

Neural Systems Governed by Nicotinic Acetylcholine Receptors: Emerging Hypotheses

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Cholinergic neurons and nicotinic acetylcholine receptors (nAChRs) in the brain participate in diverse functions: reward, learning and memory, mood, sensory processing, pain, and neuroprotection. Nicotinic systems also have well-known roles in drug abuse. Here, we review recent insights into nicotinic function, linking exogenous and endogenous manipulations of nAChRs to alterations in synapses, circuits, and behavior. We also discuss how these contemporary advances can motivate attempts to exploit nicotinic systems therapeutically in Parkinson's disease, cognitive decline, epilepsy, and schizophrenia.

Introduction

Europeans first encountered nicotinic actions when Columbus's crew sampled tobacco in 1492. After Jean Nicot, the French ambassador to Portugal, introduced tobacco to Paris, botanists honored him by naming the plant *Nicotiana*, and later its active alkaloid was named nicotine. Claude Bernard (1851) found that nicotine activates muscle when applied directly but not when applied to motor nerves; this was eventually explained by the fact that nicotine and neurally released acetylcholine activate common receptors. In 2011, we know that cholinergic actions in the brain govern various processes: cognition (attention and executive function) (Couey et al., 2007; Levin and Rezvani, 2007; Heath and Picciotto, 2009; Howe et al., 2010), learning and memory (Gould, 2006; Couey et al., 2007; Levin and Rezvani, 2007), mood (anxiety, depression) (Picciotto et al., 2008), reward (addiction, craving) (Tang and Dani, 2009), and sensory processing (Heath and Picciotto, 2009).

The discoveries of Katz and contemporaries at the nerve-muscle synapse and autonomic ganglia gave rise to the modern view that the nicotinic cholinergic synapse is an exquisite biophysical switch, specialized to function on a time scale of ~ 1 ms and a distance scale of < 1 μm (Wathey et al., 1979; Stiles et al., 1996). This picture did not, however, conform well to the view that acetylcholine functions in the brain as primarily a slow, more widespread modulatory transmitter, somewhat analogous to the biogenic amines. Until the mid-1980s, the "switch" versus "modulator" views were generally reconciled by assuming that nicotinic acetylcholine receptors (nAChRs) activated the dopaminergic system (thus explaining the feeling of well-being during smoking), while most cholinergic actions in the brain occur via muscarinic acetylcholine receptors. This assumption became untenable when specific nicotine binding, and cloned neuronal nAChRs, were found in many brain regions (Marks et al., 1983; Schwartz and Kellar, 1983; Heinemann et al., 1987). We now realize that acetylcholine liberated from cholinergic nerve terminals often activates both nAChRs and muscarinic receptors.

Well-characterized cholinergic projection neurons in the brain include those of the basal forebrain, the medial habenula, the

striatum, and the vagal nucleus. Terminals of basal forebrain neurons radiate widely and richly innervate forebrain structures. The giant cholinergic interneurons of the striatum control several aspects of basal ganglia function (Cragg, 2006; Witten et al., 2010). Specificity within the cholinergic system arises in part through its receptors. Muscarinic and nicotinic classes comprise five and fifteen subunits, respectively. Nicotinic receptors are pentamers (Figure 1); brain nicotinic receptors can exist as heteromeric combinations of $\alpha(2-10)$ and $\beta(2-4)$ subunits, and as $\alpha 7$ homopentamers (in muscle-type receptors, the non- α subunits are $\beta 1$, γ or ϵ , and δ). Each nAChR subtype exhibits distinct biophysical and pharmacological properties. Even the precise order and stoichiometry of α and β subunits in the pentamer imposes differential response profiles. A major subtype in the brain is $\alpha 4\beta 2$; the $(\alpha 4_2\beta 2_3)$ stoichiometry exhibits at least 10-fold-higher sensitivity than $(\alpha 4_3\beta 2_2)$, so that only the former has the high sensitivity (HS) that allows activation at nicotine concentrations in the 0.1–1 μM range, produced by moderate tobacco use and by the various nicotine replacement therapies. $\alpha 7$ nAChRs also respond to nicotine concentrations roughly an order of magnitude higher than $\alpha 4_2\beta 2_3$, and $\alpha 7$ nAChRs have high Ca^{2+} permeability resembling that of NMDA receptors.

Most brain HS nAChRs reside on presynaptic terminals, where they stimulate neurotransmitter release (Gotti et al., 2006; Albuquerque et al., 2009). Such presynaptic nAChR activation influences synaptic efficacy and synaptic plasticity (Mansvelder and McGehee, 2000; Dani et al., 2001), spike-timing-dependent plasticity (Couey et al., 2007), frequency-dependent filtering (Exley and Cragg, 2008; Tang and Dani, 2009; Zhang et al., 2009), and overall signal-to-noise ratio in cortex (Disney et al., 2007). Many studies also reveal the presence of somatodendritic nAChRs, but there are relatively few classically defined somatodendritic cholinergic synapses (Aznavour et al., 2005). The "volume transmission" hypothesis states that ACh released from presynaptic terminals spreads to more distant areas, reaching concentrations < 1 μM (Descaries et al., 1997), but that multiple presynaptic impulses produce enough summed release to activate receptors (Lester, 2004).

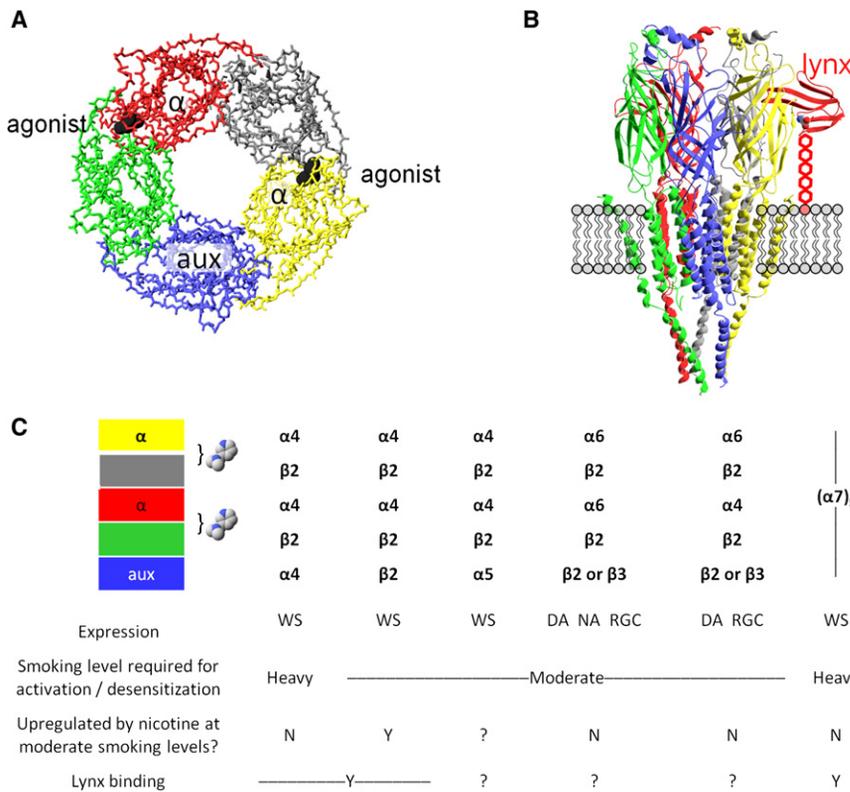


Figure 1. Major Characteristics of Some nAChRs

(A) A diagram of the symmetric or pseudosymmetric pentameric extracellular binding region, modeled by the acetylcholine receptor binding protein AChBP. The eyepoint is the cytosol; the side chains and transmembrane domains do not appear. The exemplar agonist (nicotine) is represented in black; two agonist binding sites form at the interface between subunits. The open state of the ion channel is more likely to occur when agonist molecules bind at both interfaces than at a single interface. An α subunit (red and yellow) always participates in the binding interface; the other participants are either α subunits (in $\alpha 7$ homopentameric nAChRs) or non- α subunits (in heteropentameric nAChRs such as $\alpha 4\beta 2^*$); (see the table in C). The auxiliary subunit (aux, in blue) does not participate in an agonist binding site.

(B) Depiction of a nAChR molecule in the membrane. The eyepoint is a neighboring nAChR. The receptor is Unwin's model for the *Torpedo* electric organ muscle-type AChR (Unwin, 2005). The model depicts the full extracellular region (mostly β sheets), which strongly resembles the AChBP structure shown in (A). Ribbons depict the structural elements, whereas neither backbone nor side-chain atoms appear. The model includes the full transmembrane region (mostly α -helical) and only part of the intracellular domains. The schematic also imagines a lynx molecule (red) bound at an α /non- α interface, positioned as in structures of snake α -toxins bound to AChBP (Hansen et al., 2005) or to the muscle nAChR (Dellisanti et al., 2007). Lynx binding, as indepen-

dently proposed in a recent study (Lyukmanova et al., 2011), occurs at the agonist site shown in (A). The lynx molecule, unlike toxins, is tethered to the membrane by a GPI linkage, here stretched to nearly its full extent and depicted as five hexagons.

(C) Some major nAChR subtypes found in brain. Each column represents the composition of a single pentameric receptor. The table shows our best present knowledge about the properties of detailed stoichiometries. The colored boxes correspond to the subunits of (A) and (B). The bracket and the nicotine molecules show the agonist-binding interfaces between individual subunits. Expression of each receptor subtype is wide-spread (WS), or restricted in the case of $\alpha 6^*$ nAChRs, confined largely to dopaminergic neurons (DA), noradrenergic neurons (NA), or retinal ganglion cells (RGC).

In most regions that receive cholinergic innervation, the high density of acetylcholinesterase (which can hydrolyze ACh at a rate of one per 100 μ s!) might vitiate the volume transmission mechanism. In the interpeduncular nucleus, the acetylcholinesterase density is sufficiently low to rationalize long-awaited, recent evidence that 20–50 Hz presynaptic stimulation eventually generates a postsynaptic response via volume transmission (Ren et al., 2011). As we will see below, the mystery of somatodendritic nAChRs can also be resolved by the sensitivity of $\alpha 7$ nAChRs to constant levels of another agonist, choline.

Although researchers have located the cholinergic neurons and the nicotinic receptors, the problem remains: how can changes in biophysical switches lead to widespread modulation? A series of explanations arise, because nicotinic systems are tightly balanced through a multilayered hierarchy of control mechanisms. Acetylcholinesterase efficiently hydrolyzes acetylcholine, both turning off cholinergic signaling and also reducing the likelihood of receptor desensitization. In addition, changes in subunit composition and stoichiometry can influence receptor desensitization, ligand affinity profiles, and conductance. Mutations in nicotinic receptor subunits are linked to human disease, $\alpha 4$ and $\beta 2$ in some epilepsies, $\alpha 7$ in schizophrenia, and $\alpha 5$ in nicotine addiction; and each mutation ultimately manifests itself as an imbalance in the properties of neuronal circuits. Hyperac-

tivating mutations in nAChR subunits have revealed the existence of previously underappreciated cholinergic mechanisms (Fonck et al., 2005; Drenan et al., 2008). Furthermore, posttranslational mechanisms such as upregulation can play a part in modifying the response properties of nAChRs and may underlie susceptibility toward nicotine dependence. Finally, nAChRs exist in complexes in the brain; interacting proteins engage in complexes with nAChRs and aid in the assembly and trafficking of nAChR to the plasma membrane; examples are RIC-3 (Lansdell et al., 2005), 14-3-3 proteins (Jeanclous et al., 2001), neuroligins (Cheng et al., 2009), and VILIP-1 (Lin et al., 2002).

The challenge of explaining the modulation of behavior in terms of the microscopic properties of all-or-none synapses occupies much of neuroscience; but one expects studies on nicotinic systems to lead the way, if only because of their venerability. Within the control hierarchy, especially sensitive points of regulation can have important sequelae. This review discusses three emerging hypotheses about ways that the nicotinic system can be modulated. First is the role played by lynx modulators as molecular brakes over the cholinergic system in stabilizing neural plasticity and circuitry. A second example is a critical time in neurodevelopment that controls the maturation of inhibition; misregulation of $\alpha 7$ nAChR function may lead to increased risk of schizophrenia. Lastly, we discuss how chronic nicotine

exposure due to smoking leads to nicotine dependence—and also to two inadvertent therapeutic effects.

Neuromodulation through Lynx Protein Modulators of nAChR Function

Maintaining the levels and function of nAChRs during development and in adulthood is critical for proper circuit function. An inverted U-shape characterizes an organism's response to cholinergic activators. On the extremes of this range, underactivation is associated with lower cognitive performance and dementias (Hasselmo and Sarter, 2011), whereas overactivation may be linked to epilepsy (Bertrand et al., 2002) and, in even more extreme cases, to neurodegeneration (Schwarz et al., 2006). Apparently, tight control over cholinergic systems, operating at several levels, can counteract such imbalances at both extremes. Proteins that engage nAChRs within stable complexes, such as lynx family members, provide a homeostatic influence over nicotinic receptor systems. Through functionally driven regulation of lynx expression, the inhibition exerted over the system can be released or enhanced selectively within neuronal circuits.

The Lynx Family Acts as Nicotinic Receptor Modulators

The lynx genes belong to the ly-6/PLAUR superfamily, which shares a marked structural similarity with elapid snake venom proteins such as α -bungarotoxin; all have a characteristic three-looped motif. These α -neurotoxins are secreted proteins with sub-nM affinity for nAChRs (Tsetlin et al., 2009) and other receptors (Auer et al., 2010). α -neurotoxins interact on the extracellular face of the nAChR near ligand binding sites (Figure 1B), in contrast to most other nAChR-interacting proteins, which bind to the intracellular loops. Extrapolating from these interactions, the structurally similar lynx proteins may bind at such sites as well (Lyukmanova et al., 2011). Five interfaces occur in each nAChR pentamer (Figure 1); we do not yet know which, if any, interfaces form the binding sites for various lynx paralogs (Hansen and Taylor, 2007). Most previous studies of lynx have emphasized interactions at the plasma membrane. As GPI-anchored proteins can bind to transmembrane receptors intracellularly, the interactions of lynx with nAChRs could potentially alter receptor trafficking, stoichiometry, and surface number (Lester et al., 2009).

The high level of conservation with toxins implies that lynx genes are prototoxins—evolutionary antecedents to α -neurotoxins (Miwa et al., 1999; Chimienti et al., 2003; Dessaud et al., 2006; Arredondo et al., 2007; Hruska et al., 2009). The lynx family occurs in other species, including *C. elegans* (Chou et al., 2001) and *Drosophila* (Wu et al., 2010)—and in nonvenomous snakes, where it is distinct from the neurotoxin genes. We note that, in several cases, snake toxins employ functional mimicry of proteins in normal physiological processes. Often, virulent gene variants distort endogenous pathways at sensitive or rate-limiting steps. Therefore, the evolutionary relationship between lynx modulators and the α -neurotoxins agrees with the view that lynx modulators govern critical control points in the pathway of nicotinic receptor signaling.

Lynx1, the first discovered member of this family expressed in the brain (Miwa et al., 1999), has an overall inhibitory effect on nAChR function. In an α 4 β 2* nAChR-expressing cell, coexpres-

sion of lynx1 results in reduced agonist sensitivity, accelerated onset of desensitization, and slower recovery from desensitization (Ibañez-Tallon et al., 2002). Each lynx paralog has a relative binding specificity and modulatory capability on α 4 β 2 (Miwa et al., 1999; Ibañez-Tallon et al., 2002; Levitin et al., 2008), α 3 (Arredondo et al., 2006), and α 7 (Chimienti et al., 2003; Levitin et al., 2008; Hruska et al., 2009) nAChR subtypes; some interactions actually enhance nicotinic responses (Chimienti et al., 2003; Levitin et al., 2008), or their Ca²⁺ components (Darvas et al., 2009). The actions of lynx family proteins manifest themselves at both circuit (Hruska et al., 2009) and network levels (Pfeffer et al., 2009) on nicotinic systems. The blunting effect of lynx proteins could be responsible for the paucity of synaptically driven nicotinic responses recorded in brain tissue despite the rich cholinergic innervation, as well as the different response properties in brain tissue as compared with heterologous expression systems (Quick and Lester, 2002).

Lynx Acts as a Molecular Brake on Cholinergic-Dependent Plasticity

Removal of the molecular brake provided by lynx proteins can lead to nicotinic receptor hypersensitivity—larger direct nicotinic responses, slowed desensitization kinetics (Miwa et al., 2006), and enhanced sensitivity of the EPSC frequency in the cortex to nicotine (Tekinay et al., 2009). As a consequence of nAChR hypersensitivity, lynx1 knockout mice display increased levels of Ca²⁺ in neurons, enhancements in synaptic efficacy, and improved learning and memory functions (Miwa et al., 2006; Darvas et al., 2009; Tekinay et al., 2009). Studies on such hyperactive nicotinic receptors can reveal cholinergic-dependent processes with increased clarity. For instance, adult lynx1KO mice display heightened ocular dominance plasticity after the normal close of the critical period (Morishita et al., 2010). While the role of the cholinergic system during visual processing (Disney et al., 2007) and development has been appreciated (Bear and Singer, 1986), it has been a mystery why the critical period closes in late postnatal development and remains closed despite heavy cholinergic innervation of the visual system. These findings indicate that suppression of the cholinergic system by lynx proteins stabilizes neural circuitry. Indeed, cholinergic enhancement (via cholinesterase inhibition) reopens the critical period for visual acuity in adult wild-type mice (Morishita et al., 2010), indicating that cellular mechanisms for robust plasticity are maintained in adulthood through the cholinergic system but are suppressed by the action of lynx.

Top-Down Control over the Cholinergic System through Lynx: What Regulates the Regulator?

Abolishing receptor function through null mutations or pharmacological blockers of nAChRs abolished some of the gain-of-function phenotypes in lynx mouse models, indicating that nAChRs are necessary for the expression of lynx perturbations (Miwa et al., 2006). This indicates that lynx proteins exist, genetically, as upstream modulators of nicotinic receptor function and cholinergic signaling and can exert control over cholinergic-dependent processes. Because excess activation of nAChRs damages neuronal health and brain function, organisms have a clear need to restrict the degree of nAChR activation. Yet specific enhancement of cholinergic activity in functional circuits would benefit many processes, as described above. Therefore,

regulation of lynx function that would allow the sensitivity of the cholinergic system to shift in response to environmental changes would be critical. Partial, transient, or local reductions in lynx function may produce an optimal balance; moderate cholinergic signaling would enhance synaptic plasticity, yet still protect against hyperactivation that could make neurons susceptible to excitotoxic damage. What, then, regulates the regulator? Evidence thus far indicates that the lynx family is regulated in response to relatively strong perturbations: downregulation in NKCC1 knockout mice (Pfeffer et al., 2009), in adenylyl cyclase mutant mice (Wieczorek et al., 2010), and by $\alpha 7$ nAChR blockade (Hruska et al., 2009), whereas it is upregulated at the close of the critical period in the visual cortex, and by nicotine in the lung (Sekhon et al., 2005). Through functionally driven regulation of lynx expression, cholinergic systems have the ability to exert top-down influences on circuits underlying relevant behavior via coordinated regulation of nicotinic receptors subsets. While genetic linkages of lynx family members to neurological disorders have not been found, evidence for cholinergic dysregulation has been linked to a lynx family member expressed in nonneuronal tissues and involved in human disease (Chimienti et al., 2003), and as such, alterations in lynx dosage may be useful in ameliorating cognitive decline associated with neuropsychiatric disorders.

Lynx Modulators and the Neurodevelopmental Program

The synaptic pruning of neuronal circuits takes place late in the developing brain, after a period of early sculpting of neuronal number through programmed cell death. Nicotinic receptor systems have been implicated at both these stages and evidence suggests an involvement with lynx protoxins as well. For instance, early expression of lynx1 family member, PSCA, prevents programmed cell death of parasympathetic neurons (Hruska et al., 2009). Neuronal maturation and loss of synaptic lability appear to be correlated with the onset of lynx1 expression. In the majority of cases, circuit stability would provide an adaptive advantage once sculpting of circuitry has been influenced by the patterned activity of experience. Temporal coherence of information is critical for creating a stable internal representation of our environment and provides the background for salient information to reach our attention. But what happens in cases when that program goes awry? Lynx1 is downregulated in NKCC1 KO mice (Pfeffer et al., 2009), a strain that has a delayed developmental program of GABAergic neurons, diminished inhibition, and less spontaneous network activity. The neurodevelopmental program depends in part on $\alpha 7$ signaling (Liu et al., 2006). Lynx1 upregulation during a critical neurodevelopmental period, the switch in the sign of GABAergic signaling, and coexpression of lynx with GABAergic subsets all indicate a possible role of lynx mediating the timing of such developmental transitions. Nicotinic receptor control over GABAergic neuronal development and mature activity may represent a point of convergence for diseases such as schizophrenia (see next section), some amblyopias (Bavelier et al., 2010), and some epilepsies (Klaassen et al., 2006), which distort the excitatory-inhibitory balance in general and implicate GABAergic signaling defects in particular. In such cases, interventions through lynx could be useful for reestablishing the robust plasticity of youth exhibited prior to the close of the critical period, for instance in

cases of amblyopia or brain repair in stroke. Further, manipulations of lynx activity could help to restore proper inhibitory-excitatory imbalance. Developmental changes in nAChR functions may play a role in nicotine addiction, as a central question in tobacco control is young adult smokers' marked sensitivity to developing nicotine dependence (DSM-V Nicotine Workgroup, 2010; DiFranza et al., 2000; DiFranza, 2010). Molecules, such as lynx, which have direct contacts with nAChRs are promising candidates for the control of such phenomena and sensitive periods.

An Emerging Role for $\alpha 7$ nAChRs in Schizophrenia: Pharmacotherapeutic and Developmental Perspectives

Individuals with schizophrenia have a number of elementary psychophysiological abnormalities in filtering sensory stimuli that have been hypothesized to underlie their characteristic hallucinations and delusions (Venables, 1967). Their hallucinated voices and paranoid suspicions sometimes can be triggered by background noises in the environment that most other people can ignore. For example, a common hallucination in schizophrenia is a voice from the television, perhaps combined with the paranoid delusion that the television is commanding certain actions. The breakthrough of background noises into hallucinations and delusions can be considered a nonspecific manifestation of disorganized thinking, but increasingly it has been conceptualized as more specific evidence for failure in elementary inhibitory processes that the brain uses to regulate the amount of sensory stimuli that it processes. In many persons with schizophrenia, cerebral evoked potential recording shows diminished inhibition of the response to repeated stimuli (Adler et al., 1982) (Figure 2A), and animal models of this phenomenon point to a defect in hippocampal inhibition. Recent studies provide evidence both that nicotinic signaling partially underlies these schizophrenia-related inhibitory defects and that nicotinic drugs have possible therapeutic roles.

Cerebral $\alpha 7$ nAChRs in Cortex and Thalamus

The hippocampus responds to repeated stimuli with rapid habituation, which is dependent upon cholinergic input from the medial septal nucleus, an input that is driven by the brainstem reticular formation. $\alpha 7$ nAChRs on inhibitory interneurons throughout the hippocampus and presynaptic $\alpha 7$ nAChRs on mossy fiber terminals in the dentate gyrus participate in the control of sensory response in the hippocampus (Gray et al., 1996; Alkondon et al., 1999). Nicotinic activation of inhibitory interneurons increases their activity and activates nitric oxide synthetase. The neurons release additional GABA, activating presynaptic GABA_B receptors on the excitatory inputs to pyramidal neurons, which diminish the release of glutamate onto the pyramidal neurons (Figure 2). The result is diminished pyramidal neuron response to repeated sensory stimuli. Thus, the brainstem can regulate hippocampal response in the presence of high sensory input. Although $\alpha 7$ nAChRs have both presynaptic and postsynaptic expression (Frazier et al., 1998), their postsynaptic expression in humans is especially marked on inhibitory neurons of the hippocampus (Alkondon et al., 2000). Rodents have similar expression in the hippocampus, but primates have much more expression in the interneurons of the nucleus reticularis thalamis; the selective advantage of this

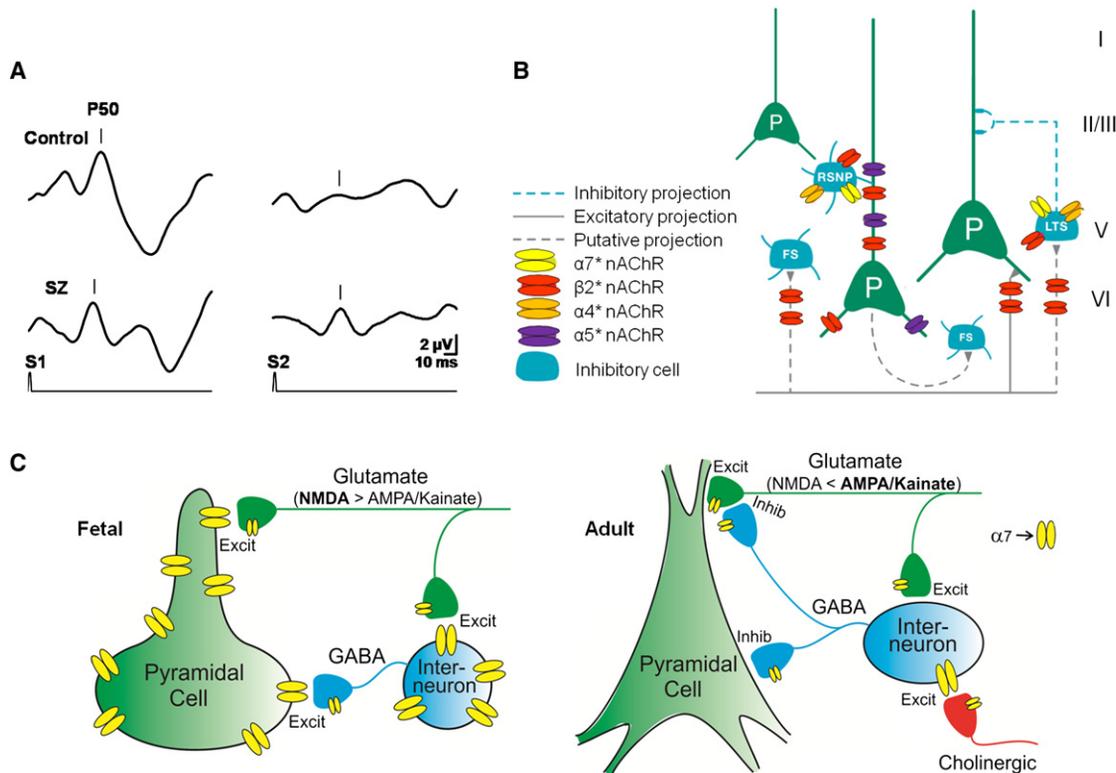


Figure 2. Aspects of nAChR Subtypes on Circuit Function

(A) Sensory inhibition deficits in schizophrenia. Cerebral evoked P50 potentials to repeated sounds (S1, S2) are inhibited in a normal (control, upper trace) but not in a schizophrenia patient (SZ, bottom trace).

(B) Differential localization of nAChRs subtypes on neurons in the prefrontal cortex. Green cells are excitatory pyramidal neurons (P) and blue cells are inhibitory interneurons. FS, fast-spiking interneurons; LTS, low threshold spiking; RSNP, regular spiking nonpyramidal neuron. Adapted with permission from Poorthuis et al. (2009).

(C) Development of $\alpha 7$ nAChRs in hippocampus. In the fetal brain before cholinergic innervation occurs (left), $\alpha 7$ nAChRs are somatodendritic and presynaptic on both GABAergic and glutamatergic neurons. In adults (right), $\alpha 7$ nAChR expression is generally reduced. Receptors are still expressed on GABAergic and glutamatergic presynaptic terminals, but only GABAergic neurons express somatodendritic $\alpha 7$ nAChRs (figure courtesy of William Proctor).

higher expression may be greater inhibitory control of sensory input to the cerebral cortex.

Three lines of evidence support the possibility that the failure of sensory inhibition in schizophrenia results from decreased expression of $\alpha 7$ nAChRs. First, postmortem studies of the hippocampus and thalamus show diminished labeling of putative inhibitory neurons by α -bungarotoxin, an antagonist of $\alpha 7$ nAChRs (Court et al., 1999). Second, the defect in inhibition is linked to the chromosome 15q14 locus of *CHRNA7*, the gene for the $\alpha 7$ nAChR subunit. Polymorphisms in the $\alpha 7$ 5' promoter and in a nearby partial duplication of the gene, *FAM7A*, are associated with both schizophrenia and the defect in inhibition (Leonard et al., 2002). It should, however, be noted that many genes have been associated with schizophrenia and there is no definitive model of its genetic transmission. Yet some of the other genes identified, such as *NRG1*, are involved in the assembly of $\alpha 7$ nAChRs, further supporting a potential link between $\alpha 7$ nAChRs and schizophrenia (Mathew et al., 2007). Third, persons with schizophrenia have the greatest rate and intensity of cigarette smoking of any identifiable subgroup in the population. Over 80% smoke, most of them multiple packs per day. Per cigarette they extract more nicotine than other

smokers with comparable cigarette consumption by inhaling more deeply and holding the smoke in their lungs. Cigarette smoking transiently improves their sensory inhibition. While it is not yet possible to know precisely how well $\alpha 7$ nAChRs are activated by smoked nicotine, one can reasonably hypothesize that the patients' higher dose of nicotine activates $\alpha 7$ nAChRs (Adler et al., 1993; Papke and Thinschmidt, 1998; Royal College of Physicians, 2007). Inhibition of the evoked response to auditory stimuli is significantly increased after patients smoke, an effect that is blocked by antagonists of $\alpha 7$ nAChRs in animal models (Luntz-Leybman et al., 1992). In schizophrenics, long-term cellular and molecular sequelae of this heavy exposure to nicotine may transcend chaperone-dependent upregulation (see next section) and also arise from the high Ca^{2+} permeability of nAChRs, especially of $\alpha 7$ nAChRs (Brunzell et al., 2003). Ca^{2+} activated signal transduction pathways reshape synaptic transmission and neural circuits, in some cases leading to gene activation (Kauer and Malenka, 2007).

If part of the genetic risk for schizophrenia involves variants in genes involved in formation of $\alpha 7$ nAChRs, then that risk has developmental significance as well. Schizophrenia generally appears in early adulthood, but long before the eruption of

hallucinations and delusions, there is neurocognitive and psychophysiological evidence for abnormalities in children with schizophrenic parents (which increase their risk of the illness). Such is the case with sensory inhibitory deficits. These are apparent at birth in some neonates with a parent who has schizophrenia (Hunter et al., 2010). Mothers who smoke during pregnancy are also likely to have a neonate with a sensory inhibitory deficit. Chronic exposure to nicotine would be expected to desensitize $\alpha 7$ nAChRs and thus lead to their dysfunction during development. Immature neurons that express $\alpha 7$ nAChRs are more likely to be injured by neonatal nicotine, whereas the expression of heteromeric $\alpha 4\beta 2^*$ nAChRs by more mature neurons may contribute to increased survival (Huang et al., 2007).

Like other nicotinic receptors, $\alpha 7$ nAChRs are thus potential targets for new therapeutic interventions for neural diseases such as schizophrenia. Several clinical trials involving schizophrenics have utilized more specific agonists for $\alpha 7$ nAChRs. 3-(2,4 dimethoxy)-benzylidene-anabaseine, derived from an alkaloid produced by nemertine worms, is a partial agonist at $\alpha 7$ nAChRs. It improves sensory inhibition in schizophrenics and also moderately improves their neuropsychological deficits in attention (Olincy et al., 2006). Clinical ratings of their negative symptoms, particularly anhedonia (absence of a sense of pleasure) and alogia (poverty of content in their speech), also improve during treatment. The atypical antipsychotic clozapine uniquely reduces smoking in schizophrenia, possibly because it releases acetylcholine in the hippocampus, activating $\alpha 7$ nAChRs (George et al., 1995). These clinical observations indicate that the patients' cognitive deficits are more amenable to treatment than many previously believed and their heavy cigarette smoking suggests that prescribed neurobiological treatment does not yet adequately address the brain pathophysiology of schizophrenia.

$\alpha 7$ nAChRs and the Development of Inhibitory Neuronal Circuitry

Like many genes expressed in the brain, the expression of $\alpha 7$ nAChRs is maximal during development. $\alpha 7$ nAChRs first appear on neuroblasts as soon as they differentiate from the neuroepithelium, and the peak expression occurs just after birth in rodents (Adams, 2003). In the third trimester, the expression of $\alpha 7$ nAChRs in the hippocampus is greater than three times the level in adults. The postsynaptic expression, confined to interneurons in adults, is prominent on fetal pyramidal neurons as well (Figure 2C). One important role for $\alpha 7$ nAChRs, in conjunction with $\alpha 3$ -containing nAChRs, is the induction of the KCC2 chloride transporter in pyramidal neurons (Liu et al., 2006). This transporter lowers the internal Cl^- concentration of the neuron and changes GABA from a depolarizing to a hyperpolarizing or inhibitory neurotransmitter. A specific role of $\alpha 7$ nAChRs was demonstrated by failure of the induction of KCC2 by treatment with $\alpha 7$ nAChR antagonists and in $\alpha 7$ KO mice (Zhang and Berg, 2007). At the time of birth, $\alpha 7$ nAChRs are involved in the transformation of glutamate neurotransmission from primarily NMDA-type receptors to kainate-aspartate receptors. $\alpha 7$ nAChRs remain embedded in the glutamate receptor-containing postsynaptic density.

Cholinergic innervation of the hippocampus occurs near the time of birth; therefore, the endogenous ligand for fetal $\alpha 7$

nAChRs cannot be synaptically released acetylcholine (Derrington and Borroni, 1990). A possible candidate is choline, which, in addition to its other development roles, activates $\alpha 7$ nAChRs at levels several fold higher than acetylcholine. Choline levels in human neonatal cord blood ($\sim 35 \mu\text{M}$) are three times higher than those in adult blood (Zeisel et al., 1980). These levels are sufficient to selectively downregulate $\alpha 7$ nAChRs on hippocampal neurons in tissue culture, perhaps reflecting a chronic low level of receptor stimulation (Alkondon et al., 1997; Uteshev et al., 2003). Brief choline treatment during gestation is associated with increased excitability and dendritic development in hippocampal pyramidal neurons (Li et al., 2004).

Choline is an essential dietary nutrient. Normally humans have adequate choline, but during pregnancy many women are thought to be deficient because the fetus makes large demands for use in the synthesis of cell membranes (Meck and Williams, 2003). In addition to poor maternal diet, choline deficiency for the fetus can occur because of maternal stress, which leads the mother to sequester choline in her own liver. Variants in the gene for phosphatidylethanolamine methyl transferase, which synthesizes phosphatidylcholine and thus provides a source of choline, are also associated with choline deficiency and with schizophrenia. Experiments in animal models suggest that choline supplementation during gestation and early postnatal development may produce a reversal of sensory inhibitory deficits that lasts through adulthood (Li et al., 2004). Clinical trials are currently in progress.

In addition to genetic risk, exposure to nicotine, and dietary deficiency, maternal infection is a risk factor for schizophrenia (Patterson, 2007). In some cases the infectious agent enters the fetus, but in most cases, like influenza, it remains in the mother's respiratory tract. It is the deleterious effect of her cytokine response to the infection on the placenta that appears to be pathogenic. $\alpha 7$ nAChRs are involved in the macrophage and placental cytokine response, which may be an additional role for genetic variants in these receptors in the pathogenesis of schizophrenia (Wang et al., 2003).

In short, schizophrenia remains a challenging and mysterious disease. Yet the perinatal development of $\alpha 7$ nAChRs, the role of the endogenous agonist choline on $\alpha 7$ nAChRs, and the consequences for maturation of inhibitory circuits provide both a partial pathophysiological role and a promising avenue for therapy of schizophrenia.

Effects of Chronic Nicotine: Role of Upregulation

"It's easy to quit smoking," Mark Twain reportedly said. "I've done it a hundred times." Nicotine dependence may be the most complex of the addictions, perhaps both because HS nAChRs occur in so many brain areas and because unlike acute opioid administration, nicotine allows a user to remain active and productive.

Maintained or repeated intake of nicotine occurs during tobacco smoking or chewing and during the use of snus, lozenges, gums, or patches. The peak and maintained nicotine concentrations during such intake are lower than those presumably associated with schizophrenics' smoking, and they primarily activate HS nAChRs (Matta et al., 2007; Royal College of Physicians, 2007). In contrast to nicotine addiction, and

somewhat surprisingly, such chronic exposure to nicotine produces inadvertent therapeutic effects in at least two other conditions, Parkinson's disease and a specific form of epilepsy.

This section discusses the status of the unifying hypothesis that these three effects of chronic nicotine exposure are explained by a common molecular and cellular phenomenon. In brief, the interaction between chronic nicotine and HS nAChRs, especially $\alpha 4\beta 2$, appears to cause selective upregulation of these nAChRs via posttranslational mechanisms.

A Pathological Effect: Brain Mechanisms of Nicotine Dependence

Nicotine-dependent people value the effects produced by the smoking-induced nicotine bolus that activates and then desensitizes nAChRs; but longer-term exposure is essential for nicotine dependence (Markou, 2008; Kalivas, 2009; Koob and Volkow, 2010). The meaning of "longer term" depends on one's definition of nicotine dependence, a lively topic in itself (DSM-V Nicotine Workgroup, 2010; DiFranza et al., 2000; DiFranza, 2010); the time required may be as brief as several days.

Some people use tobacco repeatedly because it provides a feeling of well-being, which probably begins when nicotine reaches midbrain nAChRs (Matta et al., 2007; Royal College of Physicians, 2007). Nicotine both activates and desensitizes nAChRs in midbrain dopaminergic neurons (Brodie, 1991; Pidoplichko et al., 1997), and the pleasurable effects associated with nicotine intake occur in large part via the mesolimbic dopaminergic reward system (Corrigall et al., 1992; Koob and Volkow, 2010). Recent studies also show important contributions from insular cortex (Naqvi et al., 2007). The nAChR-rich medial habenula may actually participate in aversive effects of nicotine (Fowler et al., 2011), which apparently underlie moderate smokers' (but not schizophrenics') habit of carefully titrating the nicotine dose generated by each cigarette.

In addition to alterations in reward, many nicotine-dependent people display improved declarative memory for several minutes to one hour after smoking (Myers et al., 2008). Because smokers gradually learn to exploit this effect, it is called "cognitive sensitization." However, it is not known whether the nicotine-enhanced cognitive performance exceeds the level that would occur if the person had never begun to smoke, or after remaining abstinent for one year (the usual criterion for successful smoking cessation) (Levin et al., 2006). Cognitive sensitization probably involves fore-brain-dependent processes (Xu et al., 2005; Davis and Gould, 2009; Kenny, 2011). In rodents and humans, the hippocampus is importantly implicated in cognitive sensitization, and $\alpha 4\beta 2^*$ nAChRs play key roles (Levin et al., 2006; Davis and Gould, 2009). Chronic or acute nicotine enhances LTP in several regions of hippocampus, especially dentate gyrus (Nashmi et al., 2007; Tang and Dani, 2009; Penton et al., 2011). The effects may proceed via HS receptors on both the axons of the perforant path and the intrinsic GABAergic interneurons (Gahring and Rogers, 2008).

Other nicotine-dependent people find that nicotine helps them to cope with stressors; the soldier dangling a cigarette after battle is an enduring image (Brandt, 2007). Relapse in response to environmental or contextual stimuli such as stress—even after months of abstinence—constitutes a major challenge in smoking cessation. Stress- and cue-induced reinstatement of nicotine administration is studied far less frequently than analogous

phenomena for cocaine and opioids. The VTA-nucleus accumbens system does play a role. Several additional candidate brain areas receive dopaminergic and other monoaminergic nerve terminals, and these terminals all presumably express HS nAChRs. For instance, dopamine increases in the extended amygdala during stress, fear, and nicotine withdrawal (Inglis and Moghaddam, 1999; Pape, 2005; Grace et al., 2007; Gallagher et al., 2008; Koob, 2009; Marcinkiewicz et al., 2009). We do not know whether either nAChR upregulation, or its sequelae, can account for stress- or cue-induced relapse in nicotine dependence.

nAChR Upregulation: The "Selectivity Hypothesis" and Its Functional Implications

Which molecular and cellular mechanisms could account for the widespread actions of chronic nicotine on neuronal circuit properties? This puzzle does not yet have a complete answer, but it is clear that chronic nicotine increases the number of nAChRs themselves (Marks et al., 1983; Schwartz and Kellar, 1983). In an emerging hypothesis, this "upregulation" is both necessary and sufficient for the initial stages of nicotine exposure—minutes, hours, days, and weeks. Remarkably, the upregulation shows selectivity at every level thus far examined.

At the level of whole brain, chronic nicotine causes selective upregulation of nAChRs among major brain regions. Upregulation occurs in cortex, midbrain, and hypothalamus, but not in thalamus or cerebellum (Pauly et al., 1991; Marks et al., 1992; Nguyen et al., 2003; Nashmi et al., 2007; Doura et al., 2008). In several brain regions, chronic nicotine administration produces ~50% upregulation of HS nAChRs after just two days. Continued administration then produces additional increases over one to several weeks (Marks et al., 1991; Pietilä et al., 1998).

Within individual brain regions, there is selective upregulation among cell types. In the midbrain, both DA neurons (in substantia nigra pars compacta and ventral tegmental area [VTA]) and GABAergic neurons (in substantia nigra pars reticulata and VTA) express high levels of $\alpha 4\beta 2^*$ nAChRs on their somata, but only GABAergic neurons display somatic upregulation (Nashmi et al., 2007; Xiao et al., 2009). Another example of cell-selective upregulation occurs in the projection from medial entorhinal cortex to dentate gyrus. In the medial perforant path, which mainly arises from layer II stellate cells, chronic nicotine upregulates $\alpha 4\beta 2^*$ nAChRs. However in the temporoammonic pathway, which mainly arises from layer III pyramidal neurons, $\alpha 4\beta 2^*$ nAChRs are present but are not upregulated (Nashmi et al., 2007).

Chronic nicotine also produces selective upregulation between somatodendritic versus axon terminal regions of individual neurons. In midbrain, chronic nicotine treatment elicits a general increase in $\alpha 4\beta 2^*$ nAChRs in GABAergic neurons, but only in axon terminals of DA neurons. Such "tiers of selectivity" in mesostriatal and mesolimbic upregulation have the power to explain two components of nicotine dependence: tolerance to some rewarding effects of nicotine and sensitization to others (Nashmi et al., 2007; Lester et al., 2009).

Nicotine also interacts with specific nAChR subtypes, and nicotine-induced upregulation is governed in part by these interactions at agonist binding interfaces (Figure 1). Further work is needed to understand how chronic nicotine differentially

upregulates some but not all HS nAChRs. Part of the cell selectivity in upregulation presumably arises because each neuronal type expresses a distinct repertoire of subunits. GABAergic neurons in DA brain regions express mostly $\alpha 4\beta 2$ nAChRs along with a few $\alpha 4\alpha 5\beta 2$ nAChRs (McClure-Begley et al., 2009), whereas DA neurons express at least three $\alpha 4$ -containing nAChRs ($\alpha 4\alpha 6\beta 2\beta 3$, $\alpha 4\alpha 5\beta 2$, and a few $\alpha 4\beta 2$) (Salminen et al., 2004; Gotti et al., 2007). Although $\alpha 6\beta 3^*$ nAChRs are, like $\alpha 4\beta 2$ nAChRs, highly sensitive to nicotine, several studies demonstrate that chronic nicotine treatment elicits either no change or a decrease in $\alpha 6\beta 3^*$ nAChRs in mouse brain (McCallum et al., 2006a, 2006b; Mugnaini et al., 2006). Nicotine may also subvert the coordinated regulation in place by other control mechanisms, such as lynx proteins, through the preferential upregulation of one subtype resulting in imbalance in nicotinic receptor signaling. It is too early, however, to conclude that lynx proteins influence the effects of chronic nicotine exposure.

Emerging clues to selectivity at the subunit level, especially in the context of nicotine dependence, concern the $\alpha 5$ subunit. Figure 1 shows that this subunit never participates at the agonist binding interface between α and β subunit but occupies a fifth or “auxiliary” position. In rodent brain, most $\alpha 5^*$ nAChRs are thought to be $(\alpha 4)_2(\beta 2)_2\alpha 5$ pentamers (Gotti et al., 2006; Albuquerque et al., 2009). In all known animals, the $\alpha 5$, $\alpha 3$, $\beta 4$ genes form a cluster. Indeed, $(\alpha 3)_2(\beta 4)_2\alpha 5$ pentamers are widespread in the peripheral nervous system, in the medial habenula, and in some nonneuronal cell types. [We do not emphasize $(\alpha 3)_2(\beta 4)_2\alpha 5$ nAChRs or $\alpha 3\beta 4$ nAChRs, because such nAChRs have relatively low nicotine sensitivity and relatively low susceptibility to upregulation.]

Single-nucleotide polymorphisms found in the human $\alpha 5$, $\alpha 3$, $\beta 4$ gene cluster are associated with nicotine dependence and its age-dependent onset; number of cigarettes smoked per day and “pleasurable buzz” elicited by smoking; alcoholism, sensitivity to the depressant effects of alcohol, and age of alcohol initiation; cocaine dependence; opioid dependence; lung cancer; and cognitive flexibility (Erich et al., 2010; Hansen et al., 2010; Improgo et al., 2010; Saccone et al., 2010; Zhang et al., 2010). A major “risk allele” is in a noncoding region of $\alpha 5$ and is associated with decreased expression of $\alpha 5$ subunit mRNA (Wang et al., 2009). A second “risk allele” occurs in the coding region, within the M3-M4 loop, and also produces decreased function of $(\alpha 4)_2(\beta 2)_2\alpha 5$ nAChRs (Wang et al., 2009; Kuryatov et al., 2011). Furthermore in experiments using chronic nicotine exposure in rats, $(\alpha 4)_2(\beta 2)_2\alpha 5$ nAChRs are not upregulated, but (presumptive) $(\alpha 4)_2(\beta 2)_3$ nAChRs in the same brain region are (Mao et al., 2008). Summarizing the available data, the “risk alleles” may decrease the fraction of $(\alpha 4)_2(\beta 2)_2\alpha 5$, increasing that of $\alpha 4\beta 2$ nAChRs. Because $\alpha 4\beta 2$ nAChRs are the most susceptible to nicotine-induced upregulation, the data again seem consistent with the idea that selective upregulation of $\alpha 4\beta 2$ nAChRs underlies nicotine dependence. The potential power of $\alpha 4\beta 2$ upregulation to explain the initial events of nicotine dependence thus derives from its selectivity, displayed at every level of organization: regional, neuronal, cellular, and stoichiometric.

Selective upregulation would directly result in modified neuronal excitability and neuronal interactions. As noted above,

in the context of nicotine dependence, selective upregulation presently has been studied in detail only in midbrain and in the perforant path. Thus it remains an audacious hypothesis that the initial stages of nicotine dependence can be explained solely by “selective upregulation,” with no additional mechanisms of regulation, adaptation, neuroadaptation, homeostasis, or plasticity.

Despite the selectivity described above, upregulation also displays an important *generality*. The upregulation of $\alpha 4\beta 2^*$ nAChRs by chronic nicotine treatment has been replicated many times in numerous systems—transfected cell lines, neurons in culture, brain slices, and smokers’ brains (Albuquerque et al., 2009; Fu et al., 2009; Lester et al., 2009; Srinivasan et al., 2011). Upregulation is not accompanied by an increase in nAChR subunit mRNA (Marks et al., 1992; Huang et al., 2007). Instead, the membrane-permeant nicotine molecule appears to act intracellularly, as a selective pharmacological chaperone of acetylcholine receptor and stoichiometry (SePhaChARNs) (Kuryatov et al., 2005; Sallette et al., 2005; Lester et al., 2009). SePhaChARNs arises in part from the thermodynamics of pharmacological chaperoning: ligand binding, especially at subunit interfaces, stabilizes AChRs during assembly and maturation, and this stabilization is most pronounced for the highest-affinity nAChR subunit compositions (especially $\alpha 4\beta 2^*$), stoichiometries, and functional states of nAChRs.

Upregulation Magnifies Both Activation and Desensitization

Another general aspect of upregulation is its applicability to two functional states induced by nicotine at nAChRs—activation and desensitization (Figure 3). Smoked nicotine acts differently from ACh in three ways (Lester et al., 2009). (1) Acetylcholinesterase does not hydrolyze nicotine; therefore, nicotine remains near nAChRs thousands of times longer than ACh. (2) Nicotine efficiently permeates membranes; therefore, it accumulates within cells (Putney and Borzelleca, 1971; Lester et al., 2009). (3) Nicotine activates $\alpha 4\beta 2$ nAChRs ~ 400 -fold more effectively than it activates muscle-type nAChRs, because of cation- π and H-bond interactions at the agonist binding site (Xiu et al., 2009). These factors lead nicotine to activate and desensitize the basal and nicotine-upregulated nAChRs for prolonged periods (minutes to hours). Therefore, desensitization influences actions of exogenous nicotine more than of endogenous ACh.

In summary, upregulation due to chronic nicotine can magnify either activation or desensitization by acute nicotine. While it has been debated whether the acute effects of nicotine arise from activation or from desensitization, in the contemporary view (Figure 3) (Picciotto et al., 2008) both are thought to occur at appropriate neurons and synapses.

An Inadvertent Therapeutic Effect: Parkinson’s Disease Neuroprotection

At first glance, nicotine addiction and Parkinson’s disease seem related only by the participation of neighboring dopaminergic neuron populations: the former involves dopamine release from VTA neurons, and the latter involves degeneration in the substantia nigra pars compacta. In fact, more than 50 studies document an inverse correlation between a person’s history of tobacco use and his/her risk of Parkinson’s disease (Ritz et al., 2007). The effect is remarkably large—roughly a factor of

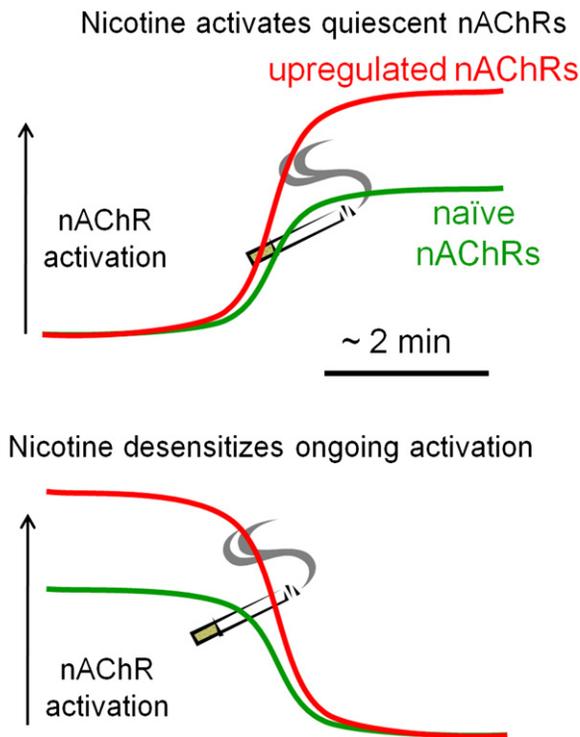


Figure 3. A Graphical View that Upregulation of nAChRs Can Amplify Both the Effects of nAChR Activation and the Effects of Desensitization

The vertical black arrow represents the level of nAChR activation at a synapse, and the x axis represents the time course of activation and/or desensitization. The cigarette represents an acute exposure to nicotine, in the context of either nicotine-naïve nAChRs (green) or nicotine-upregulated receptors (red).

(Top) Exposure to nicotine produces stronger activation at upregulated receptors than at naïve nAChRs, because upregulated receptors are both more numerous and more sensitive.

(Bottom) A synapse where ongoing endogenous ACh mediates stronger nAChR activation than at a naïve synapse. Desensitization then produces a correspondingly larger decrement of activity. The most common example of such a desensitizing response to nicotine occurs at the presynaptic terminals of nigrostriatal dopaminergic neurons (Xiao et al., 2009).

two—when one considers that it derives from retrospective epidemiological studies (Hernán et al., 2002). Some Parkinson's disease cases (~10%) are directly linked to genetic mutations. However, when all genetic factors are eliminated by studying monozygotic twins who are discordant for both tobacco use and Parkinson's disease, tobacco smoking and chewing still decrease the risk of Parkinson's disease (Tanner et al., 2002; Wirdefeldt et al., 2005). Could selective upregulation contribute to the apparent neuroprotective effects? We discuss three possible mechanisms.

One mechanism may be via regulation of nAChR-containing circuits (Nashmi et al., 2007; Xiao et al., 2009). While chronic nicotine does not change the abundance or function of $\alpha 4^*$ nAChRs in the somata of substantia nigra pars compacta dopaminergic neurons, it does suppress baseline firing rates of these DA neurons. In mice exposed to chronic nicotine, GABA neurons in substantia nigra pars reticulata have increased baseline firing rates, both in brain slices and in anesthetized animals. These contrasting effects on GABA and DA neurons are due to upregu-

lated $\alpha 4^*$ nAChR responses in GABA neurons, at both somata and synaptic terminals. Thus chronic nicotine could regularize the firing rates of substantia nigra DA neurons, preventing them from experiencing bursts that could lead to excitotoxic Ca^{2+} influx.

Another neuroprotective mechanism may occur at nerve terminals in the striatum. Chronic nicotine upregulates $\alpha 4^*$ nAChRs in dopaminergic presynaptic terminals, apparently leading to increased resting dopamine release from those terminals. This effect produces a basal decrease in the level of glutamate release from corticostriatal neurons (Xiao et al., 2009). The process may counteract the increased effectiveness of corticostriatal glutamatergic inputs during degeneration of the DA system.

A third neuroprotective mechanism may operate entirely within DA neurons. The chaperoning of nAChRs by nicotine enhances the export of $\alpha 4\beta 2$ nAChRs from the endoplasmic reticulum (ER), and this leads to a general increase in ER exit sites (Srinivasan et al., 2011). This aspect of SePhaChARNs eventually leads to plasma membrane upregulation. We hypothesize that, in addition, this process lowers the demands on the general proteostatic machinery in the ER, thereby altering ER stress, which is frequently invoked as a toxic mechanism in Parkinson's disease.

A Second Inadvertent Therapeutic Effect: ADNFLE

Autosomal-dominant nocturnal frontal lobe epilepsy (ADNFLE) is caused by missense mutations in either the $\alpha 4$ or the $\beta 2$ subunit. Several strains of knock-in mice bearing these mutations have seizure phenotypes related to ADNFLE (Klaassen et al., 2006; Teper et al., 2007; Xu et al., 2010), but $\alpha 4$ KO and $\beta 2$ KO mice display no seizure phenotypes, implying that ADNFLE has a subtle, as yet unexplained pathophysiology. ADNFLE patients who use a nicotine patch or tobacco have fewer seizures (Willoughby et al., 2003; Brodtkorb and Picard, 2006). Recent data suggest that ADNFLE mutations bias nAChR composition away from the $(\alpha 4)_2(\beta 2)_3$ stoichiometry, which is then re-established by nicotine exposure (Son et al., 2009). Thus, changes in $\alpha 4\beta 2^*$ nAChR stoichiometry, subunit composition, and sorting could contribute both to the etiology of ADNFLE and to the inadvertent therapeutic effects of nicotine. This highly penetrant monogenic disease could eventually provide important clues to the pathophysiology and therapy of complex polygenic diseases such as Parkinson's disease and nicotine dependence.

Thus, chaperoning of nascent nAChRs by smoking-relevant concentrations of nicotine represents a form of nicotine-nAChR interaction that is not directly associated with ion flux through active nAChRs. Chaperoning may provide a partial explanation for the pathological process of nicotine addiction and also for the inadvertent therapeutic effects of tobacco use in Parkinson's disease and ADNFLE. Some effects of chaperoning may actually occur at the level of nAChR stabilization in the endoplasmic reticulum, and others arise from the consequent upregulation at the plasma membrane.

Conclusions

The Introduction posed the problem of explaining how manipulations of nicotinic synapses, which have been considered all-or-none machines, can produce the graded modulation of

neuronal circuits and behaviors. Here we summarize the four (admittedly partial) explanations. First, recent evidence supports the graded “volume transmission” hypothesis (Ren et al., 2011). Second, the prototoxin lynx can function, probably both intracellularly and extracellularly, to direct the localization and activity of nAChRs. Absence of lynx has the profound modulatory effect of lengthening the critical period for ocular dominance plasticity. Third, $\alpha 7$ nAChRs can be activated in extrasynaptic regions by ambient concentrations of choline, with possible consequences for neuronal development as well as for circuit function during schizophrenia. Finally, the pharmacokinetics and stability of nicotine allow it to influence nAChRs in environments not reached by acetylcholine itself—extracellularly on somata, and intracellularly in the ER, where nicotine functions as a pharmacological chaperone to upregulate certain HS receptors. Furthermore, nicotine’s persistence leads to desensitization of nAChRs.

For more than four centuries, nicotinic systems have unfortunately played a role in drug abuse, but we have reviewed ways in which nicotinic systems can also be manipulated to provide help for neural illnesses such as Parkinson’s disease, cognitive decline, epilepsy, and schizophrenia. Nicotinic systems will continue to serve as touchstones for advances in neuroscience.

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