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High-affinity nicotinic acetylcholine receptor expression and trafficking abnormalities in psychiatric illness

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Abstract

Rationale—Nicotinic acetylcholine receptors (nAChRs) are a critical component of the cholinergic system of neurotransmission in the brain that modulates important physiological processes such as reward, cognition and mood. Abnormalities in this system are accordingly implicated in multiple psychiatric illnesses, including addiction, schizophrenia, and mood disorders. There is significantly increased tobacco use, and therefore nicotine intake, in patient populations, and pharmacological agents that act on various nicotinic receptor subtypes ameliorate clinical features of these disorders. Better understanding of the molecular mechanisms underlying cholinergic dysfunction in psychiatric disease will permit more targeted design of novel therapeutic agents.

Results—The objective of this review is to describe the multiple cellular pathways through which chronic nicotine exposure regulates nAChR expression, and to juxtapose these mechanisms with evidence for altered expression of high-affinity nAChRs in human psychiatric illness. Here we summarize multiple studies from pre-clinical animal models to human *in vivo* imaging and post-mortem experiments demonstrating changes in nAChR regulation and expression in psychiatric illness.

Conclusions—We conclude that a mechanistic explanation of nAChR abnormalities in psychiatric illness will arise from a fuller understanding of normal nAChR trafficking, along with the detailed study of human tissue, perhaps using novel biotechnological advances, such as induced pluripotent stem cells.

Keywords

Acetylcholine; nicotine; protein trafficking; schizophrenia; bipolar disorder; major depressive disorder; attention deficit hyperactivity disorder

Introduction

Psychiatric illnesses such as schizophrenia and mood disorders are chronic, devastating diseases that present a major burden to individuals, their families, and the healthcare system (Insel 2008). It is thus critical to understand the underlying pathophysiology of these disorders in order to design novel therapeutic agents for individuals whose illness is unresponsive or incompletely responsive to currently available treatment modalities (Berton and Nestler 2006; Covington et al. 2010).

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Nicotinic acetylcholine receptors (nAChR) along with muscarinic acetylcholine receptors mediate cholinergic signaling in the mammalian brain, which plays a critical role in neuromodulation, influencing a variety of brain functions including cognition and mood (Picciotto et al. 2000; Picciotto et al. 2012). Studies of the cholinergic system and its receptors in psychiatric disorders are in part motivated by the well-described phenomenon of increased cigarette smoking and nicotine use in individuals with mental illness, suggesting that the response to chronic nicotine exposure in those with psychiatric disorders differs fundamentally from those without such disorders. For example, up to 90% of individuals with schizophrenia smoke tobacco (Hughes et al. 1986). Individuals with schizophrenia also take more cigarette puffs and have greater nicotine intake per cigarette (Tidey et al. 2005; Williams et al. 2005), and these individuals have a significantly lower success rate of smoking cessation (Lasser et al. 2000). Similar findings have also been reported in major depressive disorder (Zimmerman et al. 2002), bipolar disorder (Hughes et al. 1986), and attention deficit hyperactivity disorder (ADHD, (Pomerleau et al. 1995)).

Multiple hypotheses exist to explain these observations (Mineur and Picciotto 2010; Wing et al. 2012). For example, the self-medication hypothesis suggests that individuals with psychiatric disease use nicotine to ameliorate symptoms of their mental illness. Indeed, nicotine can correct many abnormal neurophysiological deficits demonstrated in schizophrenia (Wing et al. 2012). Alternatively, the neural circuit abnormalities that underlie psychiatric disorders associated with greater risk of smoking may concomitantly increase the susceptibility for nicotine dependence. While the predilection for nicotine dependence as a comorbidity of psychiatric disease is complex and certainly multifactorial, profound abnormalities at the molecular level of nAChR expression and nAChR regulation by chronic nicotine exposure have been identified both *in vivo* and in post-mortem studies of multiple human psychiatric disorders. Given the clear involvement of the nicotinic cholinergic system in psychiatric disease, we hypothesize that correction of the molecular lesions that underlie these abnormalities represents a potential novel avenue for therapeutic design.

Understanding the regulation of nAChR trafficking and expression in cell systems is critical to provide context for the cholinergic changes observed in human psychiatric disease. The best characterized phenomenon that has been studied to reveal the complex organization of regulatory sites for nAChRs is the upregulation of high-affinity nicotine binding sites after chronic nicotine exposure, which occurs both *in vivo* in the brains of human smokers, as well as in animals and a variety of cell models (reviewed in (Corringer et al. 2006; Govind et al. 2009)). The major contributors to high-affinity nicotine binding in the brain are the α4β2-containing nAChRs ((Picciotto et al. 1995); α4β2* nAChRs, where * denotes other potentially co-assembling subunits), and consequently, most studies examining upregulation at the molecular and cellular level have focused on these receptors. Changes in the mechanisms that regulate high-affinity receptor expression may contribute to the core symptoms of various psychiatric disorders, as well as the co-morbidity of increased nicotine dependence in these individuals. We thus suggest that a logical strategy for evaluating the molecular pathophysiology of the nicotinic system in several psychiatric disorders is to dissect the previously identified cellular and molecular regulatory pathways governing regulation of high-affinity nicotinic binding sites, and to evaluate the etiology of differences between individuals with, and without, psychiatric disease.

Numerous studies have identified nicotine's effects on high-affinity nAChRs in cells and animal models. Similarly, *in vivo* imaging and post-mortem studies have found changes in high-affinity nAChR expression and endogenous acetylcholine levels in psychiatric disorders, both between non-smokers and smokers, and between individuals with and without psychiatric illness. Conversely, very few studies have bridged the two levels of examination mechanistically, mainly due to the difficulty of conducting detailed

biochemical and electrophysiological studies in human tissue. Novel biotechnology will facilitate bridging this gap. Here we survey some of the principal mechanisms by which high-affinity receptors are regulated by nicotine, with the purpose of identifying key regulatory sites of dynamic nAChR trafficking. We then discuss important studies reporting abnormalities in high-affinity nAChRs in a variety of psychiatric diseases, including schizophrenia and mood disorders. Finally, we discuss novel ways in which nAChR trafficking and expression abnormalities can be further explored in tissue from individuals with psychiatric disease. Bringing these observations together will provide the background information necessary for the basic or translational neuroscientist to ask and answer questions regarding the role of the nicotinic cholinergic system in the cause and treatment of psychiatric disease.

Nicotinic AChR upregulation by nicotine exposure

Stoichiometry and localization

The term ‘upregulation’ generally refers to an increase in high-affinity binding sites after chronic exposure to nicotine, which are frequently identified using the radiolabeled nAChR ligands nicotine or epibatidine (Breese et al. 1997). The work to elucidate the mechanism of nAChR upregulation has significantly advanced the general understanding of nAChR trafficking, and thus we briefly review selected major findings in an effort to highlight the complexity of dynamic nAChR protein trafficking (Table 1). In the mammalian brain, the major areas containing the cell bodies of cholinergic projection neurons include the basal forebrain, the medial habenula, the striatum, and the vagal nucleus, all of which project to other brain structures (Mesulam 1995). Nicotinic AChRs are pentamers, and result from the combination of α2-α5 subunits with β2-β4 subunits. Much work has focused on the $(\alpha4)_2(\beta2)_3$ nAChR, which exhibits high-affinity for nicotine, and α4β2 receptors are the most abundant high-affinity receptors in the brain (Lindstrom 2003). The homomeric α7 nAChR, whose nicotine affinity is orders of magnitude lower than α4β2 *in vitro* (although very high-affinity α7 effects have been seen in brain slice preparations (Mansvelder and McGehee 2002)), also plays an important role in brain cholinergic signaling, and is implicated in the pathophysiology of schizophrenia (Olincy and Freedman 2012).

Desensitization and cellular trafficking

Upregulation of nAChRs by chronic nicotine exposure occurs in many diverse cell types, suggesting that the general mechanism does not require cell-specific proteins and is cell autonomous. The phenomenon is post-translational, as no change in mRNA levels is typically observed with chronic nicotine exposure (Marks et al. 1992). Experiments have focused on both cell surface and intracellular sites of action. Early work suggested that nAChR desensitization might be necessary for surface receptor upregulation, since oocyte studies showed that the concentration of nicotine required for α4β2 nAChR surface upregulation is similar to that required to produce receptor desensitization, which was roughly similar to the half-maximal nicotine binding to surface nAChRs (Fenster et al. 1999). Consistent with this hypothesis, α3β4 receptors, which show a 10-fold lower affinity for nicotine, required a 10-fold higher concentration of nicotine for upregulation as compared to α4β2. Further, α4β2 nAChR mutants that do not recover readily from desensitization were upregulated by short exposure to nicotine, whereas wild-type α4β2 nAChR were not. Finally, these studies showed that nicotine affinity in homogenized total cell membranes was significantly increased compared to surface receptors in intact oocytes, revealing two pools of receptors: higher-affinity intracellular receptors, and lower-affinity surface receptors. Surprisingly, a higher concentration of nicotine was required to upregulate the intracellular receptor pool in homogenized membranes compared to the surface receptors, and the study concluded that while desensitization is important for upregulation of

surface membrane receptors, additional mechanisms must play a role in upregulation of intracellular receptors. Further study of surface receptor dynamics revealed that upregulation does not result from changes in surface endocytosis or intracellular degradation, but rather occurs earlier in the secretory pathway, likely in the endoplasmic reticulum (Darsow et al. 2005; Vallejo et al. 2005). Supporting experiments used Brefeldin A (BFA) to disrupt ER to golgi trafficking in HEK293 cells to examine the importance of forward trafficking for upregulation. BFA blocked nicotine-induced upregulation of α4β2 nAChR surface expression, but not the overall upregulation of high-affinity nAChR binding as measured by [^{3}H]-epibatidine, suggesting that upregulation occurs within the ER or earlier, and that exocytic trafficking is required for increased surface expression in the presence of nicotine. However, experiments performed by a different group found that BFA exposure reduced high-affinity binding as measured by ^{125}I-epibatidine, and that nicotine exposure did not lead to significant changes in surface receptor numbers ((Vallejo et al. 2005), discussed further below).

Pharmacological chaperoning, receptor maturation, and conformational change

The experiments cited above suggest that the early exocytic pathway represents a key site of nAChR regulation. The ER is part of the proximal exocytic pathway, and since nicotine permeates the cell membrane and can accumulate intracellularly, the ER is likely to be important for regulation of nAChR number. Indeed, experiments using optical imaging of tagged nAChR subunits revealed that chronic nicotine exposure stabilized α4β2 receptors in the $(\alpha4)_2(\beta2)_3$ stoichiometry prior to reaching the trans-Golgi network, as well as induced additional ER exit sites (Richards et al. 2011; Srinivasan et al. 2011). The authors of these studies refer to an ER “bottleneck” in the presence of chronic nicotine, whereby nicotine acts as a pharmacological chaperone, stabilizing the ER complex and eventually increasing the surface expression of the channel. Along similar lines, studies using conformation-specific monoclonal antibodies and glycosylation digestions demonstrated that nicotine promotes intracellular maturation of α4β2 receptors within the ER in both HEK293 cells and SH-SY5Y cells (Sallette et al. 2005). This concept of pharmacological chaperoning appears applicable to multiple different families of channel or related proteins, and it is hypothesized that much of the therapeutic mechanism of action for antipsychotic and antidepressant drugs might occur within the ER and *cis*-golgi rather than at the cell surface (Lester et al. 2012). Finally, an alternative mechanism for upregulation in HEK293 cells was reported, whereby chronic nicotine exposure stabilizes a high-affinity, more easily activated state of α4β2 receptors without changing surface receptor number (Vallejo et al. 2005).

Phosphorylation and the ubiquitin-proteosome system

Consistent with the identification of the ER and Golgi complex as important subcellular regulatory sites, the chaperone protein 14-3-3η interacts with the α4 subunit in the ER and Golgi, increasing both total expression and surface expression of α4β2 recombinant receptors in heterologous cells (Jeanclos et al. 2001). This interaction also occurs *in vivo* in rat brain. Phosphorylation plays a critical regulatory role, as protein kinase A activation enhances the interaction between 14-3-3η and α4 via direct α4 subunit phosphorylation, resulting in further enhancement of nAChR surface expression. Similarly, activation of protein kinase C by nicotine also promotes both subunit assembly and maturation of nAChRs (Wecker et al. 2010). Finally, the ubiquitin-proteosome system has been shown to play an important regulatory role in nicotine upregulation of nAChRs through regulation of receptor internalization and degradation (Christianson and Green 2004; Ficklin et al. 2005). Studies in rat cortical neurons examining the time course of upregulation revealed that many of the previously discussed mechanisms occur in an orchestrated fashion in response to chronic nicotine exposure, including a rapid conformational change leading to a high-affinity receptor state, and a slower process involving increased subunit assembly (Govind et

al. 2012). Taken together, the studies summarized above reveal a complex system of post-translational regulation that appears highly regulated within the exocytic pathway by multiple mechanisms, suggesting that disease states may result from perturbation at any one of a number of nodes.

Nicotinic AChR upregulation in human brain

Nicotinic AChR upregulation also occurs in human brain in response to chronic nicotine exposure. Postmortem studies of the brains of smokers demonstrate increased [^{3}H]-nicotine binding in thalamus and hippocampus compared to both non-smokers and former smokers, due to increased receptor number. [^{3}H]-nicotine binding also correlates with the amount of cigarettes previously smoked (Breese et al. 1997). *In vivo* studies of β2* nAChRs using single-photon emission computed tomography SPECT; [^{123}I]5-I-A-85380; (Mamede et al. 2007; Staley et al. 2006)) or positron-emission tomography (PET; [18F]-2-Fluoro-A85380; (Mukhin et al. 2008; Wullner et al. 2008)) reveal significant upregulation of β2* nAChRs in numerous brain regions in humans who smoke cigarettes. Furthermore, smoking appears to result in rapid saturation of nAChRs and therefore may maintain the majority of high affinity nAChRs in the desensitized state (Brody et al. 2006). Interestingly, upregulation of nAChRs in smokers may be more robust in men versus women (Cosgrove et al. 2012).

Evidence for nAChR expression or trafficking abnormalities in human psychiatric disease

As reviewed above, the effects of nicotine on nAChRs are complex and regulated at multiple points. Unfortunately, the constraints of working with human neurons have generally limited the ability to probe the molecular and cellular mechanisms regulating nAChR expression and function *in vivo*, and as a surrogate, most studies have generally looked at a few specific endpoints. In postmortem tissue, nAChR messenger RNA, total protein, or high-affinity binding sites are generally assayed, while *in vivo* imaging experiments generally assess receptor availability with radioligands, which can reflect changes in receptor occupancy, changes in protein levels, or both. In this section we review studies that have implicated changes in nAChRs or other aspects of cholinergic signaling in psychiatric disease (Table 2). We focus on studies examining nAChRs themselves, rather than making specific conclusions based on clinical findings from drugs thought to be specific for certain types of nAChRs, which have been reported in detail elsewhere (for review, see (Olincy and Freedman 2012)).

Schizophrenia

Amongst psychiatric disorders, the most evidence for abnormalities in both high-affinity (reviewed here) and low-affinity (reviewed in (Olincy and Freedman 2012)) nAChRs is available for schizophrenia. Indeed, genetic studies have implicated both α4β2 (De Luca et al. 2006; Voineskos et al. 2007) as well as α7 (Olincy and Freedman 2012) nAChRs in the etiology of schizophrenia in certain cohorts, although there are studies that have not found a significant correlation between schizophrenia and α7 nicotinic receptors in specific human populations (Neves-Pereira et al. 1998). Studies of both post-mortem brain tissue and *in vivo* imaging of individuals with schizophrenia have been performed, although studies properly accounting for smoking status are the most useful for making strong conclusions. In hippocampus, cytisine binding in tissue homogenates revealed decreased high-affinity binding sites in hippocampus from schizophrenic individuals, who were mostly smokers (Freedman et al. 1995). Similar results were also found in striatal homogenates from schizophrenic individuals compared to control subjects (Durany et al. 2000). Further, postmortem studies provided evidence for regulatory abnormalities of high-affinity nAChRs in schizophrenia (Breese et al. 2000). Post-mortem tissue from individuals with

schizophrenia was compared to controls, with the cohorts including current smokers, former smokers, and never-smokers, and homogenates from hippocampus, thalamus, and cortex (chosen because these regions receive dopaminergic innervation) were assayed for high-affinity binding with [^{3}H]-nicotine and [^{3}H]-epibatidine (Breese et al. 2000). In control subjects, as had been demonstrated previously, smoking increased the levels of [^{3}H]-nicotine binding, and quitting smoking reduced binding to the level of never-smokers. In the schizophrenia cohort, smoking did not increase [^{3}H]-nicotine binding in hippocampus and thalamus, although there was a small, but statistically significant, increase in cortex. There was also decreased [^{3}H]-nicotine and [^{3}H]-epibatidine binding in hippocampus, cortex, and caudate nucleus in schizophrenic smokers when compared directly to non-schizophrenic smokers. The authors recognized that a major confound might be the fact that essentially all of the individuals with schizophrenia were taking antipsychotic medication. To address this issue, they demonstrated in rats that chronic haloperidol treatment does not affect upregulation of epibatidine binding, suggesting that a defect in high-affinity nAChR upregulation is likely reflective of a biological difference between individuals with schizophrenia and controls, not a confound of medication. While most studies of autopsy tissue demonstrate reduced high-affinity nAChR binding in schizophrenia, one study of cortex from schizophrenic individuals and controls surprisingly found increased [^{3}H]-epibatidine and [^{3}H]-cytisine binding in the schizophrenic subjects, although this was assessed with autoradiography, and the study was unable to determine smoking status in a number of their schizophrenic cohort (Marutle et al. 2001).

Recently, imaging studies have also identified significant differences in high-affinity nAChR availability *in vivo* in the brains of individuals with schizophrenia. SPECT imaging with [^{123}I]5-IA-85380 identified decreased β2* nAChR availability in frontal cortex, parietal cortex, and thalamus of schizophrenic smokers as compared to healthy control smokers (D'Souza et al. 2012). This study was done in recently abstinent (~one week) tobacco smokers, since the nicotine from tobacco competes with the radiotracer, and previous studies have shown that there is still upregulation of nAChRs at this time point in smokers without psychiatric illness (Staley et al. 2006). The authors also showed a negative correlation between β2* nAChR availability and negative symptoms. Taken together with the postmortem studies, this suggests that schizophrenic individuals show impaired upregulation of high-affinity nAChRs *in vivo*, and that increased levels of endogenous acetylcholine, which might also cause reduced availability of high-affinity sites, is not likely to explain the decreased β2* nAChR availability.

Mood disorders

In vivo SPECT imaging was also performed in non-smoking individuals with major depressive disorder, comparing individuals with acute illness or recovered illness with controls. In addition to imaging with [^{123}I]5-I-A-85380, this study also included post-mortem binding studies in cortical brain homogenates to evaluate nAChR number after ACh washout from the tissue (Saricicek et al. 2012). In a large number of analyzed brain regions, including cortex, hippocampus, thalamus, and striatum, β2* availability was decreased in acutely ill depressed individuals compared to controls, with an intermediate decrease in those with remitted depression. However, unlike the findings in individuals with schizophrenia, post-mortem binding studies in brain homogenates revealed no significant differences in [^{123}I]5-I-A-85380 binding. As endogenous ACh is washed out in post-mortem tissues, the authors interpreted this to mean that the SPECT findings of decreased availability are due to competition from increased endogenous ACh, as opposed to decreased high-affinity nAChR protein (Esterlis et al. 2013). Taken together with the *in vivo* studies of schizophrenia, these results implicate impaired cholinergic signaling in multiple distinct psychiatric disorders, but with different mechanisms in schizophrenia and

depression. What remains unknown is whether individuals with major depressive disorder demonstrate altered upregulation of high-affinity receptors by nicotine as found in individuals with schizophrenia.

Nicotinic AChRs are also thought to play an important role in bipolar disorder through regulation of GABAergic signaling (Benes 2012), however, limited biochemical work has been published on high-affinity nAChRs in this disease. Microarray data demonstrate significant changes in mRNAs encoding multiple nAChR subunits in individuals with bipolar disorder (Benes et al. 2008). Indirect suggestion of nAChR dysfunction in bipolar disorder comes from an analysis of RIC-3, a protein shown to be involved in nAChR expression and function. This study evaluated post-mortem prefrontal cortex tissue from individuals with schizophrenia, bipolar disorder (many with history of psychosis, some without), and control subjects, and analyzed mRNA coding for RIC-3, α4 and β2 (Severance and Yolken 2007). The goal was to determine whether there was any disease-associated change in the overall quantity of message for RIC-3 and the individual nAChR subunits, and whether the ratio between RIC-3 and each subunit was altered, which might lead to abnormal nAChR function. The authors identified increased levels of mRNAs encoding RIC-3 and the α4 nAChR subunit in brain homogenates from individuals with bipolar disorder, with significantly higher levels in bipolar individuals manifesting psychosis as compared to those without psychosis. There was no correlation between RIC-3 levels and levels of the β2 nAChR subunit in bipolar individuals with psychosis. Given the significant correlation between RIC-3 and β2 nAChR subunit mRNAs in bipolar individuals without psychosis, these data suggest that chaperoning of β2 subunits may be different in individuals with or without psychosis. Most recently, SPECT imaging showed significantly lower β2* nAChR availability in subjects with bipolar depression compared to euthymic and control subjects across frontal, parietal, temporal, and anterior cingulate cortex, hippocampus, amygdala, thalamus, striatum (Hannestad et al. 2013). Similar to the study of individuals with major depressive disorder, there was no significant difference in β2* nAChR number or affinity in temporal cortex of individuals with bipolar depressed as measured in postmortem cortical homogenates following ACh washout. Thus, the authors conclude that the alteration in the cholinergic system observed during a depressive episode may resolve during euthymia.

Attention deficit hyperactivity disorder

A number of studies have identified a strong correlation between maternal smoking during pregnancy and later development of attention deficit hyperactivity disorder (ADHD) in their children (Heath and Picciotto 2009). This suggests that nAChR signaling may be disrupted in the brain in individuals with ADHD. Studies of the spontaneously hypertensive rat (SHR), an animal model of ADHD, have also revealed changes in nAChR binding and mRNA levels (Wigestrand et al. 2011). Binding of [^{3}H]epibatidine, [^{3}H]Methyllycaconitine, [^{125}I]A-85380, and [^{125}I]α-bungarotoxin were evaluated in various brain regions, as were levels of mRNAs encoding multiple nAChR subunits. When compared to the Wistar Kyoto rat as a control, there was a global reduction in high-affinity binding sites in the brains of SHRs, without any change in mRNAs encoding the α4 or β2 subunits. There was no change in levels of low-affinity nicotinic binding sites or in α7 nAChR subunit mRNA levels. Interestingly, high-affinity nicotine binding sites are not upregulated after chronic nicotine exposure in SHR brain tissue, suggesting that there may be an underlying deficit in the machinery involved in nAChR trafficking in these rats (Hohnadel et al. 2005).

Cigarette smoking and other substance use disorders

In addition to association with psychiatric illnesses such as schizophrenia and depression, smoking is also highly prevalent among individuals who use other addictive substances. As

discussed above, chronic exposure to nicotine in tobacco alters nAChR levels in the human brain, and this may contribute to the development of nicotine dependence (Staley et al 2006). A large number of studies have identified the nAChR subtypes, and critical brain circuits responsible for the addictive effects of nicotine, in particular, the ability of nAChRs in the ventral tegmental area to drive dopamine release and support nicotine reinforcement (reviewed in: Brennan et al. 2010; Buisson and Bertrand 2002; Changeux 2010; Govind et al. 2009). Nicotine dependence is also co-morbid with other substance abuse disorders, including addiction to cocaine, alcohol, cannabinoids, and opiates (Tuesta et al. 2011). These findings suggest that the cholinergic system may facilitate addiction to substances whose initial mechanisms of action are distinct from those of nicotine. system, including the nucleus accumbens. A detailed discussion of previous work in this area is beyond the scope of this review, however, recent manuscripts have explored this topic in detail (Lajtha and Sershen 2010; Tuesta et al. 2011).

Future directions and conclusions

The studies reviewed here paint an emerging picture of abnormal high-affinity nAChR regulation in multiple psychiatric conditions; however, there is a need for additional mechanistic study to characterize the molecular and cellular mechanisms underlying these abnormalities in human brain. Further understanding of the pathophysiology of some forms of psychiatric disease, as well as new avenues for targeted drug design, may come from a deeper understanding of the mechanistic details of nAChR regulation and trafficking. Since the ER is a critical node in dynamic nAChR regulation, a better understanding of ER stress responses in psychiatric disease, and in particular how this might affect nAChR stoichiometry, maturation, and trafficking, will likely be fruitful areas of study (Lester et al. 2012). Further impetus to study the ER in psychiatric disease results from the notion that the mechanism of action underlying many psychotropic medications likely involves the ER (Lester et al. 2012). The difficulty of studying these processes in salient cell types from humans with psychiatric disease remains a problem for bridging rodent and human studies. The use of induced pluripotent stem cells, although in its infancy, represents a potentially powerful method for examining nAChR trafficking deficits in psychiatric disease (Brennand and Gage 2012), especially given that the process is likely largely cell autonomous. Such an approach has already been taken to characterize the underlying pathophysiology in multiple neuropsychiatric conditions (for example, schizophrenia and Timothy syndrome, (Brennand et al. 2011; Krey et al. 2012)). Furthermore, remarkable advances in molecular imaging will continue to allow finer resolution of subcellular processes, for example, visualizing ER stress in human brain (Pysz et al. 2010). An understanding of the underlying causes of severe psychiatric illness is becoming more approachable. By elucidating the mechanistic underpinnings of phenomena such as altered nAChR expression and regulation, translational scientists may be able to design better therapeutics for treating individuals who suffer from these challenging diseases.

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Table 1

Identified mechanisms for nicotine-induced nAChR upregulation and selected references.

Mechanism of action	Selected references
Receptor desensitization	(Fenster et al. 1999)
Cellular trafficking	(Darsow et al. 2005)
Receptor maturation	(Sallette et al. 2005)
Pharmacological chaperoning and endoplasmic reticulum exit	(Jeanclos et al. 2001; Richards et al. 2011; Srinivasan et al. 2011)
Phosphorylation and the ubiquitin proteosome system	(Christianson and Green 2004; Ficklin et al. 2005; Jeanclos et al. 2001; Wecker et al. 2010)
Receptor conformational change	(Govind et al. 2012; Vallejo et al. 2005)
Upregulation in human brain	(Breese et al. 1997; Cosgrove et al. 2012; Staley et al. 2006)

Table 2

Summary of observed changes in nAChR in various psychiatric conditions.

Psychiatric condition	Finding	Reference
Schizophrenia	Decreased cytisine binding in hippocampal and striatal homogenates	(Durany et al. 2000; Freedman et al. 1995)
	Impaired nicotine and epibatidine binding upregulation in multiple brain regions	(Breese et al. 2000)
	Increased epibatidine and cytisine binding in cortex	(Marutle et al. 2001)
	Decreased β2* nAChR availability in frontal cortex, parietal cortex, and thalamus of schizophrenic smokers	(D'Souza et al. 2012)
Mood disorders	Increased endogenous ACh in multiple brain regions in major depression	(Saricicek et al. 2012)
	Transcriptional changes in bipolar disorder	(Benes et al. 2008)
	Misregulation of RIC-3 in bipolar disorder with psychosis	(Severance and Yolken 2007)
	Decreased β2* nAChR availability in subjects with bipolar depression	(Hannestad et al. 2013)
ADHD	Reduction in high-affinity binding sites in a rat model of ADHD	(Wigestrand et al. 2011)
Nicotine/other substance dependence	Changes in nAChRs in the mesocorticolimbic dopamine system	(Brennan et al. 2010; Buisson and Bertrand 2002; Changeux 2010; Govind et al. 2009; Picciotto et al. 1998; Staley et al. 2006)

Abbreviations: nAChR, nicotinic acetylcholine receptor; ADHD, attention deficit hyperactivity disorder.