell Dev Biol* 2009;20: this issue
27. Role LW. Diversity in primary structure and function of neuronal nicotinic acetylcholine receptor channels. *Curr Opin Neurobiol* 1992;2:254–62. [PubMed: 1643408]
28. Colquhoun LM, Patrick JW. Pharmacology of neuronal nicotinic acetylcholine receptor subtypes. *Adv Pharmacol* 1997;39:191–220. [PubMed: 9160116]
29. Dani J, Bertrand D. Nicotinic acetylcholine receptors and nicotinic cholinergic mechanisms of the central nervous system. *Annu Rev Pharmacol Toxicol* 2007;47:699–729. [PubMed: 17009926]
30. Karlin A. Structure of nicotinic acetylcholine receptors. *Curr Opin Neurobiol* 1993;3:299–309. [PubMed: 8369624]
31. Sargent PB. The diversity of neuronal nicotinic acetylcholine receptors. *Annu Rev Neurosci* 1993;16:403–43. [PubMed: 7681637]
32. McGehee DS, Role LW. Physiological diversity of nicotinic acetylcholine receptors expressed by vertebrate neurons. *Annu Rev Physiol* 1995;57:521–46. [PubMed: 7778876]
33. Lindstrom J. Nicotinic acetylcholine receptors in health and disease. *Mol Neurobiol* 1997;15:193–222. [PubMed: 9396010]
34. Lukas RJ, Changeux JP, Le Novère N, Albuquerque EX, Balfour DJ, Berg DK, et al. International union of pharmacology. XX Current status of the nomenclature for nicotinic acetylcholine receptors and their subunits. *Pharmacol Rev* 1999;51:397–401. [PubMed: 10353988]
35. Cordero-Erausquin M, Marubio LM, Klink R, Changeux JP. Nicotinic receptor function: New perspectives from knockout mice. *Trends Pharmacol Sci* 2000;21:211–7. [PubMed: 10838608]
36. Mongan NP, Baylis HA, Adcock C, Smith GR, Sansom MS, Sattelle DB. An extensive and diverse gene family of nicotinic acetylcholine receptor α subunits in *Caenorhabditis elegans*. *Receptors Channels* 1998;6:213–28. [PubMed: 10100329]
37. Wada E, Wada K, Boulter J, Deneris E, Heinemann S, Patrick J, et al. Distribution of $\alpha 2$, $\alpha 3$, $\alpha 4$, and $\beta 2$ neuronal nicotinic receptor subunit mRNAs in the central nervous system: A hybridization histochemical study in the rat. *J Comp Neurol* 1989;284:314–35. [PubMed: 2754038]
38. Brussaard AB, Yang X, Doyle JP, Huck S, Role LW. Developmental regulation of multiple nicotinic AChR channel subtypes in embryonic chick habenula neurons: Contributions of both the $\alpha 2$ and $\alpha 4$ subunit genes. *Pflugers Arch* 1994;429:27–43. [PubMed: 7708479]
39. McIntosh JM, Gardner S, Luo S, Garrett JE, Yoshikami D. Conus peptides: Novel probes for nicotinic acetylcholine receptor structure and function. *Eur J Pharmacol* 2000;393:205–8. [PubMed: 10771014]
40. Janes RW. α -conotoxins as selective probes for nicotinic acetylcholine receptor subclasses. *Curr Opin Pharmacol* 2005;5:280–92. [PubMed: 15907916]
41. Picciotto MR, Caldarone BJ, Brunzell DH, Zachariou V, Stevens TR, King SL. Neuronal nicotinic acetylcholine receptor subunit knockout mice: Physiological and behavioral phenotypes and possible clinical implications. *Pharmacol Ther* 2001;92:89–108. [PubMed: 11916531]
42. Picciotto MR, Zoli M, Rimondini R, Léna C, Marubio LM, Pich EM, et al. Acetylcholine receptors containing the $\beta 2$ subunit are involved in the reinforcing properties of nicotine. *Nature* 1998;391:173–7. [PubMed: 9428762]
43. Fenster CP, Rains MF, Noerager B, Quick MW, Lester RA. Influence of subunit composition on desensitization of neuronal acetylcholine receptors at low concentrations of nicotine. *J Neurosci* 1997;17:5747–59. [PubMed: 9221773]
44. Schoepfer R, Whiting P, Esch F, Blacher R, Shimasaki S, Lindstrom J. cDNA clones coding for the structural subunit of a chicken brain nicotinic acetylcholine receptor. *Neuron* 1988;1:241–8. [PubMed: 3272170]
45. Flores CM, Rogers SW, Pabreza LA, Wolfe BB, Kellar KJ. A subtype of nicotinic cholinergic receptor in rat brain is composed of $\alpha 4$ and $\beta 2$ subunits and is up-regulated by chronic nicotine treatment. *Mol Pharmacol* 1992;41:31–7. [PubMed: 1732720]
46. Shafae N, Houng M, Truong A, Viseshakul N, Figl A, Sandhu S. Pharmacological similarities between native brain and heterologously expressed $\alpha 4\beta 2$ nicotinic receptors. *Br J Pharmacol* 1999;128:1291–9. [PubMed: 10578144]

47. Picciotto MR, Zoli M, Léna C, Bessis A, Lallemand Y, Le Novère N, et al. Abnormal avoidance learning in mice lacking functional high-affinity nicotine receptor in the brain. *Nature* 1995;374:65–7. [PubMed: 7870173]
48. Marubio LM, del Mar Arroyo-Jimenez M, Cordero-Erausquin M, Léna C, Le Novère N, de Kerchove d'Exaerde A, et al. Reduced antinociception in mice lacking neuronal nicotinic receptor subunits. *Nature* 1999;398:805–10. [PubMed: 10235262]
49. Conroy WG, Vernallis AB, Berg DK. The $\alpha 5$ gene product assembles with multiple acetylcholine receptor subunits to form distinctive receptor subtypes in brain. *Neuron* 1992;9:679–91. [PubMed: 1389183]
50. Ramirez-Latorre J, Yu CR, Qu X, Perin F, Karlin A, Role L. Functional contributions of $\alpha 5$ subunit to neuronal acetylcholine receptor channels. *Nature* 1996;380:347–51. [PubMed: 8598930]
51. Wang F, Gerzanich V, Wells GB, Anand R, Peng X, Keyser K, et al. Assembly of human neuronal nicotinic receptor $\alpha 5$ subunits with $\alpha 3$, $\beta 2$, and $\beta 4$ subunits. *J Biol Chem* 1996;271:17656–65. [PubMed: 8663494]
52. Porter JT, Cauli B, Tsuzuki K, Lambolez B, Rossier J, Audinat E. Selective excitation of subtypes of neocortical interneurons by nicotinic receptors. *J Neurosci* 1999;19:5228–35. [PubMed: 10377334]
53. Cooper E, Couturier S, Ballivet M. Pentameric structure and subunit stoichiometry of a neuronal nicotinic acetylcholine receptor. *Nature* 1991;350:235–8. [PubMed: 2005979]
54. Nelson ME, Kuryatov A, Choi CH, Zhou Y, Lindstrom J. Alternate stoichiometries of $\alpha 4\beta 2$ nicotinic acetylcholine receptors. *Mol Pharmacol* 2003;63:332–41. [PubMed: 12527804]
55. Gotti C, Moretti M, Meinerz NM, Clementi F, Gaimarri A, Collins A, et al. Partial deletion of the nicotinic cholinergic receptor $\alpha 4$ or $\beta 2$ subunit genes changes the acetylcholine sensitivity of receptor-mediated 86Rb^+ efflux in cortex and thalamus and alters relative expression of $\alpha 4$ and $\beta 2$ subunits. *Mol Pharmacol* 2008;73:1796–807. [PubMed: 18337473]
56. Marks MJ, Burch JB, Collins AC. Effects of chronic nicotine infusion on tolerance development and nicotinic receptors. *J Pharmacol Exp Ther* 1983;226:817–25. [PubMed: 6887012]
57. Schwartz RD, Kellar KJ. Nicotinic cholinergic receptor binding sites in the brain: Regulation in vivo. *Science* 1983;220:214–6. [PubMed: 6828889]
58. Wonnacott S. The paradox of nicotinic acetylcholine receptor upregulation by nicotine. *Trends Pharmacol Sci* 1990;11:216–9. [PubMed: 2200178]
59. Corrigan WA, Coen KM, Adamson KL. Self-administered nicotine activates the mesolimbic dopamine system through the ventral tegmental area. *Brain Res* 1994;653:278–84. [PubMed: 7982062]
60. Maskos U, Molles BE, Pons S, Besson M, Guiard BP, Guilloux JP, et al. Nicotine reinforcement and cognition restored by targeted expression of nicotinic receptors. *Nature* 2005;436:103–7. [PubMed: 16001069]
61. Pons S, Fattore L, Cossu G, Tolu S, Porcu E, McIntosh JM, et al. Crucial role of $\alpha 4$ and $\alpha 6$ nicotinic acetylcholine receptor subunits from ventral tegmental area in systemic nicotine self-administration. *J Neurosci* 2008;28:12318–27. [PubMed: 19020025]
62. Tapper AR, McKinney SL, Nashmi R, Schwarz J, Deshpande P, Labarca C, et al. Nicotine activation of $\alpha 4^*$ receptors: Sufficient for reward, tolerance, and sensitization. *Science* 2004;306:1029–32. [PubMed: 15528443]
63. Klink R, de Kerchove d'Exaerde A, Zoli M, Changeux JP. Molecular and physiological diversity of nicotinic acetylcholine receptors in the midbrain dopaminergic nuclei. *J Neurosci* 2001;21:1452–63. [PubMed: 11222635]
64. Mansvelder H, Keath JR, McGehee DS. Synaptic mechanisms underlie nicotine-induced excitability of brain reward areas. *Neuron* 2002;33:905–19. [PubMed: 11906697]
65. Pidoplichko VI, Noguchi J, Areola OO, Liang Y, Peterson J, Zhang T, et al. Nicotinic cholinergic synaptic mechanisms in the ventral tegmental area contribute to nicotine addiction. *Learn Mem* 2004;11:60–9. [PubMed: 14747518]
66. Nashmi R, Xiao C, Deshpande P, McKinney S, Grady S, Whiteaker P, et al. Chronic nicotine cell specifically upregulates functional $\alpha 4^*$ nicotinic receptors: Basis for both tolerance in midbrain and

- enhanced long-term potentiation in perforant path. *J Neurosci* 2007;27:8202–18. [PubMed: 17670967]
67. Picciotto M, Addy NA, Mineur YS, Brunzell D. It is not “Either/or”: Activation and desensitization of nicotinic acetylcholine receptors both contribute to behaviors related to nicotine addiction and mood. *Prog Neurobiol* 2008;84:329–42. [PubMed: 18242816]
 68. Couturier S, Bertrand D, Matter JM, Hernandez MC, Bertrand S, Millar N, et al. A neuronal nicotinic acetylcholine receptor subunit ($\alpha 7$) is developmentally regulated and forms a homo-oligomeric channel blocked by α -BTX. *Neuron* 1990;5:847–56. [PubMed: 1702646]
 69. Séguéla P, Wadiche J, Dineley-Miller K, Dani JA, Patrick JW. Molecular cloning, functional properties, and distribution of rat brain $\alpha 7$: A nicotinic cation channel highly permeable to calcium. *J Neurosci* 1993;13:596–604. [PubMed: 7678857]
 70. Alkondon M, Pereira EF, Wonnacott S, Albuquerque EX. Blockade of nicotinic currents in hippocampal neurons defines methyllycaconitine as a potent and specific receptor antagonist. *Mol Pharmacol* 1992;41:802–8. [PubMed: 1569927]
 71. Zorumski CF, Thio LL, Isenberg KE, Clifford DB. Nicotinic acetylcholine currents in cultured postnatal rat hippocampal neurons. *Mol Pharmacol* 1992;41:931–6. [PubMed: 1375319]
 72. Dineley KT, Patrick JW. Amino acid determinants of $\alpha 7$ nicotinic acetylcholine receptor surface expression. *J Biol Chem* 2000;275:13974–85. [PubMed: 10788524]
 73. Anand R, Bason L, Saedi MS, Gerzanich V, Peng X, Lindstrom J. Reporter epitopes: A novel approach to examine transmembrane topology of integral membrane proteins applied to the $\alpha 1$ subunit of the nicotinic acetylcholine receptor. *Biochemistry* 1993;32:9975–84. [PubMed: 7691173]
 74. Chen D, Patrick JW. The α -bungarotoxin-binding nicotinic acetylcholine receptor from rat brain contains only the $\alpha 7$ subunit. *J Biol Chem* 1997;272:24024–9. [PubMed: 9295355]
 75. Drisdell RC, Green WN. Neuronal α -bungarotoxin receptors are $\alpha 7$ subunit homomers. *J Neurosci* 2000;20:133–9. [PubMed: 10627589]
 76. Orr-Urtreger A, Göldner FM, Saeki M, Lorenzo I, Goldberg L, De Biasi M, et al. Mice deficient in the $\alpha 7$ neuronal nicotinic acetylcholine receptor lack α -bungarotoxin binding sites and hippocampal fast nicotinic currents. *J Neurosci* 1997;17:9165–71. [PubMed: 9364063]
 77. Khiroug SS, Harkness PC, Lamb PW, Sudweeks SN, Khiroug L, Millar NS, et al. Rat nicotinic ACh receptor $\alpha 7$ and $\beta 2$ subunits co-assemble to form functional heteromeric nicotinic receptor channels. *J Physiol* 2002;540:425–34. [PubMed: 11956333]
 78. Yu CR, Role LW. Functional contribution of the $\alpha 7$ subunit to multiple subtypes of nicotinic receptors in embryonic chick sympathetic neurones. *J Physiol* 1998;509(Pt 3):651–65. [PubMed: 9596789]
 79. Alkondon M, Albuquerque EX. Initial characterization of the nicotinic acetylcholine receptors in rat hippocampal neurons. *J Recept Res* 1991;11:1001–21. [PubMed: 1753378]
 80. Alkondon M, Pereira EF, Albuquerque EX. α -bungarotoxin- and methyllycaconitine-sensitive nicotinic receptors mediate fast synaptic transmission in interneurons of rat hippocampal slices. *Brain Res* 1998;810:257–63. [PubMed: 9813357]
 81. Frazier CJ, Buhler AV, Weiner JL, Dunwiddie TV. Synaptic potentials mediated via α -bungarotoxin-sensitive nicotinic acetylcholine receptors in rat hippocampal interneurons. *J Neurosci* 1998;18:8228–35. [PubMed: 9763468]
 82. Wonnacott S. Presynaptic nicotinic ACh receptors. *Trends Neurosci* 1997;20:92–8. [PubMed: 9023878]
 83. Besson M, Granon S, Mameli-Engvall M, Cloëz-Tayarani I, Maubourguet N, Cormier A, et al. Long-term effects of chronic nicotine exposure on brain nicotinic receptors. *Proc Natl Acad Sci USA* 2007;104:8155–60. [PubMed: 17470777]
 84. Walters C, Brown S, Changeux J, Martin B, Damaj MI. The $\beta 2$ but not $\alpha 7$ subunit of the nicotinic acetylcholine receptor is required for nicotine-conditioned place preference in mice. *Psychopharmacol* 2006;184:339–44.
 85. Markou A, Paterson NE. The nicotinic antagonist methyllycaconitine has differential effects on nicotine self-administration and nicotine withdrawal in the rat. *Nicotine Tob Res* 2001;3:361–73. [PubMed: 11694204]

86. Salas R, Main A, Gangitano D, De Biasi M. Decreased withdrawal symptoms but normal tolerance to nicotine in mice null for the $\alpha 7$ nicotinic acetylcholine receptor subunit. *Neuropharmacol* 2007;53:863–9.
87. Mansvelder HD, McGehee DS. Long-term potentiation of excitatory inputs to brain reward areas by nicotine. *Neuron* 2000;27:349–57. [PubMed: 10985354]
88. Saal D, Dong Y, Bonci A, Malenka R. Drugs of abuse and stress trigger a common synaptic adaptation in dopamine neurons. *Neuron* 2003;37:577–82. [PubMed: 12597856]
89. Levin E. Nicotinic receptor subtypes and cognitive function. *J Neurobiol* 2002;53:633–40. [PubMed: 12436426]
90. Ji D, Lape R, Dani JA. Timing and location of nicotinic activity enhances or depresses hippocampal synaptic plasticity. *Neuron* 2001;31:131–41. [PubMed: 11498056]
91. Adler LE, Olincy A, Waldo M, Harris JG, Griffith J, Stevens K, et al. Schizophrenia, sensory gating, and nicotinic receptors. *Schizophrenia Bull* 1998;24:189–202.
92. Duvoisin RM, Deneris ES, Patrick J, Heinemann S. The functional diversity of the neuronal nicotinic acetylcholine receptors is increased by a novel subunit: $\beta 4$. *Neuron* 1989;3:487–96. [PubMed: 2642007]
93. Dineley-Miller K, Patrick J. Gene transcripts for the nicotinic acetylcholine receptor subunit, $\beta 4$, are distributed in multiple areas of the rat central nervous system. *Brain Res Mol Brain Res* 1992;16:339–44. [PubMed: 1337943]
94. Zoli M, Le Novère N, Hill JA, Changeux JP. Developmental regulation of nicotinic ACh receptor subunit mRNAs in the rat central and peripheral nervous systems. *J Neurosci* 1995;15:1912–39. [PubMed: 7891142]
95. Winzer-Serhan UH, Leslie FM. Codistribution of nicotinic acetylcholine receptor subunit $\alpha 3$ and $\beta 4$ mRNAs during rat brain development. *J Comp Neurol* 1997;386:540–54. [PubMed: 9378850]
96. Quick MW, Ceballos RM, Kasten M, McIntosh JM, Lester RA. $\alpha 3\beta 4$ subunit-containing nicotinic receptors dominate function in rat medial habenula neurons. *Neuropharmacol* 1999;38:769–83.
97. Covernton PJ, Kojima H, Sivilotti LG, Gibb AJ, Colquhoun D. Comparison of neuronal nicotinic receptors in rat sympathetic neurones with subunit pairs expressed in xenopus oocytes. *J Physiol* 1994;481(Pt 1):27–34. [PubMed: 7853248]
98. Conroy WG, Berg DK. Neurons can maintain multiple classes of nicotinic acetylcholine receptors distinguished by different subunit compositions. *J Biol Chem* 1995;270:4424–31. [PubMed: 7876208]
99. Sivilotti LG, McNeil DK, Lewis TM, Nassar MA, Schoepfer R, Colquhoun D. Recombinant nicotinic receptors, expressed in *Xenopus* oocytes, do not resemble native rat sympathetic ganglion receptors in single-channel behaviour. *J Physiol* 1997;500(Pt 1):123–38. [PubMed: 9097938]
100. Boulter J, O'Shea-Greenfield A, Duvoisin RM, Connolly JG, Wada E, Jensen A, et al. $\alpha 3$, $\alpha 5$, and $\beta 4$: Three members of the rat neuronal nicotinic acetylcholine receptor-related gene family form a gene cluster. *J Biol Chem* 1990;265:4472–82. [PubMed: 1689727]
101. Groot-Kormelink PJ, Luyten WH, Colquhoun D, Sivilotti LG. A reporter mutation approach shows incorporation of the “orphan” subunit $\beta 3$ into a functional nicotinic receptor. *J Biol Chem* 1998;273:15317–20. [PubMed: 9624109]
102. Perry DC, Kellar KJ. [^3H]epibatidine labels nicotinic receptors in rat brain: An autoradiographic study. *J Pharmacol Exp Ther* 1995;275:1030–4. [PubMed: 7473129]
103. Whiteaker P, Jimenez M, McIntosh JM, Collins AC, Marks MJ. Identification of a novel nicotinic binding site in mouse brain using [^{125}I]epibatidine. *Br J Pharmacol* 2000;131:729–39. [PubMed: 11030722]
104. Perry DC, Xiao Y, Nguyen HN, Musachio JL, Dávila-García MI, Kellar KJ. Measuring nicotinic receptors with characteristics of $\alpha 4\beta 2$, $\alpha 3\beta 2$ and $\alpha 3\beta 4$ subtypes in rat tissues by autoradiography. *J Neurochem* 2002;82:468–81. [PubMed: 12153472]
105. Zoli M, Léna C, Picciotto MR, Changeux JP. Identification of four classes of brain nicotinic receptors using $\beta 2$ mutant mice. *J Neurosci* 1998;18:4461–72. [PubMed: 9614223]
106. De Biasi M. Nicotinic mechanisms in the autonomic control of organ systems. *J Neurobiol* 2002;53:568–79. [PubMed: 12436421]

107. Rassadi S, Krishnaswamy A, Pié B, McConnell R, Jacob MH, Cooper E. A null mutation for the $\alpha 3$ nicotinic acetylcholine (ACh) receptor gene abolishes fast synaptic activity in sympathetic ganglia and reveals that ACh output from developing preganglionic terminals is regulated in an activity-dependent retrograde manner. *J Neurosci* 2005;25:8555–66. [PubMed: 16162937]
108. Vachon MP, Miliaressis E. Dorsal diencephalic self-stimulation: A movable electrode mapping study. *Behav Neurosci* 1992;106:981–91. [PubMed: 1472297]
109. Salas R, Pieri F, De Biasi M. Decreased signs of nicotine withdrawal in mice null for the $\beta 4$ nicotinic acetylcholine receptor subunit. *J Neurosci* 2004;24:10035–9. [PubMed: 15537871]
110. Glick SD, Maisonneuve IM, Kitchen BA, Fleck MW. Antagonism of $\alpha 3\beta 4$ nicotinic receptors as a strategy to reduce opioid and stimulant self-administration. *Eur J Pharmacol* 2002;438:99–105. [PubMed: 11906717]
111. Taraschenko OD, Shulan JM, Maisonneuve IM, Glick SD. 18-MC acts in the medial habenula and interpeduncular nucleus to attenuate dopamine sensitization to morphine in the nucleus accumbens. *Synapse* 2007;61:547–60. [PubMed: 17447255]
112. Glick SD, Ramirez RL, Livi JM, Maisonneuve IM. 18-methoxycoronaridine acts in the medial habenula and/or interpeduncular nucleus to decrease morphine self-administration in rats. *Eur J Pharmacol* 2006;537:94–8. [PubMed: 16626688]
113. Panchal V, Taraschenko OD, Maisonneuve IM, Glick SD. Attenuation of morphine withdrawal signs by intracerebral administration of 18-methoxycoronaridine. *Eur J Pharmacol* 2005;525:98–104. [PubMed: 16289028]
114. Glick SD, Maisonneuve IM, Visker KE, Fritz KA, Bandarage UK, Kuehne ME. 18-methoxycoronaridine attenuates nicotine-induced dopamine release and nicotine preferences in rats. *Psychopharmacol* 1998;139:274–80.
115. Gerzanich V, Kuryatov A, Anand R, Lindstrom J. Orphan $\alpha 6$ nicotinic AChR subunit can form a functional heteromeric acetylcholine receptor. *Mol Pharmacol* 1997;51:320–7. [PubMed: 9203638]
116. Cartier GE, Yoshikami D, Gray WR, Luo S, Olivera BM, McIntosh JM. A new α -conotoxin which targets $\alpha 3\beta 2$ nicotinic acetylcholine receptors. *J Biol Chem* 1996;271:7522–8. [PubMed: 8631783]
117. Grady SR, Meinerz NM, Cao J, Reynolds AM, Picciotto MR, Changeux JP, et al. Nicotinic agonists stimulate acetylcholine release from mouse interpeduncular nucleus: A function mediated by a different nAChR than dopamine release from striatum. *J Neurochem* 2001;76:258–68. [PubMed: 11145999]
118. Cui C, Booker TK, Allen RS, Grady SR, Whiteaker P, Marks MJ, et al. The $\beta 3$ nicotinic receptor subunit: A component of α -conotoxin MII-binding nicotinic acetylcholine receptors that modulate dopamine release and related behaviors. *J Neurosci* 2003;23:11045–53. [PubMed: 14657161]
119. Le Novère N, Zoli M, Changeux JP. Neuronal nicotinic receptor $\alpha 6$ subunit mRNA is selectively concentrated in catecholaminergic nuclei of the rat brain. *Eur J Neurosci* 1996;8:2428–39. [PubMed: 8950106]
120. Göldner FM, Dineley KT, Patrick JW. Immunohistochemical localization of the nicotinic acetylcholine receptor subunit $\alpha 6$ to dopaminergic neurons in the substantia nigra and ventral tegmental area. *Neuroreport* 1997;8:2739–42. [PubMed: 9295110]
121. Luetje CW. Getting past the asterisk: The subunit composition of presynaptic nicotinic receptors that modulate striatal dopamine release. *Mol Pharmacol* 2004;65:1333–5. [PubMed: 15155826]
122. Kulak JM, Nguyen TA, Olivera BM, McIntosh JM. α -conotoxin MII blocks nicotine-stimulated dopamine release in rat striatal synaptosomes. *J Neurosci* 1997;17:5263–70. [PubMed: 9204910]
123. Champiaux N, Gotti C, Cordero-Erausquin M, David DJ, Przybylski C, Léna C, et al. Subunit composition of functional nicotinic receptors in dopaminergic neurons investigated with knock-out mice. *J Neurosci* 2003;23:7820–9. [PubMed: 12944511]
124. Salminen O, Murphy KL, McIntosh JM, Drago J, Marks MJ, Collins AC, et al. Subunit composition and pharmacology of two classes of striatal presynaptic nicotinic acetylcholine receptors mediating dopamine release in mice. *Mol Pharmacol* 2004;65:1526–35. [PubMed: 15155845]
125. Whiteaker P, Peterson CG, Xu W, McIntosh JM, Paylor R, Beaudet AL, et al. Involvement of the $\alpha 3$ subunit in central nicotinic binding populations. *J Neurosci* 2002;22:2522–9. [PubMed: 11923417]

126. Kaiser SA, Soliakov L, Harvey SC, Luetje CW, Wonnacott S. Differential inhibition by α -conotoxin-MII of the nicotinic stimulation of [3 H]dopamine release from rat striatal synaptosomes and slices. *J Neurochem* 1998;70:1069–76. [PubMed: 9489727]
127. Wonnacott S, Sidhpura N, Balfour DJ. Nicotine: From molecular mechanisms to behaviour. *Curr Opin Pharmacol* 2005;5:53–9. [PubMed: 15661626]
128. Zhou FM, Liang Y, Dani JA. Endogenous nicotinic cholinergic activity regulates dopamine release in the striatum. *Nat Neurosci* 2001;4:1224–9. [PubMed: 11713470]
129. Gerdeman GL, Partridge JG, Lupica CR, Lovinger DM. It could be habit forming: Drugs of abuse and striatal synaptic plasticity. *Trends Neurosci* 2003;26:184–92. [PubMed: 12689769]
130. Grady S, Salminen O, Lavery DC, Whiteaker P, McIntosh J, Collins A, et al. The subtypes of nicotinic acetylcholine receptors on dopaminergic terminals of mouse striatum. *Biochem Pharmacol* 2007;74:1235–46. [PubMed: 17825262]
131. Elgoyhen AB, Johnson DS, Boulter J, Vetter DE, Heinemann S. $\alpha 9$: An acetylcholine receptor with novel pharmacological properties expressed in rat cochlear hair cells. *Cell* 1994;79:705–15. [PubMed: 7954834]
132. Elgoyhen AB, Vetter DE, Katz E, Rothlin CV, Heinemann SF, Boulter J. $\alpha 10$: A determinant of nicotinic cholinergic receptor function in mammalian vestibular and cochlear mechanosensory hair cells. *Proc Natl Acad Sci USA* 2001;98:3501–6. [PubMed: 11248107]
133. Fuchs PA. Synaptic transmission at vertebrate hair cells. *Curr Opin Neurobiol* 1996;6:514–9. [PubMed: 8794097]
134. Glowatzki E, Fuchs PA. Cholinergic synaptic inhibition of inner hair cells in the neonatal mammalian cochlea. *Science* 2000;288:2366–8. [PubMed: 10875922]
135. Schoepfer R, Conroy WG, Whiting P, Gore M, Lindstrom J. Brain α -bungarotoxin binding protein cDNAs and mAbs reveal subtypes of this branch of the ligand-gated ion channel gene superfamily. *Neuron* 1990;5:35–48. [PubMed: 2369519]
136. Keyser KT, Britto LR, Schoepfer R, Whiting P, Cooper J, Conroy W, et al. Three subtypes of α -bungarotoxin-sensitive nicotinic acetylcholine receptors are expressed in chick retina. *J Neurosci* 1993;13:442–54. [PubMed: 8426223]
137. Gotti C, Hanke W, Maury K, Moretti M, Ballivet M, Clementi F, et al. Pharmacology and biophysical properties of $\alpha 7$ and $\alpha 7\alpha 8$ α -bungarotoxin receptor subtypes immunopurified from the chick optic lobe. *Eur J Neurosci* 1994;6:1281–91. [PubMed: 7981870]
138. Mulle C, Vidal C, Benoit P, Changeux JP. Existence of different subtypes of nicotinic acetylcholine receptors in the rat habenulo-interpeduncular system. *J Neurosci* 1991;11:2588–97. [PubMed: 1869929]
139. Ishii K, Wong JK, Sumikawa K. Comparison of $\alpha 2$ nicotinic acetylcholine receptor subunit mRNA expression in the central nervous system of rats and mice. *J Comp Neurol* 2005;493:241–60. [PubMed: 16255031]
140. Forsayeth JR, Kobrin E. Formation of oligomers containing the $\beta 3$ and $\beta 4$ subunits of the rat nicotinic receptor. *J Neurosci* 1997;17:1531–8. [PubMed: 9030613]
141. Berrettini W, Yuan X, Tozzi F, Song K, Francks C, Chilcoat H, et al. $\alpha 5/\alpha 3$ nicotinic receptor subunit alleles increase risk for heavy smoking. *Mol Psychiatry* 2008;13:368–73. [PubMed: 18227835]
142. Bierut LJ, Stitzel JA, Wang JC, Hinrichs AL, Grucza RA, Xuei X, et al. Variants in nicotinic receptors and risk for nicotine dependence. *Am J Psychiatry* 2008;165:1163–71. [PubMed: 18519524]
143. Thorgeirsson TE, Geller F, Sulem P, Rafnar T, Wiste A, Magnusson KP, et al. A variant associated with nicotine dependence, lung cancer and peripheral arterial disease. *Nature* 2008;452:638–42. [PubMed: 18385739]
144. Bierut LJ, Madden PA, Breslau N, Johnson EO, Hatsukami D, Pomerleau OF, et al. Novel genes identified in a high-density genome wide association study for nicotine dependence. *Hum Mol Genet* 2007;16:24–35. [PubMed: 17158188]
145. Breitling LP, Dahmen N, Mittelstraß K, Illig T, Rujescu D, Raum E, et al. Smoking cessation and variations in nicotinic acetylcholine receptor subunits $\alpha 5$, $\alpha 3$, and $\beta 4$ genes. *Biol Psychiatry*. 2008;10.1016/j.biopsych.2008.10.004

146. Conti DV, Lee W, Li D, Liu J, Van Den Berg D, Thomas PD, et al. Nicotinic acetylcholine receptor $\beta 2$ subunit gene implicated in a systems-based candidate gene study of smoking cessation. *Hum Mol Genet* 2008;17:2834–48. [PubMed: 18593715]
147. Nestler EJ. Cellular responses to chronic treatment with drugs of abuse. *Crit Rev Neurobiol* 1993;7:23–39. [PubMed: 8385579]
148. Picciotto M, Corrigall WA. Neuronal systems underlying behaviors related to nicotine addiction: Neural circuits and molecular genetics. *J Neurosci* 2002;22:3338–41. [PubMed: 11978809]
149. Dani JA, Heinemann S. Molecular and cellular aspects of nicotine abuse. *Neuron* 1996;16:905–8. [PubMed: 8630247]
150. Gentry CL, Lukas RJ. Regulation of nicotinic acetylcholine receptor numbers and function by chronic nicotine exposure. *Curr Drug Targets CNS Neurol Disorders* 2002;1:359–85.
151. Collins AC, Romm E, Wehner JM. Nicotine tolerance: An analysis of the time course of its development and loss in the rat. *Psychopharmacol* 1988;96:7–14.
152. Breese CR, Marks MJ, Logel J, Adams CE, Sullivan B, Collins AC, et al. Effect of smoking history on [3 H]nicotine binding in human postmortem brain. *J Pharmacol Exp Ther* 1997;282:7–13. [PubMed: 9223534]
153. Quick M, Lester R. Desensitization of neuronal nicotinic receptors. *J Neurobiol* 2002;53:457–78. [PubMed: 12436413]
154. Miller RP, Rotenberg KS, Adir J. Effect of dose on the pharmacokinetics of intravenous nicotine in the rat. *Drug Metab Dispos* 1977;5:436–43. [PubMed: 20291]
155. Rotenberg KS, Adir J. Pharmacokinetics of nicotine in rats after multiple-cigarette smoke exposure. *Toxicol Appl Pharmacol* 1983;69:1–11. [PubMed: 6857681]
156. Schwartz RD, Kellar KJ. In vivo regulation of [3 H]acetylcholine recognition sites in brain by nicotinic cholinergic drugs. *J Neurochem* 1985;45:427–33. [PubMed: 4009168]
157. Collins AC, Luo Y, Selvaag S, Marks MJ. Sensitivity to nicotine and brain nicotinic receptors are altered by chronic nicotine and mecamylamine infusion. *J Pharmacol Exp Ther* 1994;271:125–33. [PubMed: 7965705]
158. Peng X, Gerzanich V, Anand R, Whiting PJ, Lindstrom J. Nicotine-induced increase in neuronal nicotinic receptors results from a decrease in the rate of receptor turnover. *Mol Pharmacol* 1994;46:523–30. [PubMed: 7935334]
159. Gopalakrishnan M, Monteggia LM, Anderson DJ, Molinari EJ, Piattoni-Kaplan M, Donnelly-Roberts D, et al. Stable expression, pharmacologic properties and regulation of the human neuronal nicotinic acetylcholine $\alpha 4 \beta 2$ receptor. *J Pharmacol Exp Ther* 1996;276:289–97. [PubMed: 8558445]
160. Whiteaker P, Sharples CG, Wonnacott S. Agonist-induced up-regulation of $\alpha 4 \beta 2$ nicotinic acetylcholine receptors in M10 cells: Pharmacological and spatial definition. *Mol Pharmacol* 1998;53:950–62. [PubMed: 9584223]
161. Sabey K, Paradiso K, Zhang J, Steinbach JH. Ligand binding and activation of rat nicotinic $\alpha 4 \beta 2$ receptors stably expressed in HEK293 cells. *Mol Pharmacol* 1999;55:58–66. [PubMed: 9882698]
162. Peng X, Gerzanich V, Anand R, Wang F, Lindstrom J. Chronic nicotine treatment up-regulates $\alpha 3$ and $\alpha 7$ acetylcholine receptor subtypes expressed by the human neuroblastoma cell line SH-SY5Y. *Mol Pharmacol* 1997;51:776–84. [PubMed: 9145915]
163. Nashmi R, Lester H. Cell autonomy, receptor autonomy, and thermodynamics in nicotine receptor up-regulation. *Biochem Pharmacol* 2007;74:1145–54. [PubMed: 17662697]
164. Fenster CP, Whitworth TL, Sheffield EB, Quick MW, Lester RA. Upregulation of surface $\alpha 4 \beta 2$ nicotinic receptors is initiated by receptor desensitization after chronic exposure to nicotine. *J Neurosci* 1999;19:4804–14. [PubMed: 10366615]
165. Benowitz NL, Porchet H, Jacob P. Nicotine dependence and tolerance in man: Pharmacokinetic and pharmacodynamic investigations. *Prog Brain Res* 1989;79:279–87. [PubMed: 2587748]
166. Katz B, Thesleff S. A study of the desensitization produced by acetylcholine at the motor end-plate. *J Physiol* 1957;138:63–80. [PubMed: 13463799]
167. Fonck C, Cohen BN, Nashmi R, Whiteaker P, Wagenaar DA, Rodrigues-Pinguet N, et al. Novel seizure phenotype and sleep disruptions in knock-in mice with hypersensitive $\alpha 4^*$ nicotinic receptors. *J Neurosci* 2005;25:11396–411. [PubMed: 16339034]

168. Green WN. Ion channel assembly: Creating structures that function. *J Gen Physiol* 1999;113:163–70. [PubMed: 9925815]
169. Millar NS, Harkness PC. Assembly and trafficking of nicotinic acetylcholine receptors. *Mol Membr Biol* 2008;25:279–92. [PubMed: 18446614]
170. Marks MJ, Pauly JR, Gross SD, Deneris ES, Hermans-Borgmeyer I, Heinemann SF, et al. Nicotine binding and nicotinic receptor subunit RNA after chronic nicotine treatment. *J Neurosci* 1992;12:2765–84. [PubMed: 1613557]
171. Gopalakrishnan M, Molinari EJ, Sullivan JP. Regulation of human $\alpha 4\beta 2$ neuronal nicotinic acetylcholine receptors by cholinergic channel ligands and second messenger pathways. *Mol Pharmacol* 1997;52:524–34. [PubMed: 9281615]
172. Rothhut B, Romano SJ, Vijayaraghavan S, Berg DK. Post-translational regulation of neuronal acetylcholine receptors stably expressed in a mouse fibroblast cell line. *J Neurobiol* 1996;29:115–25. [PubMed: 8748376]
173. Bencherif M, Fowler K, Lukas RJ, Lippiello PM. Mechanisms of up-regulation of neuronal nicotinic acetylcholine receptors in clonal cell lines and primary cultures of fetal rat brain. *J Pharmacol Exp Ther* 1995;275:987–94. [PubMed: 7473192]
174. Kuryatov A, Luo J, Cooper J, Lindstrom J. Nicotine acts as a pharmacological chaperone to up-regulate human $\alpha 4\beta 2$ acetylcholine receptors. *Mol Pharmacol* 2005;68:1839–51. [PubMed: 16183856]
175. Jia L, Flotildes K, Li M, Cohen BN. Nicotine trapping causes the persistent desensitization of $\alpha 4\beta 2$ nicotinic receptors expressed in oocytes. *J Neurochem* 2003;84:753–66. [PubMed: 12562520]
176. Sallette J, Bohler S, Benoit P, Soudant M, Pons S, Le Novère N, et al. An extracellular protein microdomain controls up-regulation of neuronal nicotinic acetylcholine receptors by nicotine. *J Biol Chem* 2004;279:18767–75. [PubMed: 14764595]
177. Sallette J, Pons S, Devillers-Thiery A, Soudant M, Prado de Carvalho L, Changeux J, et al. Nicotine upregulates its own receptors through enhanced intracellular maturation. *Neuron* 2005;46:595–607. [PubMed: 15944128]
178. Marks MJ, Grady SR, Collins AC. Downregulation of nicotinic receptor function after chronic nicotine infusion. *J Pharmacol Exp Ther* 1993;266:1268–76. [PubMed: 8371136]
179. Buisson B, Bertrand D. Chronic exposure to nicotine upregulates the human $\alpha 4\beta 2$ nicotinic acetylcholine receptor function. *J Neurosci* 2001;21:1819–29. [PubMed: 11245666]
180. Vallejo YF, Buisson B, Bertrand D, Green WN. Chronic nicotine exposure upregulates nicotinic receptors by a novel mechanism. *J Neurosci* 2005;25:5563–72. [PubMed: 15944384]
181. Mao D, Perry D, Yasuda R, Wolfe B, Kellar K. The $\alpha 4\beta 2\alpha 5$ nicotinic cholinergic receptor in rat brain is resistant to up-regulation by nicotine in vivo. *J Neurochem* 2008;104:446–56. [PubMed: 17961152]
182. Pauly JR, Marks MJ, Gross SD, Collins AC. An autoradiographic analysis of cholinergic receptors in mouse brain after chronic nicotine treatment. *J Pharmacol Exp Ther* 1991;258:1127–36. [PubMed: 1890617]
183. Nguyen HN, Rasmussen BA, Perry D. Binding and functional activity of nicotinic cholinergic receptors in selected rat brain regions are increased following long-term but not short-term nicotine treatment. *J Neurochem* 2004;90:40–9. [PubMed: 15198665]
184. Rowell PP, Wonnacott S. Evidence for functional activity of up-regulated nicotine binding sites in rat striatal synaptosomes. *J Neurochem* 1990;55:2105–10. [PubMed: 2230812]
185. Marshall DL, Redfern PH, Wonnacott S. Presynaptic nicotinic modulation of dopamine release in the three ascending pathways studied by in vivo microdialysis: Comparison of naive and chronic nicotine-treated rats. *J Neurochem* 1997;68:1511–9. [PubMed: 9084421]
186. Grilli M, Parodi M, Raiteri M, Marchi M. Chronic nicotine differentially affects the function of nicotinic receptor subtypes regulating neurotransmitter release. *J Neurochem* 2005;93:1353–60. [PubMed: 15934954]
187. Parker SL, Fu Y, McAllen K, Luo J, McIntosh JM, Lindstrom JM, et al. Up-regulation of brain nicotinic acetylcholine receptors in the rat during long-term self-administration of nicotine: Disproportionate increase of the $\alpha 6$ subunit. *Mol Pharmacol* 2004;65:611–22. [PubMed: 14978239]

188. McCallum SE, Parameswaran N, Bordia T, Fan H, Tyndale RF, Langston JW, et al. Increases in $\alpha 4^*$ but not $\alpha 3^*/\alpha 6^*$ nicotinic receptor sites and function in the primate striatum following chronic oral nicotine treatment. *J Neurochem* 2006;96:1028–41. [PubMed: 16412091]
189. Visanji NP, Mitchell SN, O'Neill MJ, Duty S. Chronic pre-treatment with nicotine enhances nicotine-evoked striatal dopamine release and $\alpha 6$ and $\beta 3$ nicotinic acetylcholine receptor subunit mRNA in the substantia nigra pars compacta of the rat. *Neuropharmacol* 2006;50:36–46.
190. Grady SR, Grun EU, Marks MJ, Collins AC. Pharmacological comparison of transient and persistent [3 H]dopamine release from mouse striatal synaptosomes and response to chronic L-nicotine treatment. *J Pharmacol Exp Ther* 1997;282:32–43. [PubMed: 9223537]
191. Walsh H, Govind AP, Mastro R, Hoda JC, Bertrand D, Vallejo Y, et al. Up-regulation of nicotinic receptors by nicotine varies with receptor subtype. *J Biol Chem* 2008;283:6022–32. [PubMed: 18174175]
192. Wang F, Nelson ME, Kuryatov A, Olale F, Cooper J, Keyser K, et al. Chronic nicotine treatment up-regulates human $\alpha 3\beta 2$ but not $\alpha 3\beta 4$ acetylcholine receptors stably transfected in human embryonic kidney cells. *J Biol Chem* 1998;273:28721–32. [PubMed: 9786868]
193. Kawai H, Berg DK. Nicotinic acetylcholine receptors containing $\alpha 7$ subunits on rat cortical neurons do not undergo long-lasting inactivation even when up-regulated by chronic nicotine exposure. *J Neurochem* 2001;78:1367–78. [PubMed: 11579145]
194. Everitt B, Robbins T. Neural systems of reinforcement for drug addiction: From actions to habits to compulsion. *Nat Neurosci* 2005;8:1481–9. [PubMed: 16251991]
195. Jacobs EH, Smit AB, de Vries TJ, Schoffelman AN. Neuroadaptive effects of active versus passive drug administration in addiction research. *Trends Pharmacol Sci* 2003;24:566–73. [PubMed: 14607079]
196. Nestler E. Is there a common molecular pathway for addiction? *Nat Neurosci* 2005;8:1445–9. [PubMed: 16251986]
197. Konu O, Kane JK, Barrett T, Vawter MP, Chang R, Ma JZ, et al. Region-specific transcriptional response to chronic nicotine in rat brain. *Brain Res* 2001;909:194–203. [PubMed: 11478936]
198. Yeom M, Shim I, Lee HJ, Hahm DH. Proteomic analysis of nicotine-associated protein expression in the striatum of repeated nicotine-treated rats. *Biochem Biophys Res Commun* 2005;326:321–8. [PubMed: 15582580]
199. Katsura M, Mohri Y, Shuto K, Hai-Du Y, Amano T, Tsujimura A, et al. Up-regulation of L-type voltage-dependent calcium channels after long term exposure to nicotine in cerebral cortical neurons. *J Biol Chem* 2002;277:7979–88. [PubMed: 11756415]
200. Hayashida S, Katsura M, Torigoe F, Tsujimura A, Ohkuma S. Increased expression of L-type high voltage-gated calcium channel $\alpha 1$ and $\alpha 2/\delta$ subunits in mouse brain after chronic nicotine administration. *Brain Res Mol Brain Res* 2005;135:280–4. [PubMed: 15857690]
201. Zhang XF, Hu XT, White FJ. Whole-cell plasticity in cocaine withdrawal: Reduced sodium currents in nucleus accumbens neurons. *J Neurosci* 1998;18:488–98. [PubMed: 9412525]
202. Zhang XF, Cooper DC, White FJ. Repeated cocaine treatment decreases whole-cell calcium current in rat nucleus accumbens neurons. *J Pharmacol Exp Ther* 2002;301:1119–25. [PubMed: 12023545]
203. Cooper DC, Moore SJ, Staff NP, Spruston N. Psychostimulant-induced plasticity of intrinsic neuronal excitability in ventral subiculum. *J Neurosci* 2003;23:9937–46. [PubMed: 14586024]
204. Hu XT, Basu S, White F. Repeated cocaine administration suppresses HVA- Ca^{2+} potentials and enhances activity of K^+ channels in rat nucleus accumbens neurons. *J Neurophysiol* 2004;92:1597–607. [PubMed: 15331648]
205. Nasif FJ, Sidiropoulou K, Hu XT, White F. Repeated cocaine administration increases membrane excitability of pyramidal neurons in the rat medial prefrontal cortex. *J Pharmacol Exp Ther* 2005;312:1305–13. [PubMed: 15574686]
206. Dong Y, Nasif FJ, Tsui JJ, Ju WY, Cooper D, Hu XT, et al. Cocaine-induced plasticity of intrinsic membrane properties in prefrontal cortex pyramidal neurons: Adaptations in potassium currents. *J Neurosci* 2005;25:936–40. [PubMed: 15673674]
207. Nasif FJ, Hu XT, White F. Repeated cocaine administration increases voltage-sensitive calcium currents in response to membrane depolarization in medial prefrontal cortex pyramidal neurons. *J Neurosci* 2005;25:3674–9. [PubMed: 15814798]

208. Vernino S, Amador M, Luetje CW, Patrick J, Dani JA. Calcium modulation and high calcium permeability of neuronal nicotinic acetylcholine receptors. *Neuron* 1992;8:127–34. [PubMed: 1370370]
209. Léna C, Changeux JP. Role of Ca^{2+} ions in nicotinic facilitation of GABA release in mouse thalamus. *J Neurosci* 1997;17:576–85. [PubMed: 8987780]
210. Ragozzino D, Barabino B, Fucile S, Eusebi F. Ca^{2+} permeability of mouse and chick nicotinic acetylcholine receptors expressed in transiently transfected human cells. *J Physiol* 1998;507(Pt 3): 749–57. [PubMed: 9508836]
211. Flavell SW, Greenberg ME. Signaling mechanisms linking neuronal activity to gene expression and plasticity of the nervous system. *Annu Rev Neurosci* 2008;31:563–90. [PubMed: 18558867]
212. Rezvani K, Teng Y, Shim D, De Biasi M. Nicotine regulates multiple synaptic proteins by inhibiting proteasomal activity. *J Neurosci* 2007;27:10508–19. [PubMed: 17898222]
213. Vezina P, McGehee DS, Green WN. Exposure to nicotine and sensitization of nicotine-induced behaviors. *Prog Neuropsychopharmacol Biol Psychiatry* 2007;31:1625–38. [PubMed: 17936462]
214. Wang F, Chen H, Steketee J, Sharp BM. Upregulation of ionotropic glutamate receptor subunits within specific mesocorticolimbic regions during chronic nicotine self-administration. *Neuropsychopharmacol* 2007;32:103–9.
215. Delibas N, Doguc DK, Sutcu R, Eroglu E, Gökalp O. Effect of nicotine on hippocampal nicotinic acetylcholine $\alpha 7$ receptor and NMDA receptor subunits 2A and 2B expression in young and old rats. *Int J Neurosci* 2005;115:1151–63. [PubMed: 16040357]
216. Wall A, Gong ZH, Johnson AE, Meyerson B, Zhang X. Cross-tolerance in drug response and differential changes in central nicotinic and N-methyl-D-aspartate receptor binding following chronic treatment with either (+)- or (–)-nicotine. *Psychopharmacol* 2000;148:186–95.
217. Levin E, Tizabi Y, Rezvani A, Caldwell DP, Petro A, Getachew B. Chronic nicotine and dizocilpine effects on regionally specific nicotinic and NMDA glutamate receptor binding. *Brain Res* 2005;1041:132–42. [PubMed: 15829222]
218. Vieyra-Reyes P, Picciotto M, Mineur YS. Voluntary oral nicotine intake in mice down-regulates GluR2 but does not modulate depression-like behaviors. *Neurosci Letts* 2008;434:18–22. [PubMed: 18261852]
219. Kane JK, Hwang Y, Konu O, Loughlin SE, Leslie FM, Li MD. Regulation of Homer and Group I metabotropic glutamate receptors by nicotine. *Eur J Neurosci* 2005;21:1145–54. [PubMed: 15813924]
220. Li SP, Park MS, Bahk JY, Kim MO. Chronic nicotine and smoking exposure decreases GABA(B1) receptor expression in the rat hippocampus. *Neurosci Letts* 2002;334:135–9. [PubMed: 12435490]
221. Li SP, Park MS, Kim JH, Kim MO. Chronic nicotine and smoke treatment modulate dopaminergic activities in ventral tegmental area and nucleus accumbens and the gamma-aminobutyric acid type B receptor expression of the rat prefrontal cortex. *J Neurosci Res* 2004;78:868–79. [PubMed: 15521060]
222. Amantea D, Tessari M, Bowery NG. Reduced G-protein coupling to the GABAB receptor in the nucleus accumbens and the medial prefrontal cortex of the rat after chronic treatment with nicotine. *Neurosci Letts* 2004;355:161–4. [PubMed: 14732456]
223. Fung YK, Schmid MJ, Anderson TM, Lau YS. Effects of nicotine withdrawal on central dopaminergic systems. *Pharmacol Biochem Behav* 1996;53:635–40. [PubMed: 8866966]
224. Le Foll B, Diaz J, Sokoloff P. Increased dopamine D3 receptor expression accompanying behavioral sensitization to nicotine in rats. *Synapse* 2003;47:176–83. [PubMed: 12494400]
225. Kenny PJ, File SE, Rattray M. Nicotine regulates 5-HT(1A) receptor gene expression in the cerebral cortex and dorsal hippocampus. *Eur J Neurosci* 2001;13:1267–71. [PubMed: 11285026]
226. Xu Z, Seidler FJ, Cousins MM, Slikker W, Slotkin TA. Adolescent nicotine administration alters serotonin receptors and cell signaling mediated through adenylyl cyclase. *Brain Res* 2002;951:280–92. [PubMed: 12270507]
227. Wewers ME, Dhatt RK, Snively TA, Tejawani GA. The effect of chronic administration of nicotine on antinociception, opioid receptor binding and met-enkephalin levels in rats. *Brain Res* 1999;822:107–13. [PubMed: 10082888]

228. Brunzell D, Russell DS, Picciotto M. In vivo nicotine treatment regulates mesocorticolimbic CREB and ERK signaling in C57BL/6J mice. *J Neurochem* 2003;84:1431–41. [PubMed: 12614343]
229. Collins SL, Wade D, Ledon J, Izenwasser S. Neurochemical alterations produced by daily nicotine exposure in periadolescent vs. adult male rats *Eur J Pharmacol* 2004;502:75–85.
230. Li S, Kim KY, Kim JH, Kim JH, Park MS, Bahk JY, et al. Chronic nicotine and smoking treatment increases dopamine transporter mRNA expression in the rat midbrain. *Neurosci Letts* 2004;363:29–32. [PubMed: 15157990]
231. Awtry T, Werling L. Acute and chronic effects of nicotine on serotonin uptake in prefrontal cortex and hippocampus of rats. *Synapse* 2003;50:206–11. [PubMed: 14515338]
232. Houdi AA, Dasgupta R, Kindy MS. Effect of nicotine use and withdrawal on brain preproenkephalin A mRNA. *Brain Res* 1998;799:257–63. [PubMed: 9675304]
233. Isola R, Zhang H, Tejwani G, Neff N, Hadjiconstantinou M. Dynorphin and prodynorphin mRNA changes in the striatum during nicotine withdrawal. *Synapse* 2008;62:448–55. [PubMed: 18361441]
234. Mathieu-Kia AM, Besson MJ. Repeated administration of cocaine, nicotine and ethanol: Effects on preprodynorphin, preprotachykinin A and preproenkephalin mRNA expression in the dorsal and the ventral striatum of the rat. *Brain Res Mol Brain Res* 1998;54:141–51. [PubMed: 9526067]
235. Katsura M, Ohkuma S, Xu J, Hibino Y, Tsujimura A, Kuriyama K. Continuous treatment with nicotine increases diazepam binding inhibitor (DBI) and its mRNA in the mouse brain. *Brain Res Mol Brain Res* 1998;55:345–9. [PubMed: 9582452]
236. Yamazaki Y, Jia Y, Wong J, Sumikawa K. Chronic nicotine-induced switch in SRC-family kinase signaling for long-term potentiation induction in hippocampal CA1 pyramidal cells. *Eur J Neurosci* 2006;24:3271–84. [PubMed: 17156388]
237. Pandey SC. Effects of chronic nicotine treatment on the expression of phospholipase C isozymes and the α subunit of gq/11 protein in the rat brain. *Neurosci Letts* 1996;212:127–30. [PubMed: 8832655]
238. Nisell M, Nomikos GG, Chergui K, Grillner P, Svensson TH. Chronic nicotine enhances basal and nicotine-induced fos immunoreactivity preferentially in the medial prefrontal cortex of the rat. *Neuropsychopharmacol* 1997;17:151–61.
239. Pich EM, Pagliusi SR, Tessari M, Talabot-Ayer D, Hooft van Huijsduijnen R, Chiamulera C. Common neural substrates for the addictive properties of nicotine and cocaine. *Science* 1997;275:83–6. [PubMed: 8974398]
240. Marttila K, Raattamaa H, Ahtee L. Effects of chronic nicotine administration and its withdrawal on striatal fosB/ Δ fosB and c-fos expression in rats and mice. *Neuropharmacol* 2006;51:44–51.
241. Pluzarev O, Pandey S. Modulation of CREB expression and phosphorylation in the rat nucleus accumbens during nicotine exposure and withdrawal. *J Neurosci Res* 2004;77:884–91. [PubMed: 15334606]
242. Pandey SC, Roy A, Xu T, Mittal N. Effects of protracted nicotine exposure and withdrawal on the expression and phosphorylation of the CREB gene transcription factor in rat brain. *J Neurochem* 2001;77:943–52. [PubMed: 11331423]
243. Nuutinen S, Barik J, Jones IW, Wonnacott S. Differential effects of acute and chronic nicotine on Elk-1 in rat hippocampus. *Neuroreport* 2007;18:121–6. [PubMed: 17301675]
244. Blum M, Wu G, Mudó G, Belluardo N, Andersson K, Agnati LF, et al. Chronic continuous infusion of (–)nicotine reduces basic fibroblast growth factor messenger RNA levels in the ventral midbrain of the intact but not of the 6-hydroxydopamine-lesioned rat. *Neuroscience* 1996;70:169–77. [PubMed: 8848122]
245. Kenny PJ, File SE, Rattray M. Acute nicotine decreases, and chronic nicotine increases the expression of brain-derived neurotrophic factor mRNA in rat hippocampus. *Brain Res Mol Brain Res* 2000;85:234–8. [PubMed: 11146126]
246. Kane JK, Konu O, Ma JZ, Li MD. Nicotine coregulates multiple pathways involved in protein modification/degradation in rat brain. *Brain Res Mol Brain Res* 2004;132:181–91. [PubMed: 15582157]
247. Liu JJ, Mohila CA, Gong Y, Govindarajan N, Onn SP. Chronic nicotine exposure during adolescence differentially influences calcium-binding proteins in rat anterior cingulate cortex. *Eur J Neurosci* 2005;22:2462–74. [PubMed: 16307589]

Figure 1. Homeostatic model of $\alpha 4\beta 2^*$ nAChR upregulation

The diagram shows a hypothetical scheme of a "cholinergic synapse" in the context of a neuronal circuit under five different conditions. The first three conditions represent the synapse under normal conditions (left), during acute (center) and prolonged (right) application of nicotine. Through upregulation of nAChRs, the synapse adapts to the prolonged presence of nicotine. The right two conditions illustrate possible states of the synapse following withdrawal from nicotine, with receptors either functionally upregulated (left) or downregulated (right). Any of the states that are not homeostatically balanced are potentially capable of initiating downstream changes that could underlie behavioral tolerance and sensitization.

Table 1

$\alpha 3\beta 4^*$ (type 3)	$\alpha 2\beta 4^*$ (type 4)	$\alpha 6\beta 2\beta 3^*$
$[\alpha 4-6, \beta 3]$ cytisine	$[\alpha 3-6, \beta 3]$ DMPP (relative to nicotine)	[none]
MHB	IPN	MLA
withdrawal, secondary reward axis	secondary reward axis	regulation of dopamine release

Table 2
Downstream molecular changes following chronic in vivo nicotine treatment

Parameter	Model	Regulation	Brain Region	Method	Reference
Non-ligand gated ion channels					
TWIK-1 mRNA	Adult male rats (225–250g)—self-administration (0.03 mg/kg/injection)—22–23 hrs/day—21 days	↓	AMG	cDNA microarray RT-PCR	[197]
TWIK-1 mRNA	Adult male rats (225–250g)—self-administration (0.03 mg/kg/injection)—22–23 hrs/day—21 days	↑	VTA	cDNA microarray RT-PCR	[197]
THIK-1 protein and mRNA	Adult male rats (250–280g)—twice daily injections (0.4 mg/kg s.c.)—7 days	↓ (3dwd w/nic inj. on d11)	Str	2-D electrophoresis MALDI-TOF MS RT-PCR	[198]
L-type VDCC binding & protein	Mice (ddY) (30g)—1 mg/kg s.c. three times/day—7 days	↑	CTX	[³ H]verapamil binding [199]; Western blot [200]	[199,200]
Ligand-gated ion channels					
NMDAR: NR2A & NR2B protein	Adult male rats (250–350g)—self-administration (0.03 mg/kg i.v.; FR1 schedule) 23 h/day—18 days [214]; Adult rats (250–300g)—0.45mg/kg s.c. twice daily—28 days [215]	↑	PFC [214]; Hipp [215]	Western blot	[214,215]
NMDAR binding	Adult male rats (150–205g)—2 mg/kg twice daily—21 days*(+)-nicotine treatment [216]; Adult female rats—5mg/kg/day Alzet 2004—4 weeks [217]	↑	Hipp (CA1) [216]; Hipp [217]	[³ H]MK801 binding	[216,217]
GluR2/3 protein	Adult male rats (250–350g)—self-administration (0.03 mg/kg i.v.; FR1 schedule) 23 h/day—18 days	↑	VTA	Western blot	[214]
GluR2 protein	Adolescent C57BL/6J mice—13–28 mg/L in sucrose water-80 days treatment -no withdrawal-adults at time of measure	↓	NAcc core & shell; VTA	Western blot	[218]
G-protein signaling					
mGluR1 mRNA	Adult male rats (250–350g)—4.0 mg/kg/day i.p. injection—7 days, 14 days	↑	VTA	Quantitative RT-PCR	[219]
GABA _B mRNA	Adult male rats (250g)—3 mg/kg/day orally—4 weeks	↓	Hipp; PFC	In situ hybridization; RNase protection assay	[220,221]
GABA _B binding	Adult male rats (180–200g)—0.4 mg/kg once daily—14 days	No change	NAcc core & shell; PFC; VTA; CPu; Parietal CTX	[³ H]GABA binding in the presence of isoguvacine	[222]
Dopamine D1R protein	Adult male rats (250g)—3 mg/kg/day orally—4 weeks	↓	NAcc	Western blot	[221]

Parameter	Model	Regulation	Brain Region	Method	Reference
Dopamine D2R binding	Adult male rats (180–200g)—1.5 mg/kg/day Alzet 2002 pumps—14 days	↓ (24 hrs wd)	NAcc	[³ H]spiperone binding	[223]
Dopamine D3R binding & mRNA	Adult male rats (180–200g)—0.5 mg/kg once daily—5 days	↑	NAcc Shell	[¹²⁵ I]7-OH-PIPAT binding; in situ hybridization	[224]
5HT1A mRNA	Adult male rats (240–400g)—0.5 mg/kg i.p. twice daily—7 days	↓	CTX	In situ hybridization	[225]
5HT1A mRNA	Adult male rats (240–400g)—0.5 mg/kg i.p. twice daily—7 days	No change from control 24–72 hrs wd	CTX	In situ hybridization	[225]
5HT2R binding	PN30-47.5 rats, 6 mg/kg/day Alzet 1002	↓	Hipp; CTX	[³ H]ketanserin binding	[226]
5HT2R binding	PN30-47.5 rats, 6 mg/kg/day Alzet 1002	↑	Midbrain	[³ H]ketanserin binding	[226]
MOR binding	Adult male (250–300g) & female (205–240g) rats—0.4 mg/kg three times daily—14 days	↑	Str	[³ H]DAMGO binding	[227]
NT enzymes/transporters					
TH protein	Adult male rats (250g)—3 mg/kg/day orally—4 weeks [221]; 6–10 wk old C57BL/6J mice—200 µg/mL in saccharin water—4 weeks [228]	↑	VTA [221]; PFC [228]; AMG [228]	Western blot	[221,228]
TH protein	Adult male rats (250g)—3 mg/kg/day orally—4 weeks	↓	SNC	Western blot	[221]
DAT binding [229]; DAT mRNA [230]	PN30 male rats—0.4 mg/kg/day i.p. once daily—7 days [229]; Adult male rats (250g)—3 mg/kg/day orally—4 weeks [230]	↑	CPu [229]; NAcc Core & Shell [229]; VTA [230]; SNC [230]	[¹²⁵ I]RTI-121 binding [229]; In situ hybridization [230]	[229,230]
SERT binding	PN30 male rats—0.4 mg/kg/day i.p. once daily—7 days	↓	CPu; NAcc Core	[¹²⁵ I]RTI-55 binding	[229]
SERT binding	Adult male rats (225–325g)—0.7 mg/kg s.c. twice daily—10 days	↑	PFC; Hipp	[³ H]paroxetine binding	[231]
Neuropeptides					
Met-enkephalin protein	Adult male (250–300g) & female (205–240g) rats—0.4 mg/kg three times daily—14 days	↓	Str; midbrain	radioimmunoassay	[227]
Preproenkephalin A mRNA	Adult male rats (300+/- 25g)—0.6 mg/kg s.c. twice daily—14 days	↓ (2 hrs wd)	Str; Hipp	Northern blot	[232]
Preproenkephalin A mRNA	Adult male rats (300+/- 25g)—0.6 mg/kg s.c. twice daily—14 days	↑ (24 hrs wd)	Str; Hipp	Northern blot	[232]

Parameter	Model	Regulation	Brain Region	Method	Reference
Dynorphin protein	Male swiss-webster mice (25–30g) —2 mg/kg s.c. 4 times daily—14 days	↓ (0.5–72 hrs wd)	Str	radioimmunoassay	[233]
Prodynorphin mRNA	Male swiss-webster mice (25–30g) —2 mg/kg s.c. 4 times daily—14 days	↑ (8–96 hrs wd)	Str	Northern blot	[233]
Prodynorphin mRNA	Male swiss-webster mice (25–30g) —2 mg/kg s.c. 4 times daily—14 days	↑ (24–72 hrs wd)	Str; NAcc	In situ hybridization	[233]
Preprodynorphin mRNA	Male rats—0.4 mg/kg free base s.c. 3 times daily—14 days	↓	NAcc ventral shell	In situ hybridization	[234]
DBI protein & mRNA	Male ddY mice (30g)—1 mg/kg s.c. three times/day—7 days	↑ (24 hrs wd)	CTX	Radioimmunoassay; RNA blot hybridization	[235]
Enzymes (kinases, phosphatases, etc.)					
ERK1 protein	6–10wk old C57BL/6J mice—200 µg/mL in saccharin water—4 weeks	↓	AMG	Western blot	[228]
pERK1 protein	6–10wk old C57BL/6J mice—200 µg/mL in saccharin water—4 weeks	↓	AMG	Western blot	[228]
pERK1 protein	6–10wk old C57BL/6J mice—200 µg/mL in saccharin water—4 weeks	↑	PFC	Western blot	[228]
ERK2 protein	6–10wk old C57BL/6J mice—200 µg/mL in saccharin water—4 weeks	↓	AMG	Western blot	[228]
pERK2 protein	6–10wk old C57BL/6J mice—200 µg/mL in saccharin water—4 weeks	↑	PFC	Western blot	[228]
CNP1 protein	Adult male rats (250–280g)—twice daily injections (0.4 mg/kg s.c.)—7 days	↑ (3dwd w/nic inj. on d11)	Str	2-D electrophoresis MALDI- TOF MS	[198]
DNase113 protein & mRNA	Adult male rats (250–280g)—twice daily injections (0.4 mg/kg s.c.)—7 days	↑ (3dwd w/nic inj. on d11)	Str	2-D electrophoresis MALDI- TOF MS RT-PCR	[198]
PTEN mRNA	Adult male rats (225–250g)—self- administration (0.03 mg/kg/ injection)—22–23 hrs/day—21 days	↑	AMG	cDNA microarray RT-PCR	[197]
Src protein	Rats—1 mg/kg twice daily—10 days	↑	Hipp	Western blot	[236]
PLC-β1 protein	Adult male rats (225–250g)—0.7 mg/kg base s.c. twice daily—10 days	↑	CTX	Western blot	[237]
Transcription					
Fos-like immunoreactivity	Adult male rats (250–300g)—0.5 mg/kg once daily—12 days	↑	mPFC	immunohistochemistry	[238]
Fos-related antigen expression	Adult rats self-administration (0.03 mg/kg/injection)—2 weeks	↑	NAcc	immunoblot	[239]

Parameter	Model	Regulation	Brain Region	Method	Reference
FosB/ Δ FosB	5-week old male NMRI mice—nicotine in drinking water for 7 weeks; Adult male rats (270–370g)—0.5 mg/kg s.c. once daily—5 days	↑	NAcc	immunohistochemistry	[240]
c-Fos	5-week old male NMRI mice—nicotine in drinking water—7 weeks	↑	CPu	immunohistochemistry	[240]
c-Fos	Adult male rats (270–370g)—0.5 mg/kg s.c. once daily—5 days	↑	NAcc	immunohistochemistry	[240]
CREB, p-CREB protein	Adult male rats (225–250g)—0.7 mg/kg twice a day—10 days [241, 242]	↓ (18 hrs wd)	NAcc Shell [241]; Cingulate gyrus, parietal CTX, piriform CTX, medial & basolateral AMG [242]	Gold immunolabelling [241, 242]	[241,242]
CREB protein	6–10wk old C57BL/6J mice—200 μ g/mL in saccharin water—4 weeks	↑ (0 & 24 hrs wd)	NAcc	Western blot	[228]
pCREB protein	6–10wk old C57BL/6J mice—200 μ g/mL in saccharin water—4 weeks	↑ (24 hrs wd)	VTA	Western blot	[228]
CREB protein	6–10wk old C57BL/6J mice—200 μ g/mL in saccharin water—4 weeks	↓	PFC	Western blot	[228]
pCREB protein	6–10wk old C57BL/6J mice—200 μ g/mL in saccharin water—4 weeks	↓	NAcc	Western blot	[228]
ZBP89 protein & mRNA	Adult male rats (250–280g)—twice daily injections (0.4 mg/kg s.c.)—7 days	↑ (3dwd w/nic inj. on d11)	Str	2-D electrophoresis MALDI-TOF MS RT-PCR	[198]
Elk-1 protein	Adult male rats (250–350g)—4 mg/kg/day free base Alzet pumps—14 days	↑	Hipp (CA1)	immunohistochemistry	[243]
Pre/post synaptic proteins					
Homer 1b/c mRNA	Adult male rats (250–350g)—4.0 mg/kg/day i.p. injection—14 days	↑	NAcc	Quantitative RT-PCR	[219]
Homer 2a/b mRNA	Adult male rats (250–350g)—4.0 mg/kg/day i.p. injection—14 days	↑	VTA	Quantitative RT-PCR	[219]
Growth factors					
FGF-2 mRNA	Adult male rats (150–250g)—0.72, 3.0, 7.2 mg/kg/day Alzet 2002 pumps—14 days	↓	SNC, VTA	RNase protection assay	[244]
BDNF protein & mRNA	Adult male rats (250–280g)—twice daily injections (0.4 mg/kg s.c.)—7 days	↓ (3dwd w/nic inj. on d11)	Str	2-D electrophoresis MALDI-TOF MS RT-PCR	[198]
BDNF mRNA	Adult male rats (250–400g)—twice daily 0.5 mg/kg i.p. injections—7 days	↑ (2 hrs wd)	Hipp (CA1)	In situ hybridization	[245]

Parameter	Model	Regulation	Brain Region	Method	Reference
Other					
BRAL-1 protein	Adult male rats (250–280g)—twice daily injections (0.4 mg/kg s.c.)—7 days	↓ (3dwd w/nic inj. on d11)	Str	2-D electrophoresis MALDI-TOF MS	[198]
DEDD protein & mRNA	Adult male rats (250–280g)—twice daily injections (0.4 mg/kg s.c.)—7 days	↓ (3dwd w/nic inj. on d11)	Str	2-D electrophoresis MALDI-TOF MS RT-PCR	[198]
Ubiquitin mRNA	Adult males rats (250–350g)—4.0 mg/kg/day i.p. injection—14 days	↑	PFC	cDNA microarray RT-PCR	[246]
Calretinin protein	Male rats (175–200g) 7.3–61.3 mg/kg/day nicotine salt in water—21 days	↑	Anterior cingulate CTX	immuno	[247]
Parvalbumin protein	Male rats (175–200g) 7.3–61.3 mg/kg/day nicotine salt in water—21 days	↑	Anterior cingulate CTX	immuno	[247]

Abbreviations: AMG, amygdala; CPu, caudate putamen; CTX, cortex; Hipp, hippocampus; i.p., intraperitoneal; i.v., intravenous; NAcc, nucleus accumbens; PFC, prefrontal cortex; SNc, substantia nigra; Str, striatum; s.c., subcutaneous; VTA, ventral tegmental area; wd, withdrawn; ↑, increased; ↓, decreased.