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REVIEW

Muscarinic and Nicotinic Acetylcholine Receptor Agonists and Allosteric Modulators for the Treatment of Schizophrenia

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Muscarinic and nicotinic acetylcholine (ACh) receptors (mAChRs and nAChRs) are emerging as important targets for the development of novel treatments for the symptoms associated with schizophrenia. Preclinical and early proof-of-concept clinical studies have provided strong evidence that activators of specific mAChR (M_1 and M_4) and nAChR (α_7 and $\alpha_2\beta_4$) subtypes are effective in animal models of antipsychotic-like activity and/or cognitive enhancement, and in the treatment of positive and cognitive symptoms in patients with schizophrenia. While early attempts to develop selective mAChR and nAChR agonists provided important preliminary findings, these compounds have ultimately failed in clinical development due to a lack of true subtype selectivity and subsequent dose-limiting adverse effects. In recent years, there have been major advances in the discovery of highly selective activators for the different mAChR and nAChR subtypes with suitable properties for optimization as potential candidates for clinical trials. One novel strategy has been to identify ligands that activate a specific receptor subtype through actions at sites that are distinct from the highly conserved ACh-binding site, termed allosteric sites. These allosteric activators, both allosteric agonists and positive allosteric modulators, of mAChR and nAChR subtypes demonstrate unique mechanisms of action and high selectivity *in vivo*, and may provide innovative treatment strategies for schizophrenia.

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INTRODUCTION

Schizophrenia is a complex psychiatric illness that affects approximately 1% of the population worldwide and is characterized by three broad clusters of symptoms that result in lifelong disability. These symptom domains include positive symptoms, such as delusions, thought disorders, and hallucinations; negative symptoms, including anhedonia, blunted affect, and social withdrawal; and cognitive impairments in sensory information processing, attention, working memory, and executive functions (American Psychiatric Association, 2000; Nuechterlein *et al*, 2004). Clinically available typical (eg, haloperidol) and atypical (eg, clozapine, risperidone) antipsychotic medications provide relief for the positive symptoms, but have little or no effect on the negative symptoms or cognitive impairments (Keefe *et al*, 2007; Swartz *et al*, 2008). Moreover, poor social and occupational outcomes in individuals with schizophrenia are directly linked with the impairments in normal cognitive function (Green *et al*, 2004). Effective treatment for schizophrenia is further complicated by marked heterogeneity in the onset of treatment response, with subpopulations of schizophrenic patients showing either delayed onsets of antipsychotic drug action (Kaplan *et al*, 1990; Garver *et al*, 1991; McDermott *et al*, 1991) or rapid responses within hours to days of initiating treatment (Agid *et al*, 2003, 2008; Kapur *et al*, 2005; Leucht *et al*, 2005; Raedler *et al*, 2007). Other limitations for successful treatment of this disorder include partial responsiveness or treatment resistance to currently available antipsychotic medications (Lieberman *et al*, 2003) and adverse drug effects, including extrapyramidal motor side effects, metabolic syndrome, and agranulocytosis (Gerlach *et al*, 1975; Idänpään-Heikkilä *et al*, 1975; Parsons *et al*, 2009). While the etiology of schizophrenia is unknown, imbalances in

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several neurotransmitter systems have been implicated in the pathophysiology of this illness, including structural and functional abnormalities in the dopaminergic, glutamatergic, γ-amino butyric acid (GABA)ergic, and cholinergic systems (Carlsson, 1977; Jentsch and Roth, 1999; Guan *et al*, 1999; Krystal *et al*, 2002; Lewis and Moghaddam, 2006; Severance and Yolken, 2008; Scarr *et al*, 2009; Howes and Kapur, 2009; Beneyto and Lewis, 2011). Taken together, there remains a tremendous unmet need to develop novel therapies to more effectively and safely address the complex symptoms associated with schizophrenia.

The coordination of different cognitive and affective functions requires proper signaling through both muscarinic and nicotinic acetylcholine (ACh) receptors (mAChRs and nAChRs) and disruptions in mAChR and nAChR signaling have been implicated in the symptoms observed in schizophrenic patients (Guan *et al*, 1999; Severance and Yolken, 2008; Scarr *et al*, 2009). For example, mAChR and nAChR antagonists, such as scopolamine and mecamylamine, have shown potent amnesiac properties in animals and humans (Domer and Schuller, 1960; Pazzagli and Pepeu, 1965; Rusted and Warburton, 1988; Decker and Majchrzak, 1992; Newhouse *et al*, 1992, 1994; Terry *et al*, 1996), whereas mAChR and nAChR agonists and acetylcholinesterase inhibitors (AChEIs) have augmented normal cognition and/or ameliorated impairments induced by lesions of cholinergic circuitry or antagonism of cholinergic receptors (Aigner and Mishkin, 1986; Elrod *et al*, 1988; Rupniak *et al*, 1989; Matsuoka *et al*, 1991; Levin *et al*, 1998, 2006; Newhouse *et al*, 2004; Sarter *et al*, 2009). Furthermore, mAChR and nAChR antagonists have exacerbated existing positive and cognitive symptoms in schizophrenic patients and/or induced psychosis in normal human volunteers (Harington and Kincaid-Smith, 1958; Osterholm and Camoriano, 1982; Hamborg-Petersen *et al*, 1984; Tandon *et al*, 1991), whereas mAChR and nAChR agonists and AChEIs have improved certain aspects of the positive and/or negative symptoms, and attentional and memory-related deficits (Janowsky *et al*, 1973; Smith *et al*, 2006; Harris *et al*, 2004; Edelstein *et al*, 1981; Kirrane *et al*, 2001; Shekhar *et al*, 2008). Overall, these preclinical and clinical findings support the hypothesis that imbalances in mAChR and/or nAChR signaling may underlie the symptoms associated with schizophrenia.

Unfortunately, all cholinergic ligands used in early preclinical and clinical studies, including AChEIs (eg, Forette *et al*, 1999; Feldman *et al*, 2007), mAChR agonists (Bodick *et al*, 1997a, b; Shekhar *et al*, 2008), and nAChR agonists (Ingram *et al*, 2005), lacked true receptor subtype selectivity, resulting in numerous dose-limiting adverse effects and failure in clinical development (Bruno *et al*, 1986; Bodick *et al*, 1997a, b; Shekhar *et al*, 2008). For several decades, the lack of subtype-selective ligands for the mAChRs and nAChRs has also prevented a more comprehensive understanding of the fundamental roles of these different receptor subtypes in the central nervous system and in the clinical efficacy observed with AChEIs and non-selective mAChR and nAChR agonists. The interpretation of the effects of non-selective mAChR and nAChR agonists and antagonists in animals and clinical populations is further complicated by the fact that these ligands activate or antagonize both pre- and postsynaptically expressed receptor subtypes. For example, in contrast to the action of the mAChR antagonist scopolamine on postsynaptic mAChRs, antagonism of presynaptic mAChRs results in enhanced release of ACh and subsequent activation of other mAChRs and nAChRs (Bymaster *et al*, 1993; Quirion *et al*, 1994; Carey *et al*, 2001). In addition, recent studies have indicated that there is a critical balance in cholinergic neurotransmission required for normal cognitive and motivational functions that appears to be both brain region- and task-specific (Hasselmo and Sarter, 2010). These findings suggest that tonic enhancement of cholinergic neurotransmission by a mAChR or nAChR agonist may not appropriately normalize cholinergic neurotransmission for the improvement of different cognitive and affective disturbances in schizophrenic patients. However, the state of cholinergic signaling in different brain regions and during different tasks remains unknown in schizophrenia. Potential evidence for regional alterations in cholinergic signaling has been demonstrated in reductions of choline acetyltransferase (ChAT) activity, the catalytic enzyme involved in the synthesis of ACh, in the nucleus accumbens and pontine tegmentum of postmortem brain tissue from schizophrenic patients (Bird *et al*, 1977; Karson *et al*, 1993). Moreover, reductions in cortical and pontine tegmentum ChAT activity have been directly correlated with decreased cognitive performance in schizophrenic patients (Karson *et al*, 1996; Powchik *et al*, 1998). Using magnetic resonance spectroscopy, elevated levels of free choline and phosphocholines, precursors of ACh and membrane phospholipids, respectively, have also been detected in thalamic, anterior cingulate, and caudate brain regions of antipsychotic-naive individuals with schizophrenia (Bustillo *et al*, 2002). These alterations are constistent with abnormalities in phospholipid membrane synthesis and integrity, but may also reflect impaired ChAT function. By contrast, other studies have found no changes or regional elevations in ChAT activity, indicating the potential for variability and/or artifacts with the use of postmortem brain tissues (eg, differences in tissue quality, postmortem interval, patient medication history, appropriate age-matched controls, and/or small cohort sizes) (Domino *et al*, 1973; McGeer and McGeer, 1977). Limitations with these early pharmacological and postmortem studies further support the need to develop subtype-selective mAChR and nAChR ligands. However, the high conservation of the ACh-binding site across the different mAChR or nAChR subtypes has presented a major obstacle to the development of highly selective ACh orthosteric-site ligands.

Over the last decade, a novel approach has been undertaken in the discovery of mAChR and nAChR ligands that activate a particular receptor subtype by actions at sites that are topographically distinct and less highly conserved than

the orthosteric binding site of ACh, termed allosteric sites. The development of allosteric activators is already a well validated approach with benzodiazepines, which are allosteric activators of $GABA_A$ receptors and provide a safe, effective treatment approach for anxiety disorders without inducing adverse effects of direct-acting $GABA_A$ receptor agonists (Ehlert *et al*, 1983). Allosteric activators of mAChRs and nAChRs possess high subtype selectivity and can show different modes of action. Allosteric agonists can activate the receptor subtype directly and do not require the presence of ACh. Positive allosteric modulators (PAMs), on the other hand, do not directly activate the receptor, but bind to an allosteric site distinct from the ACh-binding site and potentiate the effects of endogenous ACh. One potential advantage of the use of mAChR and nAChR PAMs is that these ligands have no intrinsic activity and can only exert their effects in the presence of ACh at a given synapse, thereby maintaining some level of activity dependence of endogenous receptor activation. In the case of nAChRs, another approach has been the development of selective partial nAChR agonists, most notably for the α_7 nAChR, which provide receptor activation with less desensitization. To date, these novel agonists and allosteric activators of the different mAChR and nAChR subtypes have shown robust efficacy in preclinical models of antipsychotic-like activity and/or enhancement of cognitive function, and possess suitable physiochemical properties for optimization as potential clinical candidates. In this review, we will provide a brief overview of cholinergic neurotransmission, circuitry, and mAChR and nAChR structure and function. We will next describe the evidence for the roles of the muscarinic and nicotinic cholinergic systems in the pathophysiology of schizophrenia. We will then review the preclinical and clinical breakthroughs in the development of highly subtype-selective allosteric agonists and PAMs for the mAChRs, most notably M_1 and M_4, and the partial agonists and PAMs for the α_7 and $\alpha_4\beta_2$ nAChRs, and their potential advantages and limitations for the treatment of schizophrenia.

ACh AND CHOLINERGIC TRANSMISSION

Central cholinergic transmission is coordinated through projection neurons and interneurons. Cholinergic projection neurons are organized into relatively discrete cell groups, Ch1–Ch6, with distinct projection patterns. The basal forebrain cholinergic projection neurons (Ch1–Ch4) in the medial septum, diagonal band of Broca, and the nucleus basalis of Meynert are the major source of ACh to the cortical and hippocampal regions (Mesulam *et al*, 1983). The cholinergic projection neurons with the cell bodies in the pedunculopontine tegmantal nucleus (Ch5) and the laterodorsal tegmental nucleus (Ch6) project to the thalamus, pontine reticular formation, and the dopamine (DA) neurons in the ventral tegmental area (VTA) and the substantia nigra (SN) (Satoh and Fibiger, 1986). In the caudate-putamen and nucleus accumbens, large cholinergic interneurons are the only source of Ach (Kimura *et al*, 1980; Bolam *et al*, 1984; Phelps *et al*, 1985). Thus, the central cholinergic system is strategically positioned to modulate brain function at sites thought to be impacted by schizophrenia, especially through key functional interactions with dopaminergic and glutamatergic systems.

ACh modulates a host of physiological processes in the central and peripheral nervous systems. Centrally, ACh regulates motor function, sensory perception, cognitive processing, arousal, sleep/wake cycles, and nociception, while in the periphery it controls heart rate, gastrointestinal tract motility, and smooth muscle activity (Abrams *et al*, 2006). ACh mediates its effects through activation of two functionally and structurally distinct families of cell-surface receptors, the mAChRs and nAChRs. A schematic representation of a hypothetical cholinergic synapse illustrating the general synaptic localization and the function of the mAChRs and nAChRs is shown in Figure 1. The nAChRs, members of the ligand-gated ion channel superfamily that includes $GABA_A$ and $GABA_C$ receptors, are divided into muscle nAChRs at the skeletal neuromuscular junction and neuronal nAChRs, which mediate fast synaptic neurotransmission throughout the nervous system (Harvey and Dryden, 1974; Mulle *et al*, 1991). The mAChRs are members of the Family-A G-protein-coupled receptors (GPCRs) and provide slower and more sustained synaptic responses through second messenger systems (Dutar and Nicoll, 1988). Both muscarinic and nicotinic receptors exist in various subtypes offering numerous ways to pharmacologically alter cholinergic transmission.

Muscarinic Receptor Subtypes

Structure of mAChRs. To date, five molecularly distinct mammalian subtypes of mAChRs, M_1–M_5, have been cloned (Bonner *et al*, 1987, 1988; Liao *et al*, 1989). Each of the five mAChR subtypes is a seven-transmembrane (TM) protein that can be further divided into two major functional classes based on G-protein coupling (see Figure 2). The M_1, M_3, and M_5 mAChRs selectively couple to the Gq/G11-type G-proteins, which leads to the generation of inositol-1,4,5-trisphosphate and 1,2-diacylglycerol through activation of phosphoinositide-specific phospholipase-Cβ, and subsequent increases of intracellular calcium levels (Felder, 1995; Espada *et al*, 2009). The M_2 and M_4 mAChRs preferentially activate Gi/Go-type G-proteins, resulting in the inhibition of adenylyl cyclase and prolongation of the opening of potassium, non-selective cation, and transient receptor potential channels (Felder, 1995; Migeon *et al*, 1995). All mAChR subtypes show a high homology sequence for the orthosteric ACh-binding site, which is thought to account for the past difficulties in developing subtype-selective ligands for muscarinic receptors.

Distribution and function of mAChRs. Neuroanatomical studies using subtype-specific antibodies for the different mAChRs have established distinct patterns of expression for

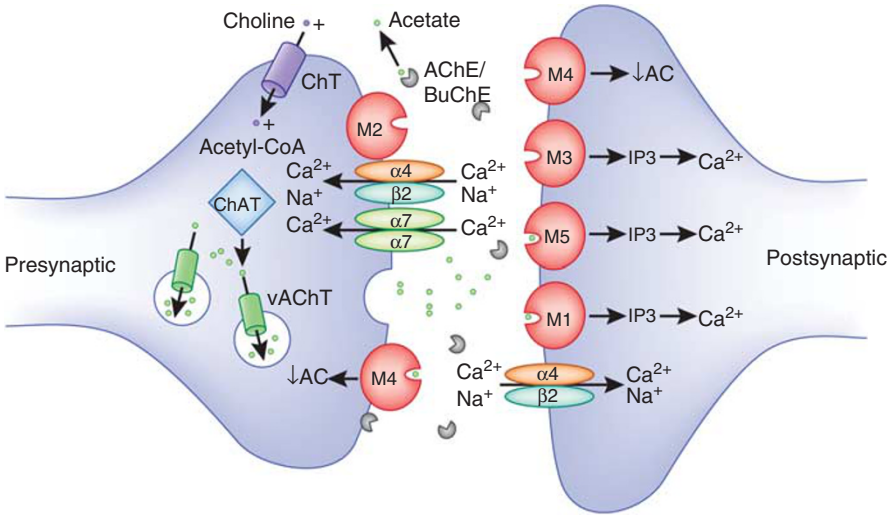

Figure 1. Schematic representation of a hypothetical cholinergic synapse illustrating general synaptic localization and function of cholinergic receptors relevant to schizophrenia. mAChR subtypes have diverse synaptic localization patterns and function pre- and postsynaptically to modulate neurotransmitter release and postsynaptic excitability, respectively. For instance, the M_2 and M_4 mAChRs serve as autoreceptors on cholinergic terminals to suppress ACh release and inhibit cholinergic neurotransmission at select synapses in the central nervous system (left neuron). The mAChRs located on non-cholinergic neurons act as heteroceptors controlling the release of other neurotransmitters, such as DA (not shown). M_1, M_3, M_5, but also M_4 mAChRs that are located postsynaptically facilitate slow cholinergic synaptic neurotransmission relative to nAChR subtypes. The α_7 and $\alpha_4\beta_2$ nAChR subtypes mediate fast synaptic transmission and also use-dependent changes required for neuronal plasticity. These nAChR subtypes can have both pre- and postsynaptic localization. The endogenous ligand of these cholinergic receptors, ACh, is synthesized in cholinergic neurons (left neuron) by the enzyme ChAT through the transfer of acetyl-CoA onto choline. Choline uptake is mediated by presynaptic high-affinity choline transporters (ChT). After synthesis, ACh is packaged into synaptic vesicles by the vesicular ACh transporter (vAChT). After neuronal activation-mediated release into the synaptic cleft, ACh can bind to pre- and postsynaptic receptors, or it can be inactivated through hydrolysis by the AChE enzymes, a process that can be inhibited by different substances (eg, organophosphates, AChE inhibitors) to increase synaptic ACh levels. Once ACh is hydrolyzed, choline is transported through the ChTs into the presynaptic terminal, where it is again synthesized into ACh.

the different mAChR subtypes within key forebrain and limbic structures (Levey *et al*, 1991, 1994, 1995a, b). More recently, the development and characterization of knockout (KO) mice for each of the mAChR subtypes have provided a clearer understanding of the central and peripheral functions of each subtype (see Wess *et al*, 2007).

As the predominant subtype in the central nervous system, the M_1 mAChR is expressed in the striatum, throughout all layers of the cortex, and postsynaptically on the cell bodies and dendrites of hippocampal pyramidal neurons and granule cells (Levey *et al*, 1991, 1995b; Marino *et al*, 1998; Rouse *et al*, 1998, 1999). One of the most important effects of M_1 mAChR activation in the hippocampus and other forebrain regions is the potentiation of currents through the *N*-methyl-D-aspartate receptor (NMDAR) of the glutamatergic system (Marino *et al*, 1998). Owing to the major contribution of NMDAR signaling to the regulation of cognitive function and neural circuits thought to be disrupted in schizophrenia (Tsai and Coyle, 2002; Marino *et al*, 1998), ligands that selectively activate M_1 mAChRs are postulated to alleviate some of the psychotic and cognitive deficits observed in schizophrenia through enhancement of NMDAR neurotransmission. In addition, recent studies by Shirey *et al* (2009) have shown that activation of M_1 mAChRs markedly increases the synaptic excitation of pyramidal cells in the medial prefrontal cortex (mPFC) and enhances mPFC-mediated cognitive functions, including performance in attentional set shift tasks. By contrast, M_1-KO mice showed specific deficits in tasks requiring mPFC function (Anagnostaras *et al*, 2003). Other changes observed in M_1-KO mice, particularly increased spontaneous locomotor activity and enhanced amphetamine-induced hyperactivity coupled with a twofold increase in extracellular striatal DA (Gerber *et al*, 2001; Miyakawa *et al*, 2001), indicate a role for the M_1 mAChRs in the regulation of the dopaminergic system.

The M_2 mAChRs are expressed presynaptically on cholinergic terminals throughout the brain, particularly in the cortex, basal forebrain, hippocampus, and striatum, and function as autoreceptors to inhibit ACh release (Rouse *et al*, 2000; Zhang *et al*, 2002; Tzavara *et al*, 2003), suggesting that selective M_2 mAChR antagonists may be beneficial for cognition. M_2 mAChRs are also localized on the axon terminals of non-cholinergic neurons and serve as heteroceptors involved in the presynaptic regulation of release of other neurotransmitters (Rouse *et al*, 2000). The deficits of M_2-KO mice in behavioral flexibility, working memory, passive avoidance learning, and hippocampal short- and long-term potentiation (LTP) (Gomeza *et al*, 1999b; Tzavara *et al*, 2003; Seeger *et al*, 2004) are consistent with the interpretation that blockade of all M_2 mAChRs on cholinergic and non-cholinergic terminals may actually have detrimental effects on cognition. To date, it is unclear whether M_2 mAChR antagonists will actually be beneficial

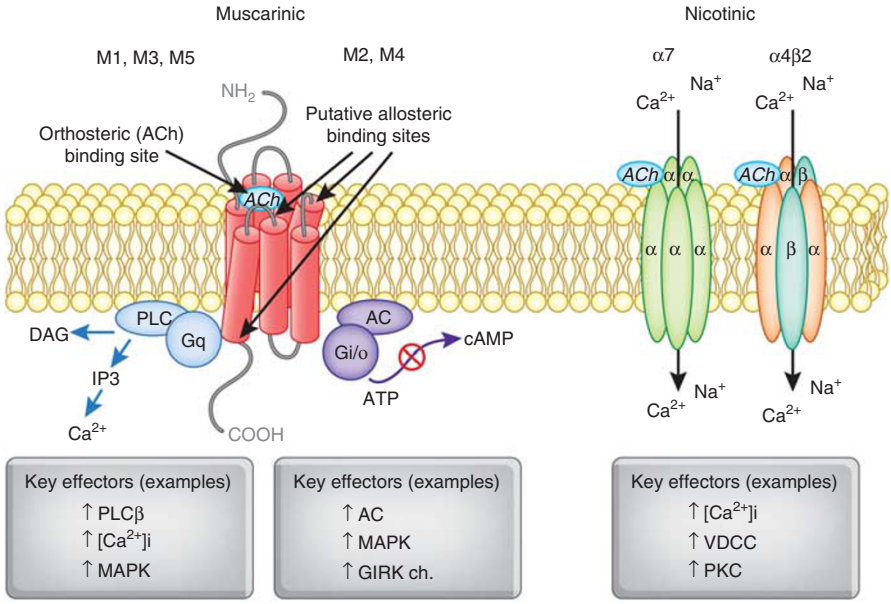

Figure 2. The structure and signaling pathways of mAChRs and nAChRs. Each mAChR subtype is a seven-transmembrane protein, which belongs to two major functional classes based on G-protein coupling. The M_1, M_3, and M_5 mAChRs selectively couple to the Gq/G11-type G-proteins resulting in the generation of inositol-1,4,5-trisphosphate (IP3) and 1,2-diacylglycerol (DAG) through activation of the phosphoinositide-specific phospholipase-Cβ leading to increased intracellular calcium levels. The M_2 and M_4 mAChRs preferentially activate Gi/Go-type G-proteins, thereby inhibiting adenylate cyclase, reducing intracellular concentration of cAMP, and prolonging potassium channel opening. All mAChR subtypes show a high sequence homology across species, particularly in the orthosteric ACh-binding sites. Neuronal nAChRs are pentameric ligand-gated ion channels. The most abundant neuronal subunits are α_4, β_2, and α_7, with the heteromeric $\alpha_4\beta_2$ receptor subtype in highest abundance. The heteromeric $\alpha_4\beta_2$ receptor subtype can exist in two different forms: $(\alpha4)_2(\beta2)_3$ receptors show low Ca^{2+} permeability and high affinity to ACh and nicotine, whereas $(\alpha4)_3(\beta2)_2$ receptors have high Ca^{2+} permeability. By contrast, the α_7 nAChR also shows high permeability to Ca^{2+} relative to the heteromeric $\alpha_4\beta_2$ nAChRs. The action of $\alpha_4\beta_2$ nAChRs can enhance intracellular levels of Ca^{2+} by secondary activation of VOCCs, whereas α_7 nAChRs preferentially increase Ca^{2+} release from ryanodine-sensitive intercellular stores through CICR. The capacity of these different nAChR subtypes to couple to VOCC or CICR mechanisms results in distinct patterns of Ca^{2+} signaling that can provide a broader control of synaptic plasticity and neurotransmitter release, as well as gene transcription.

for the cognitive deficits and other symptoms observed in schizophrenia.

Compared with the other mAChR subtypes, relatively little is known about the role of the neuronal M_3 mAChRs, which are expressed at low levels throughout the central nervous system. M_3-KO mice are lean and hypophagic, with decreased serum leptin levels, a phenotype that appeared to be hypothalamus-driven rather than from decreased salivary flow or gastrointestinal motility (Yamada *et al*, 2001b). More recently, M_3-deficient mice were shown to have severe deficits in hippocampus-mediated contextual fear conditioning, suggesting that selective M_3 mAChR activators may be beneficial for cognition (Poulin *et al*, 2010).

The M_4 mAChRs are located across many brain regions, including the cortex and hippocampus, but are most prominent in the striatum (Levey *et al*, 1991; Hersch *et al*, 1994; Rouse *et al*, 1999) where they are enriched in cholinergic interneurons and striatal projection neurons, particularly those projecting directly to the SN (Ince *et al*, 1997). The M_4 mAChRs can function as autoreceptors in the striatum and midbrain (Zhang *et al*, 2002; Tzavara *et al*, 2004), and as postsynaptic modulatory receptors in the striatum, neocortex, and hippocampus (Levey *et al*, 1991; Zang and Creese, 1997). M_4-KO mice showed increased locomotor activity, enhanced DA D1 receptor-mediated effects (Gomeza *et al*, 1999a), and increased basal and psychostimulant-induced DA levels in the nucleus accumbens (Tzavara *et al*, 2004), which are recapitulated in mice with targeted KO of M_4 mAChRs in neurons expressing DA D1 receptors (Jeon *et al*, 2010). Taken together, this hyper-dopaminergic phenotype suggests that facilitation of M_4 mAChR function may be beneficial for the treatment of schizophrenia. For example, stimulation of M_4 autoreceptors, located on the terminals of cholinergic neurons in the caudal midbrain, is predicted to decrease the activity of dopaminergic VTA neurons, leading to a reduction of nucleus accumbal DA release.

By contrast, the M_5 mAChRs have only been found postsynaptically on the dopaminergic neurons in the VTA and SN pars compacta, the two brain regions that provide dopaminergic innervation to the dorsal striatum, nucleus accumbens, and mPFC (Vilaró *et al*, 1990; Weiner *et al*, 1990). M_5-KO mice showed deficits of pre-pulse inhibition (PPI) of the acoustic startle reflex, indicating abnormal sensorimotor gating (Thomsen *et al*, 2007) and reduced sensitivity to the locomotor and/or rewarding effects of cocaine (Fink-Jensen *et al*, 2003; Thomsen *et al*, 2005) and opiates (Basile *et al*, 2002;

Yamada *et al*, 2003; Steidl and Yeomans, 2009), two strong activators of mesolimbic DA signaling. These findings suggest that selective M_5 mAChR antagonists may be useful for controlling the hyperactive mesolimbic dopaminergic circuitry that is reported in schizophrenia. Non-neuronal M_5 mAChRs are localized to the cerebrovasculature and control cerebral vasodilation and blood flow (Yamada *et al*, 2001a; Araya *et al*, 2006). The neuronal atrophy and impairments in novel object recognition observed in M_5-KO mice (Araya *et al*, 2006) may be due to dysfunction of the cerebrovasculature and therefore of non-neuronal origin.

Nicotinic Receptor Subtypes

Structure of nAChRs. Neuronal nAChRs are pentameric ligand-gated ion channels (see Figure 2). To date, molecular cloning has identified nine α (α_2–α_{10}) and three β-subunits (β_2–β_4) (Conti-Tronconi *et al*, 1982). Homomeric nAChRs are composed only of α-subunits (α_7–α_9 subtype), whereas heteromeric nAChRs have different combinations of α and β-subunits (eg, the $\alpha_4\beta_2$ subtype). The most abundant neuronal subunits are α_4, β_2, and α_7, with the heteromeric $\alpha_4\beta_2$ receptor subtype comprising over 90% of all neuronal nAChRs (Corriveau and Berg, 1993; Perry *et al*, 2002). The $\alpha_4\beta_2$ nAChR is composed of α_4 and β_2 subunits that can be expressed in two different stoichiometries, the $(\alpha_4)_2(\beta_2)_3$ and $(\alpha_4)_3(\beta_2)_2$ receptor subtypes (Tapia *et al*, 2007). The $(\alpha_4)_2(\beta_2)_3$ receptors show low Ca^{2+} permeability and high affinity to ACh and nicotine, whereas the $(\alpha_4)_3(\beta_2)_2$ receptors have high Ca^{2+} permeability with low sensitivity to nicotine (Anderson *et al*, 2009; Carbone *et al*, 2009). In contrast to the $\alpha_4\beta_2$ nAChR subtype, the α_7 nAChR shows relatively low ACh affinity and rapid desensitization kinetics in the presence of 100 μM ACh or higher (Fenster *et al*, 1997). The α_7 nAChR also shows high permeability to Ca^{2+} relative to the heteromeric $\alpha_4\beta_2$ nAChRs (Seguela *et al*, 1993). Whereas $\alpha_4\beta_2$ nAChRs can further augment the intracellular levels of Ca^{2+} by secondary activation of voltage-operated calcium channels (VOCCs), α_7 nAChRs preferentially mobilize Ca^{2+} release from ryanodine-sensitive intracellular stores through a Ca^{2+}-induced Ca^{2+} release (CICR) (Dajas-Bailador *et al*, 2002). The capacity of these different nAChR subtypes to couple to VOCC or CICR mechanisms results in distinct patterns of Ca^{2+} signaling that may deliver a broader control of synaptic signaling and neurotransmitter release.

There are four traditional conformation states of activation for all nAChRs: resting (closed channel with an unoccupied agonist-binding site), active (open channel), desensitized (closed channel with high-affinity agonist binding), and an inactive state that is a more prolonged desensitized state (Changeux *et al*, 1984). With acute exposure to high concentrations of ACh or non-selective nAChR agonists such as nicotine, the equilibrium between these conformation states shifts to an active state, allowing signal transduction followed by subsequent desensitization of the receptor. However, under sustained exposure to low concentrations of agonists, the desensitized conformational state of the receptor can be stabilized and become refractory to agonist activation. The ACh-binding site for activation of nAChRs is located at the interface between the α-subunit and an adjacent subunit (Blount and Merlie, 1989). The $\alpha_4\beta_2$ nAChR contains two identical ACh-binding sites, whereas the homomeric α_7 nAChR contains up to five possible ACh-binding sites (Palma *et al*, 1996). Although the α_7 nAChR shows lower sensitivity to ACh activation and rapid desensitization, it has been speculated that the five ACh-binding sites provide a more versatile range of sensitivity and signaling than other nAChR subtypes.

Distribution and function of α_7 and $\alpha_4\beta_2$ nAChRs. The α_7 and $\alpha_4\beta_2$ nAChR subtypes are expressed on postsynaptic membranes (Schoepfer *et al*, 1990; Gotti and Clementi, 2004; Perry *et al*, 2002) or presynaptically to regulate the release of ACh and other neurotransmitters (Wonnacott, 1997; Li *et al*, 1998; Sher *et al*, 2004) in key corticolimbic circuits shown to be disrupted in schizophrenia. In the hippocampus, the nAChRs are expressed predominantly on GABAergic interneurons, with moderate expression on pyramidal neurons (Fabian-Fine *et al*, 2001; Ji *et al*, 2001). The majority of these neurons express the α_7 nAChR subtype, whereas a fraction of interneurons are also mecamylamine-sensitive indicating the presence of non-α_7 nAChRs (Ji and Dani, 2000; McQuiston and Madison, 1999). The rapid desensitization of the α_7 nAChR subtype provides a critical feedback mechanism for cholinergic signaling, especially for the autoregulation of neurotransmission at cholinergic synapses where fast desensitization can avoid the potential for uncontrolled increases in response. While extensive behavioral characterization of α_7-KO mice demonstrated that the α_7-subunit is not required for a number of normal behavioral responses (Paylor *et al*, 1998), these mutant mice did lack rapidly desensitizing nicotinic currents in the hippocampal neurons, suggesting the involvement of α_7-containing nAChRs in hippocampus-mediated synaptic plasticity (Orr-Urtreger *et al*, 1997; Ji *et al*, 2001). In addition, the nicotine activation of presynaptic α_7 nAChRs induces long-term enhancement of glutamatergic transmission in the VTA (Mansvelder and McGehee, 2000), whereas stimulation of non-α_7 nAChR postsynaptic receptors can enhance GABAergic signaling (Mansvelder *et al*, 2002). For example, the $\alpha_4\beta_2$ nAChRs localized on somatodendritic regions of interneurons facilitate inhibitory GABA signaling (see Albuquerque *et al*, 2009). Several studies have also reported anatomical and pharmacological evidence for functional presynaptic $\alpha_4\beta_2$ nAChRs that modulate DA release from nigrostriatal terminals (Soliakov *et al*, 1995; Soliakov and Wonnacott, 1996; Luo *et al*, 1998; Wonnacott *et al*, 2000) and loss of nicotine-induced stimulation of nucleus accumbal DA release in β_2 KO mice. Interestingly, the β_2-subunit-containing nAChRs may provide an important role for neuron survival and maintenance of normal cognitive

functions during aging as aged β_2-KO mice at 22–24 months show disruptions in spatial learning tasks, cortical and hippocampal neuronal atrophy, gliosis, and an increase of serum corticosterone levels (Zoli *et al*, 1999). In α_4-KO mice, high-affinity nicotine-induced physiological responses were absent in the thalamic and raphe magnus neurons, and the antinociceptive effects of nicotine were diminished (Marubio *et al*, 1999).

ROLE OF MUSCARINIC RECEPTOR SUBTYPES IN SCHIZOPHRENIA

Multiple lines of evidence suggest that alterations in central muscarinic cholinergic neurotransmission are involved in the underlying pathophysiology of schizophrenia. Early validation came from extensive preclinical and clinical studies with non-selective mAChR ligands. Non-selective mAChR antagonists (eg, scopolamine) robustly impaired multiple cognitive functions, such as sensory information processing, attention, learning, working and short-term memory, and executive tasks, whereas direct- and indirect-acting (eg, AChEIs) mAChR agonists enhanced aspects of normal cognition and/or reversed cognitive impairments induced by mAChR antagonists or cholinergic circuit lesions (Aigner and Mishkin, 1986; Rusted and Warburton, 1988; Rupniak *et al*, 1989; Matsuoka *et al*, 1991; Decker and Majchrzak, 1992). Moreover, mAChR antagonists induced psychotic-like symptoms and cognitive impairments in healthy subjects and/or exacerbated existing positive and cognitive symptomatology in schizophrenic patients (Osterholm and Camoriano, 1982; Hamborg-Petersen *et al*, 1984; Tandon *et al*, 1991). Non-selective mAChR agonists have been reported, albeit in many cases anecdotally, to provide moderate efficacy for the symptoms in schizophrenic patients (Pfeiffer and Jenny, 1957; Edelstein *et al*, 1981). Unfortunately, interpretations of these early studies are confounded by the lack of true subtype-selective ligands *in vivo*. Nevertheless, postmortem, clinical imaging, and genetic approaches have further implicated mAChR expression and function in the underlying pathophysiology of schizophrenia.

Several postmortem [^{3}H]pirenzepine-binding studies have demonstrated decreased levels of M_1/M_4 mAChRs in specific brain regions of schizophrenic patients, including the prefrontal and anterior cingulate cortices, superior temporal gyrus, hippocampus, and dorsal striatum (Dean *et al*, 1996, 2002; Crook *et al*, 1999, 2000, 2001; Katerina *et al*, 2004; Deng and Huang, 2005). These changes in mAChR expression appear to be specific to schizophrenia, as similar decreases were not observed in patients with bipolar disorder or major depression (Zavitsanou *et al*, 2004). Others have found decreased levels of M_1 mAChR mRNA and/or protein in the superior prefrontal gyrus and dorsolateral PFC in individuals with schizophrenia (Mancama *et al*, 2003; Dean *et al*, 2002). In addition, *in vivo* mAChR occupancy was decreased by 20–33% in a group of unmedicated schizophrenic patients relative to controls (Raedler *et al*, 2003), and although the use of a pan-mAChR SPECT ligand for these studies does not provide information on which mAChR subtype(s) are decreased, the results are consistent with the postmortem studies. Taken together, these anatomical studies suggest that decreases in mAChR levels may be both region- and subtype-specific in schizophrenic patients. However the interpretation of these findings may be confounded by the lack of subtype-selective radioligands and the possible effects of atypical antipsychotics. Interestingly, a polymorphism of the M_1 mAChR gene (*CHRM1*) was associated with improved performance on the Wisconsin Card Sorting Test in schizophrenic patients (Liao *et al*, 2003). Other cholinergic receptor genes have also been linked to schizophrenia. For example, the M_5 mAChR (*CHRM5*) and α_7 nAChR (*CHRNA7*) genes on 15q13 were also found to confer susceptibility to schizophrenia (De Luca *et al*, 2004).

Breakthrough with the M_1/M_4 mAChR Agonist Xanomeline

Over the last two decades, mAChR agonists developed for the cognitive impairment associated with Alzheimer's disease (AD) and other dementias have failed during clinical trials owing to dose-limiting adverse effects from non-selective activation of peripheral mAChR subtypes (Bruno *et al*, 1986; Bodick *et al*, 1997a, b). However, in one large multicenter trial on the effects of the M_1/M_4 mAChR agonist xanomeline in AD patients, significant effects were observed on the behavioral disturbances with a trend toward improvement in cognition (Bodick *et al*, 1997a, b). In particular, xanomeline produced a robust dose-dependent reduction in vocal outbursts, suspiciousness, delusions, agitation, and hallucinations, while improving blunted affect and other AD-related behavioral disturbances that share similarities to those observed in schizophrenia. This surprising finding raised the possibility that xanomeline might provide a novel approach for the treatment of schizophrenia.

In preclinical models predictive of antipsychotic-like activity, xanomeline was shown to produce an efficacy profile similar to atypical antipsychotics like clozapine (Stanhope *et al*, 2001; Perry *et al*, 2001; Jones *et al*, 2005). For example, xanomeline induced Fos expression and increased monoamine turnover in the PFC and nucleus accumbens but not in the dorsal striatum (Perry *et al*, 2001). After acute and chronic dosing, xanomeline inhibited VTA, but not SN, DA cell firing similar to clozapine (Shannon *et al*, 2000). In rodent behavioral studies, xanomeline dose dependently inhibited conditioned avoidance responding, amphetamine-induced hyperlocomotion, and apomorphine-induced climbing without induction of catalepsy as seen with the typical antipsychotic haloperidol (Shannon *et al*, 2000). Moreover, xanomeline dose dependently reversed the apomorphine-induced disruption of PPI, a preclinical model of sensory information processing deficits. Many of the preclinical

findings with xanomeline were also confirmed in non-human primates (Andersen *et al*, 2003). Based on these preclinical studies, a subsequent 4-week, double-blind, placebo-controlled outcome trial in subjects with schizophrenia ($n = 20$) was performed to evaluate the potential antipsychotic efficacy of xanomeline (Shekhar *et al*, 2008). In these studies, xanomeline treatment led to marked improvements in schizophrenic patients as compared with the placebo group, as measured by the Brief Psychiatric Rating Scale (BPRS), Positive and Negative Syndrome Scale (PANSS), and Clinical Global Impression (Shekhar *et al*, 2008). The response to xanomeline was superior to that previously reported in studies using typical and atypical antipsychotics with a delayed onset of action (Kaplan *et al*, 1990, Garver *et al*, 1991; McDermott *et al*, 1991). In particular, the xanomeline group showed a statistically significant change in BPRS scores after 1 week of treatment, which continually improved throughout the study, as well as statistically significant improvements in total PANSS scores. In a test battery that addressed multiple domains of cognition, including visuospatial learning, verbal learning, attention/vigilance, speed of processing, and working memory, xanomeline significantly improved verbal learning and short-term memory, indicating efficacy in some aspects of cognition (Shekhar *et al*, 2008). Adverse effects, specifically gastrointestinal, were observed in the xanomeline treatment group. Although these side effects did not lead to any discontinuation, the dose limitations prevented xanomeline from long-term clinical use. These studies, however, provided important clinical validation of the potential of mAChR agonists as novel therapeutic agents for treatment of psychosis as well as cognitive disturbances in schizophrenia patients. These findings also raised the question of whether activation of M_1, M_4, or both receptors is critical for the observed clinical effects.

Allosteric Modulation of Muscarinic Receptors

M_1 allosteric modulators. Remarkable progress has been achieved in the discovery of M_1 allosteric activators that provide tools to further the understanding of the relative contributions of M_1 to the preclinical and clinical efficacy of xanomeline (see Table 1). The first generation of M_1 mAChR allosteric activators includes brucine, a selective, but weak PAM, increasing ACh affinity only two-fold and most notably, AC-42, the first M_1 compound with demonstrated action through binding at an allosteric site on the M_1 mAChR. Through a systematic evaluation of chimeric receptors, AC-42 was shown to activate M_1 mAChRs within only the 1 and 7 TM domains, without interactions at other TM domains that contribute to the orthosteric ACh-binding site (Spalding *et al*, 2002; Langmead *et al*, 2006). Although these first-generation M_1 PAMs provided an important demonstration of allosteric activity in recombinant systems, these ligands suffered from unsuitable physiochemical properties to advance into studies in animal models. Interestingly, previous studies have demonstrated that

TABLE 1 Effects of Selective mAChR Allosteric Agonists and Positive Allosteric Modulators *In Vivo*

Symptom domain	Compound	Pharm.	Species	Model	Effect	Ref.
Positive	TBPB	M_1 allosteric agonist	Rat	Amphetamine-induced hyperlocomotion	Reversed	Jones *et al* (2008)
	TBPB	M_1 allosteric agonist	Rat	Fos staining	Induced in nucleus accumbens and PFC, but not striatum	Jones *et al* (2008)
	BQCA	M_1 PAM	Mouse	Amphetamine-induced hyper-locomotion	Reversed	Ma *et al* (2009)
	LY2033298 (+oxo)	M_4 PAM	Rat	Conditioned avoidance response	Reduced avoidance responses	Chan *et al* (2008)
	LY2033298 (+oxo)	M_4 PAM	Mouse	Conditioned avoidance response	Reduced avoidance responses	Leach *et al* (2010)
	VU0152099	M_4 PAM	Rat	Amphetamine-induced hyper-locomotion	Reversed	Brady *et al* (2008)
	VU0152100	M_4 PAM	Rat	Amphetamine-induced hyper-locomotion	Reversed	Brady *et al* (2008)
Cognition						
Sensory processing	LY2033298 (+oxo)	M_4 PAM	Rat	Apomorphine-induced disruption of PPI	Reversed deficits	Chan *et al* (2008)
Memory	BQCA	M_1 PAM		Novel object recognition in a Y-maze apparatus	Prevented time-dependent deficits	Chambon *et al* (2011)
	VU0357017	M_1 allosteric agonist	Rat	Scopolamine-induced deficits in contextual fear conditioning	Reversed deficits	LeBois *et al* (2010)
	BQCA	M_1 PAM	Mouse	Set shifting—Tg2576 Alzheimer's disease model	Restored impairment in reversal learning	Shirey *et al* (2009)
	BQCA	M_1 PAM	Mouse	Single unit recordings in an auditory detection task	Increased spontaneous firing rate of mPFC cells	Shirey *et al* (2009)
Other						
CBF	BQCA	M_1 PAM	Rat	Laser Doppler flowmetry (anesthetized)	Increased cerebral blood flow	Ma *et al* (2009)
Sleep	BQCA	M_1 PAM	Rat	Sleep EEG	Promoted arousal; inhibited delta sleep	Ma *et al* (2009)
Neuronal firing	77-LH-28-1	M_1 allosteric agonist	Rat	Hippocampal CA1 firing (anesthetized)	Enhanced neuronal activity	Langmead *et al* (2008)

Abbreviations: CBF, cerebral blood flow; DMTS, delayed matching to sample; mAChR, muscarinic acetylcholine receptor; oxo, oxotremorine; PAM, positive allosteric modulator; PFC, prefrontal cortex; PPI, pre-pulse inhibition.

N-desmethylclozapine (NDMC), the biologically active metabolite of the atypical antipsychotic clozapine, possesses high selectivity for M_1 relative to other mAChR subtypes and can activate M_1 mAChRs containing a mutation in the ACh-binding site (Sur *et al*, 2003). Like clozapine itself, NDMC can potentiate NMDAR activity and induce Fos expression in specific rat forebrain regions, suggesting that allosteric activation of M_1 mAChRs may account for the distinct clinical efficacy of clozapine (Natesan *et al*, 2007; Young *et al*, 1998).

Recently, several systemically active, second-generation M_1 allosteric agonists and PAMs are proving to be useful tools for the study of selective activation of M_1 mAChRs in native tissue preparations and in animal models predictive of antipsychotic-like activity and enhancement of cognition. To date, several M_1 allosteric agonists have been discovered, including TBPB, 77-LH-28-1, and AC260584. TBPB is a potent ($EC_{50} = 280$ nM) and selective (>30 μM *vs* M_2–M_5) M_1 allosteric agonist in cell-based systems (Jones *et al*, 2008). Point mutations in the ACh-binding site that diminish orthosteric agonist activity did not alter the cellular response to TBPB. A Schild analysis of the inhibition of TBPB effects with the orthosteric antagonist atropine established that TBPB interacts with the orthosteric site in a non-competitive manner (Jones *et al*, 2008). These data are consistent with the interpretation that TBPB may act as an allosteric M_1 agonist as described by the allosteric ternary complex model for the actions of two molecules that interact with distinct sites on a receptor (Christopoulos and Mitchelson, 1997; Jacobson *et al*, 2010). Nevertheless, future studies are still needed to rule out the possibility that TBPB may function as a bitopic agonist, like the novel M_1 agonist 77-LH-28-1, by binding to a site on the M_1 mAChR, which overlaps with the orthosteric site and with an allosteric site that modulates the affinity of the ACh site (Avlani *et al*, 2010). M_1 mAChR activation by TBPB potentiated NMDAR currents in CA1 hippocampal pyramindal cells, a physiological response that is linked to the facilitation of learning and memory (Jones *et al*, 2008). Functional anatomical studies with TBPB revealed a Fos expression pattern that was similar to the profile of the atypical antipsychotic clozapine, with increased numbers of Fos cells in the mPFC and nucleus accumbens but not the dorsal striatum. In addition, TBPB dose dependently reversed amphetamine-induced hyperlocomotion within a dose range that did not induce side effects associated with the non-selective stimulation of peripheral mAChRs as measured using a modified Irwin test battery or catalepsy, a preclinical measure of extrapyramidal motor symptoms (Jones *et al*, 2008).

A second systemically active M_1 allosteric agonist, 77-LH-28-1, was identified from a series of AC-42 analogs (Langmead *et al*, 2008) and shown to have high selectivity for M_1 but weak M_3 agonist activity (Heinrich *et al*, 2009). Initial *in vitro* studies demonstrated that the orthosteric antagonist scopolamine produced parallel rightward shifts in the 77-LH-28-1 concentration response curve (CRC), which led to the interpretation that 77-LH-28-1 may bind at an orthosteric site of M_1 (Langmead *et al*, 2008). Further functional and site-directed mutagenesis studies have confirmed an allosteric mode of agonist action for this ligand, which, interestingly, suggest that 77-LH-28-1 may function as a 'bitopic' agonist as discussed above as a potential mode of action for TBPB (Avlani *et al*, 2010). 77-LH-28-1 also demonstrated a number of important physiological effects thought to facilitate synaptic plasticity, including increased hippocampal CA1 pyramidal cell firing *in vitro* and *in vivo* (Langmead *et al*, 2008; Buchanan *et al*, 2010; Jo *et al*, 2010), and induction of synchronous network activity through increased CA3 hippocampal gamma oscillations alone or in combination with the clinically available AChEI donepezil (Langmead *et al*, 2008; Spencer *et al*, 2010).

A structural analog of AC-42, AC-260584, has also been shown to produce effects in animal models of antipsychotic-like activity after systemic dosing (Vanover *et al*, 2008; Bradley *et al*, 2010). AC-260584 has been shown to be a potent M_1 mAChR allosteric agonist devoid of agonist activity at the M_3 mAChR subtype; however, some M_2 mAChR agonism has been reported (Bradley *et al*, 2010). In microdialysis studies, AC-260584, like xanomeline, increased ACh and DA levels in both the PFC and hippocampus, effects that are thought to be beneficial in schizophrenia (Perry *et al*, 2001; Li *et al*, 2007). Systemic administration of AC-260584 induced extracellular-signal regulated kinase-1 (ERK1)/2 activation in the CA1 region of the hippocampus, an effect that was absent in M_1-KO mice (Bradley *et al*, 2010). AC-260584 also reversed the hyper-locomotion induced by amphetamine and the non-competitive NMDAR antagonist MK-801, and suppressed apomorphine-induced climbing without inducing catalepsy or changes in spontaneous locomotor activity (Vanover *et al*, 2008). AC-260584 also produced efficacy in two preclinical models of learning and memory, the Morris water maze and novel object recognition task (Vanover *et al*, 2008; Bradley *et al*, 2010). Unfortunately, interpretation of the enhancement of cognition and antipsychotic-like activity observed with AC-260584 is complicated by off-target actions at other GPCRs, including serotonin 5-HT_{2A}, DA D_2, and adrenergic α1A receptors (Heinrich *et al*, 2009).

In the last 2 years, a third generation of potent (EC_{50} values 150–200 nM), selective (>50 μM *vs* M_2–M_5), and systemically active M_1 allosteric agonists, VU0186470 and VU0357017, was developed at the Vanderbilt Center for Neuroscience Drug Discovery (Lebois *et al*, 2010). In contrast to other allosteric modulators of M_1 that act at an allosteric site within the seventh TM domain, *in vitro* studies have demonstrated that VU0186470 and VU0357017 act at a novel allosteric site on the third extracellular loop of the M_1 mAChR (Lebois *et al*, 2010). These novel allosteric agonists potentiate NMDAR currents and also reverse scopolamine-induced deficits in the acquisition of hippocampus-mediated contextual fear conditioning (Lebois *et al*, 2010). The evaluation of potential

preclinical antipsychotic-like activity for these ligands is ongoing.

For the development of M_1 PAMs, a major breakthrough was the discovery of BQCA (benzyl quinolone carboxylic acid). Through extensive *in vitro* studies, BQCA has been shown to be a potent (human M_1 $EC_{50} = 845$ nM, 129-fold leftward shift of the ACh CRC), highly selective M_1 PAM with no other activity (eg, PAM, agonist, or antagonist) across the other mAChR subtypes when screened at up to 100 μM (Ma *et al*, 2009). BQCA does not bind at the orthosteric ACh-binding site, but increased the affinity of the M_1 mAChR for ACh. Site-directed mutagenesis experiments have identified an allosteric binding site for BQCA involving residues Y179 and W400, which are located on the second (o2) and third (o3) extracellular loops of the receptor (Ma *et al*, 2009). In *in vitro* electrophysiology studies, BQCA potentiated the effects of the non-selective mAChR agonist carbachol to induce inward currents in mPFC pyramidal cells and spontaneous excitatory post-synaptic currents, and these effects were not observed in M_1-KO mice (Shirey *et al*, 2009). Moreover, systemic treatment with BQCA robustly increased firing rates *in vivo* as measured by single unit recordings in mPFC pyramidal cells (Shirey *et al*, 2009). BQCA also has favorable pharmacokinetics and central nervous system exposure for *in vivo* studies. For example, BQCA reversed deficits in mPFC-dependent discrimination reversal learning in a transgenic mouse model of AD (Shirey *et al*, 2009). In addition, BQCA reversed the scopolamine-induced deficits in the acquisition of contextual fear conditioning similar to the effects observed with M_1 allosteric agonists; improved novel object recognition in a Y-maze task; and altered sleep architecture by enhancing wakefulness states and decreasing delta sleep (Ma *et al*, 2009; Chambon *et al*, 2011). Comparable to the preclinical profile of xanomeline and clozapine, BQCA increased Fos expression in the forebrain and dose dependently reversed amphetamine-induced hyper-locomotion in mice (Ma *et al*, 2009). Surprisingly, BQCA enhanced blood flow in the cerebral cortex, a function previously associated with activation of non-neuronal M_5 mAChRs (Yamada *et al*, 2001a, 2003). This finding further highlights the need for subtype-selective tools to clarify the functions of the different mAChR subtypes *in vivo*. Collectively, selective activation of M_1 by both M_1 allosteric agonists and PAMs is sufficient to mimic some of the effects of xanomeline and clinical available antipsychotics in animal models that are relevant to clinical efficacy. These studies also support the idea that M_1 activation may have a critical role in mPFC-dependent cognitive functions and suggest that M_1 allosteric activators may serve as an important approach for the treatment of the prefrontal cortical deficits observed in schizophrenic patients.

M_4 positive allosteric modulators. Significant advancement has also been made in the discovery of M_4 allosteric activators with central penetration and suitable physiochemical properties for preclinical studies, allowing for further delineation of the relative contributions of M_1 and M_4 to the preclinical and clinical efficacy of xanomeline (see Table 1). Thiochrome, an oxidation product and metabolite of thiamine, was the first M_4 PAM to be reported in the literature but possessed unsuitable properties for studies *in vivo* (Lazareno *et al*, 2004). A major advance in the development of selective M_4 mAChR allosteric activators was the discovery of LY2033298, a highly selective M_4 PAM with no detectable activity at any of the other mAChR subtypes and suitable properties for *in vivo* dosing (Chan *et al*, 2008). LY2033298 does not directly activate M_4 mAChRs, but, based on site-directed mutagenesis studies, robustly potentiates the response of ACh through binding at residue F186 in the third extracellular loop (o3) of the receptor (Nawaratne *et al*, 2010). However, when the *in vitro* potency of LY2033298 was assessed by [^{3}H]oxotremorine-M potentiation in rat M_4 mAChR membranes, there was a 5- to 6-fold reduction in comparison with studies completed in human M_4 mAChR (hM_4) membranes (hM_4 $EC_{50} = 8$ nM; Chan *et al*, 2008). Interestingly, LY2033298 had no effects when administered alone in preclinical studies, but robustly potentiated the effects of a sub-threshold dose of the non-selective mAChR agonist oxotremorine to reverse the apomorphine-induced disruption of PPI and the inhibition of conditioned avoidance responding (Chan *et al*, 2008; Leach *et al*, 2010). In early neurochemical studies using *in vivo* microdialysis techniques, LY2033298 also potentiated oxotremorine-stimulated DA release in the PFC but not nucleus accumbens. The lower potency of LY2033298 at the rat M_4 mAChR was speculated as a potential explanation for the lack of efficacy observed with the compound alone when used *in vivo*. However, the reported effects of LY2033298 in animal models predictive of antipsychotic-like activity provided important support for further development of other M_4 PAMs.

Recently, the discovery of the highly selective M_4 PAM VU0010010 with an EC_{50} of 400 nM for potentiation of ACh effects at the rat M_4 and a 47-fold leftward shift in the functional ACh response curve (>30 μM *vs* M_1–M_3, M_5) was reported (Shirey *et al*, 2008). Based on a number of *in vitro* pharmacological studies, VU0010010 was shown to act through an allosteric site to increase the efficiency of the coupling of the M_4 mAChR to G-proteins and the affinity of the M_4 mAChR for ACh (Shirey *et al*, 2008). In electrophysiological studies, VU100010 potentiates the carbachol-induced depression of synaptic transmission at excitatory but not inhibitory hippocampal CA1 synapses (Shirey *et al*, 2008). While VU100010 represented an important tool for cell-based and slice physiology studies, this ligand had unsuitable physicochemical properties for formulation and *in vivo* dosing (Shirey *et al*, 2008). Chemical optimization of VU100010 resulted in the discovery of VU0152099 and VU0152100, two related M_4 PAMs with rat M_4 EC_{50} values for potentiation of ACh responses of approximately 400 nM (30- to 70-fold leftward shift of the ACh CRC). Both M_4 PAMs showed high mAChR subtype selectivity for M_4 (>30 μM *vs* M_1–M_3, and M_5) relative to the other mAChRs

and further functional selectivity in a screen of 15 other GPCRs that are highly expressed in the brain (Brady *et al*, 2008). VU0152099 and VU0152100 enhanced M_4 receptor affinity for ACh, without competing for the orthosteric ACh-binding site (Brady *et al*, 2008). Most importantly, these novel M_4 PAMs are centrally penetrant, with pharmacokinetic properties ideal for studies in animal models of psychosis and cognition. Both VU0152100 and VU0152099 produced a robust reversal of amphetamine-induced hyper-locomotion (Brady *et al*, 2008) and VU0152100 reversed amphetamine-induced disruptions in the acquisition of contextual fear conditioning in rats (Byun *et al*, 2011). The *in vivo* effects of these M_4 PAMs were observed when administered alone, indicating that there is sufficient endogenous ACh within the circuitry mediating these behaviors to observe modulation by an M_4 PAM mechanism. The studies with novel M_4 PAMs provide critical support for the hypothesis that selective activation of M_4 is also a viable target for the development of novel antipsychotic treatments.

M_5 positive allosteric modulators. As highlighted earlier from KO mice studies, the M_5 mAChR represents another compelling target for the development of novel antipsychotics. However, the lack of subtype-selective ligands has limited our current understanding of the function of this mAChR, relative to M_1 and M_4, in circuitry thought to be disrupted in schizophrenia. Recently, chemical optimization of VU0119498, a pan-M_1/M_3/M_5 mAChR PAM, resulted in the discovery of VU0238429, the first M_5-preferring mAChR PAM (Bridges *et al*, 2009). VU0238429 shows moderate potency, with a 14-fold shift in ACh the CRC (M_5 $EC_{50} = 1.1\,\mu M$) and *in vitro* selectivity ($>30\,\mu M$ *vs* M_1–M_4). It also enhances the affinity of the M_5 mAChR for ACh, but does not compete for the ACh-binding site (Bridges *et al*, 2009). More recently, further optimization of this first M_5 PAM has produced VU0400265, a fully selective M_5 PAM in recombinant systems with comparable potency *in vitro* (M_5 $EC_{50} = 1.9\,\mu M$) (Bridges *et al*, 2010). Future *in vivo* studies with these M_5-selective PAMs hold promise for a better appreciation of the role of M_5 in preclinical models of antipsychotic-like activity and cognitive enhancement.

Potential Challenges with mAChR Allosteric Activators for the Treatment of Schizophrenia

As discussed under the Introduction, optimal cholinergic signaling for affective and cognitive functions may be region- and task-specific. While the regional levels of cholinergic signaling in schizophrenic patients remain unclear, tonic enhancement of cholinergic neurotransmission by an mAChR agonist may actually limit improvements in the different symptoms observed in schizophrenic patients. If this is the case, mAChR PAMs may provide a more physiologically relevant modulation of regional and temporal endogenous cholinergic signaling for effective treatment. On the other hand, if the levels of cholinergic neurotransmission are decreased in schizophrenic patients, similar to dementia patients, then there may not be sufficient cholinergic tone to observe efficacy with mAChR PAMs. Future clinical studies with mAChR agonists and PAMs are needed to confirm which of these approaches will be more effective for the treatment of the different symptom clusters associated with schizophrenia.

While the preclinical characterization of subtype-selective allosteric mAChRs suggests a potential therapeutic approach for the symptoms associated with schizophrenia, there are several potential challenges to this approach that merit additional evaluation. First, the degree of subtype selectivity of these mAChR allosteric activators must be confirmed further in animal models as well as in the clinic. Extensive studies in recombinant and native system preparations have demonstrated that these mAChR allosteric activators show greater subtype selectivity relative to orthosteric mAChR agonists. However, mAChR allosteric activators, as reported with the M_4 mAChR PAM thiochrome, may bind with comparable affinities to allosteric sites on multiple mAChR subtypes and show subtype selectivity through selective cooperativity with other orthosteric site ligands (Lazareno *et al*, 2004; Conn *et al*, 2009). Under these circumstances, the selectivity of thiochrome and other mAChR allosteric modulators may fluctuate based on the system in which their effects are assessed. To clarify this important issue, detailed binding studies using radioligands selective for the allosteric sites, site-directed mutagensis, and measurements of the effects on the binding of orthosteric site ligands will be important for a complete characterization of these novel mAChR allosteric activators.

An additional concern with the observed selectivity of mAChR PAMs is the potential for differential selectivity in the presence of different orthosteric agonists, or probe dependence (Kenakin, 2008). As reported previously by our group and others using the M_4 mAChR PAM LY2033298 and analogs, the selectivity of these ligands at the five mAChR subtypes is different when used in combination with the endogenous ligand ACh and other orthosteric agonists, like oxotremorine (Digby *et al*, 2010). As shown with LY2033298, co-administration of a sub-threshold dose of the orthosteric mAChR agonist oxotremorine was required to observe efficacy in animal models and may also be necessary for clinical efficacy. While not all mAChR PAMs require combination treatment with an orthosteric mAChR agonist for efficacy, this issue points to the importance of broadly characterizing the functional selectivity of mAChR PAMs with different chemical classes of orthosteric mAChR agonists.

Previous studies have reported that allosteric mAChR activators can differentially activate specific signaling pathways and demonstrate clear ligand-directed trafficking of receptor signaling (LDTRS), comparable to the action of some orthosteric mAChR agonists (see Digby *et al*, 2010). For example, in a previous study, the M_1-selective mAChR PAMs VU0029767 and VU0090157 were reported to robustly potentiate ACh-induced calcium mobilization

(Marlo *et al*, 2009). In addition, VU0090157 also potentiated the ACh-induced activation of phospholipase-D (PLD) and phosphatidylinositol (PI) hydrolysis, whereas VU0029767 produced little to no effect on M_1-induced increases in PLD activity and PI hydrolysis. These findings are consistent with the interpretation that VU0029767 may induce M_1 receptor conformation changes that cannot efficiently form signaling complexes with non-$G\alpha_q$ signaling partners (Marlo *et al*, 2009). An allosteric mAChR agonist or PAM-induced LDTRS may provide tremendous functional selectivity for various effector systems that could translate in the future to effective strategies for the treatment of different symptoms in schizophrenia. However, this phenomenon also raises the potential for more complicated efficacy profiles with these allosteric ligands. Thus, it will be increasingly important to use a multi-assay approach for a complete characterization of the *in vitro* and *in vivo* effects of these novel allosteric mAChR activators.

To date, the assessment of *in vivo* efficacy with allosteric mAChR ligands has been conducted after an acute, single dose administration of a compound alone or in combination with a low dose of an orthosteric agonist. As the action of mAChR PAMs depends on the activation of the mAChR subtype by ACh, these ligands may preserve a level of activity dependence in contrast to the sustained activation with an agonist. Under chronic dosing regimes, direct-acting mAChR receptor agonists can produce receptor desensitization and tolerance, whereas mAChR PAMs are postulated to not produce these effects, or at least not to the same degree. However, it should be noted that benzodiazepines, which act as allosteric potentiators of $GABA_A$ receptors, can also induce tolerance after repeated dosing in some preclinical models and in the clinic (File, 1985; Costa *et al*, 1995). Yet, GABA can produce robust desensitization of the $GABA_A$ receptor within milliseconds whereas benzodiazepines do not. Moreover, because of the fundamentally different natures of these classes of receptors, it may not be valid to directly compare the actions of PAMs at ion channels with the action of mAChR PAMs, which signal through G-proteins. Thus, future studies are needed to evaluate the efficacy of mAChR allosteric activators, both agonists and PAMs, after chronic dosing in multiple preclinical models.

ROLE OF NICOTINIC RECEPTOR SUBTYPES IN SCHIZOPHRENIA

Numerous clinical and preclinical findings suggest that disruptions in central nicotinic cholinergic transmission may be associated with the symptoms observed in individuals with schizophrenia. There is a significantly higher incidence of cigarette smoking among individuals with schizophrenia relative to the general population or in individuals with other psychiatric disorders (Lohr and Flynn, 1992; De Leon *et al*, 1995; Campo-Arias *et al*, 2006). Smoking behavior in schizophrenia is also reported to be independent of antipsychotic treatment and frequently observed prior to the initiation of antipsychotic therapies (De Leon *et al*, 2002). While the underlying causes for the higher rates of smoking in schizophrenia remain unclear, acute nicotine exposure has been shown to improve cognition, particularly in the domains of attention and vigilance, in animals, healthy volunteers, and in smoking and non-smoking schizophrenic patients (Levin *et al*, 1992; Rezvani and Levin, 2001; Levin, 2002; Avila *et al*, 2003; Sacco *et al*, 2005; AhnAllen *et al*, 2008; Barr *et al*, 2008; Jubelt *et al*, 2008). For example, in schizophrenic patients and their relatives, nicotine can transiently improve deficits in P50 auditory gating performance and smooth pursuit eye movements, two clinical measures of sensory information processing (Klein and Andersen, 1991; Adler *et al*, 1992, 1993). Moreover, a positive correlation has been reported between disruptions in P50 auditory gating function and the severity of cognitive impairments in schizophrenic patients (Erwin *et al*, 1998). In addition, several polymorphisms in the α_7 nAChR gene (*CHRNA7*) have been linked to P50 gating deficits in individuals with schizophrenia (Freedman *et al*, 1997, 2001; Stassen *et al*, 2000; Leonard *et al*, 2002). In a recent study by Mexal *et al* (2010), both *CHRNA7* mRNA and protein expression were reported to be decreased in schizophrenic non-smokers relative to controls; however, in schizophrenic smokers, the levels of *CHRNA7* mRNA and protein expression were normal relative to controls. Similar auditory gating deficits observed in the DBA/2 mouse strain have been correlated with decreased levels of hippocampal α_7 nAChRs and a polymorphism associated with their α_7 nAChR gene (Stevens *et al*, 1996; Leonard *et al*, 1998). The nicotine-mediated normalization of auditory gating deficits in DBA/2 mice can be blocked by α-bungarotoxin, a selective α_7 nAChR antagonist, but not by mecamylamine, a selective $\alpha_4\beta_2$ nAChR antagonist (Stevens and Wear, 1997; Stevens *et al*, 1998).

Sensorimotor gating studies in schizophrenic patients have revealed an association between cigarette smoking and enhanced PPI (George *et al*, 2006; Rabin *et al*, 2009; Woznica *et al*, 2009). Smoking cessation, on the other hand, selectively impaired visuospatial working memory and attentional deficits in schizophrenia patients; the improvements in performance following smoking reinstatement were prevented by pretreatment with mecamylamine (Sacco *et al*, 2005). However, in non-smoking individuals with schizophrenia or controls, mecamylamine did not alter performance on tests of attention, working memory, and executive function (Sacco *et al*, 2006). Unfortunately, the effects of nicotine are highly transient and limited with repeated dosing; thus nicotine is unsuitable as a treatment for schizophrenic patients (Adler *et al*, 1992, 1993; Stevens and Wear, 1997). Nicotine also has other limitations, including high abuse liability and undesirable effects on the cardiovascular system (Benowitz, 1988; Benowitz and Gourlay, 1997).

Recently, deletions at the α_7 nAChR locus were shown to be linked to high risk (odds ratio 11.54) for schizophrenia and psychosis. However, this genetic finding was identified

in less than 0.2% of the schizophrenic patients tested, suggesting that only a small proportion of individuals with schizophrenia carry deletions in 15q13.3 (Stefansson *et al*, 2008). To date, genetic linkage studies for $\alpha_4\beta_2$ nAChRs and schizophrenia have been even less reproducible, with no associations found between the individual *CHRNA4* and *CHRNB2* genes and this illness; only one study reported a significant combined α_4 and β_2-subunit gene interaction with schizophrenia (De Luca *et al*, 2006; Kishi *et al*, 2008).

The connection between schizophrenia and nAChRs, especially α_7 nAChRs, is supported by postmortem immunohistochemical and binding studies that revealed reduced α_7 nAChR levels in many brain regions thought to be affected in schizophrenia, including the hippocampus, the thalamic reticular nucleus, and the cingulate cortex (Freedman *et al*, 1995; Guan *et al*, 1999; Court *et al*, 1999; Marutle *et al*, 2001). However, similar changes were not reproducibly observed with α_4 or β_2-subunits (Breese *et al*, 2000; Martin-Ruiz *et al*, 2003). While nicotine exposure increases high-affinity and low-affinity nAChR binding, such increases cannot explain the decreased α_7 nAChR levels observed in schizophrenic patients or the lack of effects on $\alpha_4\beta_2$ nAChRs (Breese *et al*, 1997; Court *et al*, 1998; Gopalakrishnan *et al*, 1997). Overall, preclinical and clinical findings, along with neuroanatomical and genetic data, support the interpretation that neuronal nAChR signaling is altered in schizophrenia patients. These studies also suggest that the development of selective nAChR subtype activators may lead to important potential therapeutic interventions for this illness.

Preclinical Studies of Full and Partial α_7 nAChR Agonists

Over the last decade, important progress has been made in the discovery of multiple full and partial α_7 nAChR agonists (see Table 2). DMXB-A (GTS-21) was the first partial α_7 nAChR agonist with systemic activity to be reported and has been followed by the characterization of numerous α_7 nAChR agonists as listed in Table 2. While early α_7 nAChR ligands, such as DMXB-A, AR-R17779, and ABBF (EVP-6124), had less favorable physiochemical properties and showed a lack of true subtype selectivity with off-target effects at hERG, 5-HT_3, and/or $\alpha_4\beta_2$ nAChR (Briggs *et al*, 1997; Mullen *et al*, 2000; Boess *et al*, 2007), recently disclosed α_7 nAChR agonists possess improved selectivity and properties for oral dosing, including TC-5619 ($EC_{50} = 33$ nM, $K_i = 0.3$ nM at rat α_7 nAChRs, with little to no activity on $\alpha_4\beta_2$ nAChRs in electrophysiology studies) (Hauser *et al*, 2009). Despite the limitations of early α_7 nAChR agonists, the characterization of these novel ligands has provided exciting opportunities for critical proof-of-concept studies in animal models and, in some cases, clinical trials for schizophrenia and other neurological disorders.

In recombinant systems and native tissue preparations, studies with DMXB-A and other α_7 nAChRs agonists have demonstrated that selective activation of α_7 nAChRs regulates a number of cellular, physiological, and neurochemical responses thought to facilitate synaptic plasticity, learning, and memory. In both *in vitro* and *in vivo* studies, α_7 nAChR agonists enhanced hippocampal LTP, and these effects were blocked by α_7 nAChR antagonists at concentrations that have no effect on LTP when administered alone (Mann and Greenfield, 2003; Biton *et al*, 2007; Lagostena *et al*, 2008; Söderman *et al*, 2011). Activation of α_7 nAChR agonists also enhanced hippocampal theta oscillation network activity, a physiological function that is disrupted in schizophrenia (Siok *et al*, 2006). Moreover, deficits in theta burst stimulation-induced LTP, elicited by fimbria–fornix lesions of the cholinergic innervation of the hippocampus, were reversed by the α_7 nAChR agonist AZD0328 within a dose range that also improved cognitive performance in preclinical working memory tasks (Sydserff *et al*, 2009). α_7 nAChR agonists have also been shown to increase ERK phosphorylation and cAMP response element-binding protein phosphorylation in certain brain regions, including the cortex and hippocampus, after acute administration within a dose range that enhanced performance in animal models of cognition, including monkey delayed matching-to-sample, rat social recognition, and mouse inhibitory avoidance (Bitner *et al*, 2007). These studies are consistent with the interpretation that activation of α_7 nAChRs increases intracellular calcium and the downstream stimulation of calcium-dependent ERK signal transduction, a cellular pathway that regulates LTP. Finally, several studies have shown enhancement of release of neurotransmitters by α_7 nAChRs activation in key brain circuits impaired in schizophrenia. Selective activation of α_7 nAChRs in the VTA also increased glutamate-mediated DA release in the PFC, a region implicated in the cognitive and negative symptoms of schizophrenic patients (Nanri *et al*, 1998; Sydserff *et al*, 2009). Moreover, the α_7 nAChR agonist SSR180711 dose dependently elevated the extracellular levels of ACh in the hippocampus as well as DA in the PFC in microdialysis studies (Biton *et al*, 2007; Pichat *et al*, 2007). It is important to note that enhanced release of ACh and DA in the PFC is consistent with the ability of α_7 nAChRs agonists to potentially enhance cognitive performance; however, such augmentation of DA release in the nucleus accumbens might worsen the positive symptoms and lead to increased abuse liability.

Many of these novel α_7 nAChR agonists have been evaluated in animal models of cognitive enhancement in the domains of sensory information gating, attention, memory, and/or executive functions. To date, one of the most robust and reproducible findings with partial and full α_7 nAChR agonists has been the reversal of auditory gating deficits in DBA/2 mice, which, unlike with nicotine, can be observed after repeated dosing (Stevens *et al*, 1998, 2010; Feuerbach *et al*, 2009). Moreover, the effects of α_7 nAChR agonists on gating deficits can be blocked by α_7 nAChR antagonists, but not $\alpha_4\beta_2$ receptor antagonists (eg, mecamylamine), indicating that the effects are mediated through α_7 nAChR

TABLE 2 Effects of Selective α_7 nAChR Full and Partial Agonists *In Vivo*

Symptom domain	Compound	Pharm.	Species	Model	Effect	Ref.
Positive	PNU-282987	Full	Rat	Fos	Induced in nucleus accumbens shell and PFC, but not striatum	Hansen *et al* (2007)
Negative						
	AR-R17779	Full	Rat	Social recognition	Increased memory	Van Kampen *et al* (2004)
	SSR180711	Partial	Rat	Neonatal PCP-induced impairments in social novelty discrimination	Normalized impairments	Pichat *et al* (2007)
	JN403	Partial	Mouse	Social recognition	Increased time with novel mouse	Feuerbach *et al* (2009)
	JN403	Partial	Rat	Social exploration	Increased	Feuerbach *et al* (2009)
	ABBF	Partial	Rat	Social recognition	Improved	Boess *et al* (2007)
	TC-5619	Full	Mouse	Social recognition in th(tk−)/th(tk−) transgenics	Improved	Hauser *et al* (2009)
Cognition						
Sensory processing	PHA-543613	Partial	Rat	Amphetamine-induced disruption of auditory gating	Normalized deficit	Wishka *et al* (2006)
	JN403	Partial	Mouse	Auditory gating deficits in DBA/2 mice	Increased	Feuerbach *et al* (2009)
	SEN12333	Full	Rat	Apomorphine-induced disruption of PPI	Normalized deficit	Roncarati *et al* (2009)
	TC-5619	Full		Apomorphine-induced disruption of PPI	Reversed	Hauser *et al* (2009)
	SSR180711	Partial	Rat	MK801-induced persistent latent inhibition	Reversed	Barak *et al* (2009)
	SSR180711	Partial	Rat	Neonatal -induced L-NoArg-induced persistent latent inhibition	Reversed	Barak *et al* (2009)
	SSR180711	Partial	Rat	Amphetamine-induced latent inhibition disruption	Reversed	Barak *et al* (2009)
	GTS-21/DMXB-A	Partial	Mouse	Auditory gating deficits in DBA/2 mice	Improved	Stevens *et al* (1998)
	GTS-21/DMXB-A	Partial	Mouse	Auditory gating deficits in DBA/2 mice	Improved (gastric administration)	Simosky *et al* (2001)
	GTS-21/DMXB-A	Partial	Mouse	Auditory gating deficits in DBA/2 mice	Improved (7-d dosing)	Stevens *et al* (2010)
	MEM3454	Partial	Rat	Apomorphine-induced disruption of PPI	Improved	Wallace *et al* (2011)
Attention	MEM3454	Partial	Rat	Visual signal detection task	Improvement in % hit accuracy	Rezvani *et al* (2009)
	GTS-21/DMXB-A	Partial	Monkey	Delayed matching to sample ketamine disruption	Attenuated decreases in accuracies	Buccafusco and Terry (2009)
Executive function	MEM3454	Partial	Rat	Repeated PCP-induced deficits in attentional set shifting	Improved	Wallace *et al* (2011)
	MEM3454	Partial	Rat	Repeated PCP-induced deficits in attentional set shifting	Improved	Wallace *et al* (2011)
Memory	AZD0328	Full	Monkey	Spatial delayed response working memory task	Improved at low doses	Castner *et al* (2011)
	AR-R17779	Full	Rat	Eight-arm radial arm maze—normal and septohippocampal lesion	Improved both	Levin *et al* (1999)
	SSR180711	Partial	Rat	PCP-induced deficit in a linear maze	Partially reversed	Pichat *et al* (2007)
	SSR180711	Partial	Mouse	Scopolamine-induced deficits in the Y-maze task	Improved deficits	Redrobe *et al* (2009)
	SSR180711	Partial	Mouse	Repeated PCP-induced deficits in the modified Y-maze	Reversed deficits (acute and repeated dosing)	Thomsen *et al* (2009)
	SSR180711	Partial	Mouse	Neonatal PCP-induced deficits in the modified Y-maze	Reversed deficits	Thomsen *et al* (2009)
	SSR180711	Partial	Rat	Novel object recognition	Increased memory	Pichat *et al* (2007)
	SSR180711	Partial	Rat	MK-801-induced deficits in novel object recognition	Reversed deficits	Pichat *et al* (2007)
	SSR180711	Partial	Rat	Acute PCP-induced deficits in novel object recognition—PCP-sensitized	Prevented deficits by acute PCP challenge	Pichat *et al* (2007)
	SSR180711	Partial	Mouse	Novel object recognition	Increased memory	Pichat *et al* (2007)

TABLE 2 Continued

Symptom domain	Compound	Pharm.	Species	Model	Effect	Ref.
	SSR180711	Partial	Mouse	Repeated PCP-induced deficits in novel object recognition	Improved deficits	Hashimoto *et al* (2008)
	SSR180711	Partial	Rat	MK-801-induced deficits in the Morris water maze task	Attenuated deficits	Pichat *et al* (2007)
	PNU-282987	Full	Rat	Eight-arm radial maze	Improved	Chan *et al* (2007)
	PNU-282987	Full	Mouse	Morris water maze	Increased retention	Vicens *et al* (2011)
	A-582941	Full	Mouse	Scopolamine-induced deficits in the V-maze task	Improved deficits	Redrobe *et al* (2009)
	PHA-543613	Partial	Rat	Novel object recognition	Increased memory	Wishka *et al* (2006)
	ABBF	Partial	Mouse	Novel object recognition	Increased memory	Boess *et al* (2007)
	ABBF	Partial	Rat	Morris water maze	Improved	Boess *et al* (2007)
	SENI2333	Full	Rat	Novel object recognition	Increased memory	Roncarati *et al* (2009)
	SENI2333	Full	Rat	Scopolamine-induced deficits in novel object recognition	Restored memory	Roncarati *et al* (2009)
	SENI2333	Full	Rat	MK801-induced deficits in novel object recognition	Restored memory	Roncarati *et al* (2009)
	SENI2333	Full	Rat	Scopolamine-induced deficits in passive avoidance	Normalized	Roncarati *et al* (2009)

Abbreviations: 5-CSRTT, 5-Choice Serial Reaction Time Task; L-NoArg, N^{ω}-nitro-L-arginine; nAChR, nicotinic acetylcholine receptor.

activation *in vivo* (Simosky *et al*, 2001). α_7 nAChR agonists have also reversed auditory gating deficits induced by amphetamine or fimbria–fornix lesions of cholinergic innervation of the hippocampus in rats (Wishka *et al*, 2006). More recently, the partial α_7 nAChR agonist SSR180711 was shown to reverse NMDAR antagonist-induced impairments in latent inhibition and novelty discrimination, two other gating and attentional tasks, and these effects were blocked by α_7 nAChR antagonists (Barak *et al*, 2009). Similar to the effects of mAChR activators, α_7 nAChR agonists have been shown to reverse the apomorphine-induced disruption of PPI (Hauser *et al*, 2009; Roncarati *et al*, 2009; Wallace *et al*, 2011).

Selective activation of α_7 nAChRs by full and partial α_7 nAChR agonists also produced efficacy across a number of preclinical models of learning and memory, including improvements in passive avoidance responding, novel object recognition, and maze tasks in young and aged rats (Redrobe *et al*, 2009; Roncarati *et al*, 2009). Again, the effects of α_7 nAChR agonists on memory functions were blocked by co-administration with α_7 nAChR antagonists (Roncarati *et al*, 2009). In non-human primates, α_7 nAChR agonists have also enhanced delayed matching-to-sample task and spatial delayed responding (Buccafusco and Terry, 2009; Castner *et al*, 2011). In contrast to the lack of studies with muscarinic activators in preclinical models of NMDAR hypofunction, α_7 nAChR agonists such as SSR180711 produced robust efficacy in reversing both acute and chronic NMDAR antagonist-induced deficits in hippocampal and non-hippocampal memory tasks, including object recognition, Morris water maze, and Y-maze in rodents (Wishka *et al*, 2006; Boess *et al*, 2007; Pichat *et al*, 2007; Hashimoto *et al*, 2008; Thomsen *et al*, 2009). More recently, the partial α_7 nAChR agonist MEM3454 was also reported to reverse the chronic PCP-induced impairments of extra-dimensional attentional set-shifting in rats (Wallace *et al*, 2011). The effects of SSR180711 and MEM3454 in these NMDAR disruption models were blocked by α_7 nAChR antagonists (Pichat *et al*, 2007; Wallace *et al*, 2011; Thomsen *et al*, 2009). Interestingly, α_7 nAChR agonists have also produced effects in social recognition tasks, a potential preclinical model of the negative symptoms in schizophrenia (Feuerbach *et al*, 2009; Boess *et al*, 2007; Hauser *et al*, 2009). Finally, in a preclinical model of antipsychotic-like activity, the α_7 nAChRs agonist PNU-282987 revealed a pattern of Fos induction in forebrain regions similar to atypical antipsychotics (Hansen *et al*, 2007; Thomsen *et al*, 2010). Taken together, these preclinical findings suggest that selective activation of α_7 nAChRs may provide efficacy across the different symptoms domains of schizophrenia.

Based on the favorable preclinical findings of enhanced cognitive function, antipsychotic-like effects, and safety for several α_7 nAChR agonists, these ligands were advanced into clinical trials for schizophrenia, as well as for the cognitive impairments associated with ADHD and mild-to-moderate dementia in AD (see Table 3). To date, there have been only three clinical studies reported in the literature using the

TABLE 3 Effects of Selective $\alpha_4\beta_2$ and α_7 nAChR Full Agonists and Partial Agonists in Clinical Studies

Disease	Compound	Pharm.	Test	Effect	Ref.
Schizophrenia	GTS-21/DMXB-A	α_7 partial agonist	Computerized test battery; attention, working memory, episodic recognition memory, visual tracking, overnight face recognition tests	Significantly enhanced attention, working memory, and episodic secondary memory in healthy volunteers	Kitagawa *et al* (2003)
	GTS-21/DMXB-A	α_7 partial agonist	Repeatable Battery for the Assessment of Neuropsychological status score and P50 inhibitory gating	Significant neurocognitive improvement and P50 inhibition	Olincy *et al* (2006)
	GTS-21/DMXB-A	α_7 partial agonist	P50 auditory evoked potential inhibition; MATRICS battery	Improved (first arm only)	Freedman *et al* (2008)
	GTS-21/DMXB-A	α_7 partial agonist	SANS; attention/vigilance and working memory	Improved	Freedman *et al* (2008)
	GTS-21/DMXB-A	α_7 partial agonist	fMRI: hippocampal activation in smooth pursuit eye movement task	Decreased activity	Tregellas *et al* (2010)
	GTS-21/DMXB-A	α_7 partial agonist	fMRI: Default mode network	Improved in non-smoking schizophrenics; reduced in posterior cingulate, inferior parietal cortex, medial frontal gyrus, and increased in precuneus	Tregellas *et al* (2011)
	Varenicline	$\alpha_4\beta_2/\alpha_7$ agonist	PANSS; RBANS, including learning and memory; virtual Morris water maze	No change in PANSS scores; improved verbal learning and memory; no affect on visuospatial memory or attention	Smith *et al* (2009)
	Varenicline	$\alpha_4\beta_2/\alpha_7$ agonist	P50 auditory evoked potential inhibition	No change	Waldo *et al* (2010)
Age-associated memory impairment	Isproniclne/TC-1734/ AZD3480	$\alpha_4\beta_2$ agonist	Cognitive Drug Research computerized tests in episodic memory and attention	Improved	Dunbar *et al* (2011)

Abbreviations: nAChR, nicotinic acetylcholine receptor; PANSS, Positive and Negative Syndrome Scale; RBANS, Repeatable Battery for the Assessment of Neuropsychological Status; SANS, Scale for the Assessment of Negative Symptoms.

α_7 nAChR partial agonist DMXB-A. In an initial phase-I randomized, double-blind, placebo-controlled study (18 healthy, non-smoking male volunteers), DMXB-A was administered perorally three times daily over 5 days (Kitagawa *et al*, 2003). Across all doses, DMXB-A significantly improved performance on a number of cognitive tasks, including immediate and delayed word recall, and correct detection during digit vigilance with increases in reaction time (Kitagawa *et al*, 2003). DMXB-A also dose dependently enhanced performance in spatial and numeric working memory tasks at doses of up to approximately 1.9 mg/kg t.i.d. that were well-tolerated over a 5-day treatment session. Next, the effects of DMXB-A were evaluated in a phase-II randomized, double-blind, placebo-controlled cross-over trial with 12 non-smoking schizophrenic patients on concurrent neuroleptic treatment (Olincy *et al*, 2006). In this trial, DMXB-A significantly reduced P50 auditory gating deficits and enhanced total performance scores on the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) test, with the largest improvement in attention functions (Olincy *et al*, 2006). Based on the positive results from the first two DMXB-A trials, another randomized, double-blind, placebo-controlled, phase-II trial was performed (31 non-smoking schizophrenic patients) over a 4-week period (Freedman *et al*, 2008). In this cross-over trial design, there were no significant changes in cognitive measures between DMXB-A- and placebo-treated patients over the three treatment arms on the MATRICS Consensus Cognitive Battery. However, there were significant improvements at the higher DMXB-A dose (150 mg) on the Scale for the Assessment of Negative Symptoms (SANS), with a trend toward enhancement on BPRS, particularly in the anhedonia and alogia subscales (Freedman *et al*, 2008). While it is tempting to conclude that the loss of efficacy over time was due to the possible tachyphylaxis effects of DMXB-A, the findings in this study, like many others, were confounded by a significant practice effect observed across all groups, especially within the MATRICS tasks. During this phase-II trial, patients were also included in functional magnetic resonance imaging studies to determine whether DMXB-A could normalize changes in default mode network and hippocampal activity, two biomarkers of antipsychotic treatment efficacy (Tregellas *et al*, 2010, 2011). Changes in default mode network activity were evaluated in the context of a polymorphism in *CHRNA7*, which was previously found to be associated with schizophrenia. DMXB-A treatment was associated with changes in default mode activity as compared with placebo, with reductions in the posterior cingulate, inferior parietal cortex, and medial frontal gyrus, and an increase in precuneus activity. The most robust difference, specifically, reductions posterior cingulate activity, was influenced by the *CHRNA7* genotype (Tregellas *et al*, 2010, 2011). These results suggest normalization of default mode function, but future studies will need to include control subjects for verification of the normal default mode network. In addition, DMXB-A

TABLE 4 Effects of Selective α7 nAChR Allosteric Positive Allosteric Modulators *In Vivo*

Symptom domain	Compound	Pharm.	Species	Model	Effect	Ref.
Negative						
	NS-1738	Type-I	Rat	Social recognition test	Improved performance	Timmermann *et al* (2007)
Cognition						
Sensory processing	PNU-120596	Type-II	Rat	Amphetamine-induced deficits in auditory gating	Improved	Hurst *et al* (2005)
	XY-4083	Type-I	Mouse	Deficits in auditory-evoked potentials in DAB/2 strain	Reversed	Ng *et al* (2007)
	A-867744	Type-II	Mouse	Deficits in auditory-evoked potentials in DAB/2 strain	Reversed	Faghih *et al* (2009)
Memory	NS-1738	Type-I	Rat	Scopolamine-induced deficit in Morris water-maze	Improved deficit in acquisition	Timmermann *et al* (2007)
	XY-4083	Type-I	Rat	Eight-arm radial maze	Improved	Ng *et al* (2007)

significantly decreased hippocampal activation during a smooth pursuit eye movement task. These findings are consistent with the previously established function of α_7-nicotinic receptors on the inhibitory interneurons in the hippocampus (Tregellas *et al*, 2010, 2011).

Preclinical Studies of α_7 nAChR Positive Allosteric Modulators

While the development of full and partial α_7 nAChR agonists has led to a greater understanding of the functional significance of selective activation of α_7 nAChRs in preclinical models and in clinical populations, these studies have also raised many questions. One key concern involves the possibility of limited or diminished efficacy after chronic dosing owing to rapid desensitization of α_7 nAChRs *in vivo* and adverse effects due to off-target activity at other nAChRs. Similar to the challenges observed with orthosteric mAChR agonists, the development of subtype-selective allosteric modulators for the nAChRs may provide several advantages for therapeutic development. To date, a number of α_7-selective nAChR PAMs have been reported; they can be divided into two functionally distinct types based on the way in which these ligands affect the time course of agonist-evoked currents in electrophysiology studies (Gronlien *et al*, 2007) (see Table 4). In general, both types of nAChR PAMs increase the potency and efficacy of agonists. Type-I nAChR PAMs potentiate the ACh-induced peak current, while having little or no effect on ACh-induced desensitization and deactivation processes. Examples of type-I PAMs include NS-1738 (eg, $EC_{50} = 3.4\,\mu M$; 2- to 3-fold shift) and XY-4083 (Ng *et al*, 2007; Timmermann *et al*, 2007). Type-II nAChR PAMs potentiate ACh-induced peak current and prolong the time course of the agonist-evoked response by suppressing the extent of fast desensitization and by increasing the contribution of a slow desensitizing current. Two representative type-II PAMs include PNU-120596 (eg, $EC_{50} = 1.5\,\mu M$; fourfold shift) and A-867744 (Hurst *et al*, 2005; Gronlien *et al*, 2007; Malysz *et al*, 2009; Faghih *et al*, 2009). Interestingly, both type-I and II α_7 nAChR PAMs produced efficacy in preclinical models, including reversal of auditory gating deficits in DBA/2 mice or after amphetamine challenge; reversal of MK-801-induced deficits in PPI and other pharmacological disruptions in novelty-induced exploratory activity; Morris water maze; and social interaction (Hurst *et al*, 2005; Timmermann *et al*, 2007; Faghih *et al*, 2009). Interestingly, these preliminary findings have been observed with administration of the nAChR PAMs alone, indicating that there is sufficient cholinergic tone on α_7 nAChRs for a viable allosteric modulator approach *in vivo*. There are now several α_7 nAChR PAMs under clinical development for the treatment of schizophrenia. One of the important considerations for the viability of PAMs in the clinic will be to more fully understand the issue of whether sustained Ca^{2+} entry resulting from delayed α_7 nAChR desensitization by type-II PAMs will result in Ca^{2+}-induced cytotoxicity as there have been conflicting results in the literature (Ng *et al*, 2007; Hu *et al*, 2009).

Preclinical Studies of $\alpha_4\beta_2$ nAChR Agonists

Beyond the clear role of α_7 nAChRs in the underlying auditory gating deficits observed in schizophrenic patients, there is accumulating evidence suggesting that altered $\alpha_4\beta_2$ nAChR function may also have a role in the symptoms of this illness. To date, there have been several novel selective $\alpha_4\beta_2$ nAChR agonists developed for the treatment of cognitive deficits associated with schizophrenia and other neurological disorders (see Table 5), including ABT-418, ABT-089, ABT-594, 5-iodo-A-85380, and TC-1734 (AZD3480; isopronicline) (Arneric *et al*, 1994; Decker *et al*, 1994b; Lin *et al*, 1997; Mukhin *et al*, 2000; Obinu *et al*, 2002). Preliminary preclinical studies have shown that selective activation of $\alpha_4\beta_2$ improves auditory gating deficits

TABLE 5 Effects of Selective $\alpha_4\beta_2$ Agonists, nAChR Full Agonists, and Partial Agonists *In Vivo*

Symptom domain	Compound	Pharm.	Species	Model	Effect	Ref.
Cognition						
Sensory processing	ABT-418	Full	Mouse	Auditory-evoked sensory gating in DBA/2 mice	Normalized (second dose ineffective)	Stevens and Wear (1997)
	ABT-418	Full	Rat	Auditory-evoked sensory gating—fimbria-fornix lesion	Normalized (second dose ineffective)	Stevens and Wear (1997)
	5-Iodo-A-85380	Full	Mouse	Auditory-evoked sensory gating—DBA/2 mice	Normalized	Wildeboer and Stevens (2008)
Attention	ABT-418	Full	Rat	5-CSRTT	Increased accuracy (transient); reduced response latency	Hahn *et al* (2003)
	SIB-1765F	Partial	Rat	5-CSRTT	Increased correct responding	Grottick and Higgins (2000)
	SIB-1765F	Partial	Rat	5-CSRTT	Increased performance speed	Grottick *et al* (2003)
Memory	ABT-089	Full	Monkey	DMTS	Improved performance in mature and aged	Decker *et al* (1997)
	ABT-418	Full	Monkey	DMTS	Improved performance	Buccafusco *et al* (1995)
	ABT-418	Full	Monkey	Delayed recall test—after distractions	Increased accuracy; reduced distractibility	Prendergast *et al* (1998)
	ABT-418	Full	Monkey	Delayed recall test, aged *vs* young (transdermal *vs* i.m.)	Improved performance (patch prolonged improvement in the young)	Prendergast *et al* (1997)
	TC-1734/isopronicline/ AZD3480		Mouse	Novel object recognition	Improved	Obinu *et al* (2002)
	ABT-594	Partial	Monkey	DMTS	Increased accuracy	Buccafusco *et al* (2007)
	ABT-418	Full	Rat	Morris water maze—septal lesion	Attenuated deficits	Decker *et al* (1994b)
	ABT-418	Full	Mouse	Contextual fear conditioning	Enhanced	Kenney *et al* (2010)
	ABT-418	Full	Mouse	Inhibitory avoidance	Improved retention	Decker *et al* (1994a)
	ABT-089	Full	Rat	Morris water maze—septal lesion	Attenuated deficits (repeated dosing)	Decker *et al* (1997)
	ABT-089	Full	Rat	Inhibitory avoidance	No effect on young or old	Decker *et al* (1997)

Abbreviations: 5-CSRTT, 5-Choice Serial Reaction Time Test; DMTS, Delayed Matching to Sample; nAChR, nicotinic acetylcholine receptor; PPI, pre-pulse inhibition.

in both DBA/2 mice and in rats (Stevens and Wear, 1997; Wildeboer and Stevens, 2008). In preclinical studies, activation of $\alpha_4\beta_2$ receptors by selective agonists has no effect on PPI deficits similar to the lack of effects with α_7 nAChR partial and full agonists (Radek *et al*, 2010). However, unlike α_7 nAChR agonists, $\alpha_4\beta_2$ nAChR agonists produced robust effects in preclinical models of other attentional functions, such as in the five-choice serial reaction time task, and these effects were only blocked by $\alpha_4\beta_2$ antagonists such as DhβE but not α_7 antagonists (Blondel *et al*, 2000; Grottick *et al*, 2000; Hahn *et al*, 2003). $\alpha_4\beta_2$ agonists such as ABT-418 also significantly improved performance in several preclinical models of working memory in rodents and monkeys, and, again, these effects were blocked by $\alpha_4\beta_2$ antagonists (Buccafusco *et al*, 1995, 2007; Decker *et al*, 1997; Prendergast *et al*, 1997, 1998; Obinu *et al*, 2002; Levin, 2002; Chan *et al*, 2007). Furthermore, ABT-418 was shown to enhance hippocampus-mediated tasks, including contextual fear conditioning as well as water maze performance in rats with septal lesions (Decker *et al*, 1994a, b; Kenney *et al*, 2010). Currently, there have been no reported clinical findings with $\alpha_4\beta_2$ nAChRs in patients with schizophrenia. However, a number of ongoing clinical trials are evaluating the effects of $\alpha_4\beta_2$ nAChR agonists for the cognitive deficits in schizophrenia, ADHD, and mild-to-moderate dementias in AD patients. In a recent randomized, placebo-controlled study, the $\alpha_4\beta_2$ agonist TC-1734 (AZD3480; isopronicline) was well-tolerated and robustly improved age-associated memory impairments as measured by assessment through the Cognitive Drug Research computerized test battery and a Subject Global Impression Scale of Cognition (SCI-Cog) (Dunbar *et al*, 2011) (see Table 3). Finally, varenicline, a clinically approved treatment for smoking cessation, with partial $\alpha_4\beta_2$ agonist and full α_7 agonist activity (Mihalak *et al*, 2006), significantly improved scores on verbal learning and memory tests, but not performance on visual–spatial or attentional performance or PANSS scores; these findings were consistent with previous preclinical data (Smith *et al*, 2009). However, in a more recent pilot study ($n=6$), varenicline did not improve P50 auditory gating in schizophrenia patients as compared with placebo, but elicited central side effects (Waldo *et al*, 2010), which were in line with the potential exacerbation of neuropsychiatric conditions (Kuehn, 2008). Clearly more clinical studies are needed with highly selective nAChR ligands to further elucidate the respective roles of these subtypes in previously reported clinical effects.

Potential Challenges with nAChR Partial Agonists and Allosteric Activators for the Treatment of Schizophrenia

Current clinical and preclinical studies with α_7 and $\alpha_4\beta_2$ nAChR agonists and PAMs suggest that this approach will continue to be successful as a novel treatment strategy for schizophrenia. Many of the concerns raised under the potential challenges of mAChR allosteric activators are relevant to the development of nAChR partial agonists and modulators as well. For example, it remains unclear whether the recombinant systems used to develop more subtype-selective nAChR agonists and PAMs sufficiently reflect the expression levels and stoichiometries of the α_7 and $\alpha_4\beta_2$ nAChR subunits comprising these two subtypes found in native systems, particularly in clinical populations with chronic nicotine intake. Such potential differences could lead to the development of nAChR ligands with inadequate subtype selectivity for schizophrenic patients. The development of biomarkers for α_7 and $\alpha_4\beta_2$ nAChR target engagement and/or target-related efficacy will also be crucial to confirm clear α_7 or $\alpha_4\beta_2$ nAChR-mediated effects in future clinical trials for schizophrenia. Moreover, future clinical studies are needed to further understand the broader clinical utility of the selective α_7 and $\alpha_4\beta_2$ nAChR ligands for the treatment of the different symptom domains in schizophrenia, beyond the observed clinical efficacy on auditory gating and attentional functions.

While early studies with repeated administration of nicotine revealed diminished efficacy of some clinical endpoints due presumably to rapid receptor desensitization (Griffith *et al*, 1998; Harris *et al*, 2004), more recent preclinical and clinical data with α_7 and $\alpha_4\beta_2$ nAChR partial agonists and PAMs suggest that efficacy can be sustained with repeated dosing. However, there are lingering questions regarding the underlying mechanism of action of α_7 and $\alpha_4\beta_2$ nAChR activators, and whether some or most of the observed effects with these compounds are due to receptor activation or desensitization. For example, the rapid desensitization of α_7 nAChR in response to repeated dosing of nicotine and analogs combined with the observations that, at low doses, some nicotinic antagonists induce agonist-like responses suggest that α_7 nAChR desensitization, rather than activation, may explain some of the observed efficacy with α_7 agonists (Buccafusco *et al*, 2009; Picciotto *et al*, 2008). Furthermore, there are substantial inconsistencies between the high concentrations of nAChR ligands required to activate nAChR-mediated *in vitro* responses and the considerably lower dose ranges needed to induce behavioral responses in animals and humans. In electrophysiology studies, nicotine and the nAChR antagonists *d*-tubocurarine and α-bungarotoxin enhanced hippocampal neuronal excitation (Ropert and Krnjević, 1982). At low doses nAChR antagonists, such as mecamylamine, enhanced performance comparable to nicotine in several learning and memory tasks, including delayed matching-to-sample accuracy in monkeys (Buccafusco and Jackson, 1991; Terry *et al*, 1999), and delayed stimulus discrimination, radial arm maze, and T-maze alternation tasks in rats (Moran, 1993; Levin *et al*, 1997; Terry *et al*, 1999). In a recent study by Buccafusco *et al* (2009), the efficacy of nicotine, cotinine, a major nicotine metabolite, and two analogs of choline, JWB1-84-1 and JAY2-22-33, were compared side-by-side in the delayed matching-to-sample task in monkeys. Interestingly, the performance levels in this cognitive task directly correlated with the degree of

receptor desensitization induced by each of these nAChR agonists and similar to responses in rodents (Sood *et al*, 2007). If this desensitization hypothesis is correct, then the development of ligands that desensitize the nAChRs, especially α_7 nAChR, without activation would be a more tractable approach for the treatment of cognitive deficits in schizophrenic patients. However, other findings, including rapid upregulation of α_7 nAChRs upon their activation, support the interpretation that α_7 nAChR agonists produce their effects through activation of the receptors. Future *in vivo* studies using novel nAChR PAMs, such as Sazetidine-A, that directly desensitize nAChRs without activation (Xiao *et al*, 2006) are needed to better understand the mechanism of action of nAChR agonists in animal models and in the clinic. Finally, future studies are also needed to assess the possible interactions between the different mAChR and nAChR subtypes in the modulation of the schizophrenia-related circuitry, as well as possible synergistic effects with clinically available antipsychotics.

FUTURE RESEARCH DIRECTIONS

Preclinical and clinical findings with subtype-selective mAChR and nAChR activators are providing important validation for the cholinergic hypothesis of schizophrenia and novel approaches for the treatment of the cognitive, positive, and negative symptoms. In addition, these subtype-selective mAChR and nAChR activators are serving as critical tools to better understand the relative roles of the different receptor subtypes in the observed efficacy of non-selective muscarinic and nicotinic receptor agonists *in vivo*. For the modulation of mAChRs, current data suggest that selective M_1 and M_4 allosteric agonists and PAMs may be efficacious for the treatment of the cognitive impairments as well as the positive symptoms. However, additional studies are needed to further understand the effects of these compounds in preclinical models of the negative symptoms and different aspects of PFC- and hippocampus-mediated cognition. For the nicotinic cholinergic system, preclinical and clinical studies with selective agonists of the α_7 and $\alpha_4\beta_2$ nAChRs suggest that central activation of these receptors may be especially efficacious for the cognitive deficits observed in individuals with schizophrenia.

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DISCLOSURE

The authors declare no conflict of interest.

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