Review

Nicotinic Acetylcholine Receptor Ligands, Cognitive Function, and Preclinical Approaches to Drug Discovery

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Abstract

Interest in nicotinic acetylcholine receptor (nAChR) ligands as potential therapeutic agents for cognitive disorders began more than 30 years ago when it was first demonstrated that the tobacco alkaloid nicotine could improve cognitive function in nicotine-deprived smokers as well as non-smokers. Numerous animal and human studies now indicate that nicotine and a variety of nAChR ligands have the potential to improve multiple domains of cognition including attention, spatial learning, working memory, recognition memory, and executive function. The purpose of this review is to (1) discuss several pharmacologic strategies that have been developed to enhance nAChR activity (eg, agonist, partial agonist, and positive allosteric modulator) and improve cognitive function, (2) provide a brief overview of some of the more common rodent behavioral tasks with established translational validity that have been used to evaluate nAChR ligands for effects on cognitive function, and (3) briefly discuss some of the topics of debate regarding the development of optimal therapeutic strategies using nAChR ligands. Because of their densities in the mammalian brain and the amount of literature available, the review primarily focuses on ligands of the high-affinity $\alpha 4\beta 2^*$ nAChR ("*" indicates the possible presence of additional subunits in the complex) and the low-affinity $\alpha 7$ nAChR. The behavioral task discussion focuses on representative methods that have been designed to model specific domains of cognition that are relevant to human neuropsychiatric disorders and often evaluated in human clinical trials.

Implications: The preclinical literature continues to grow in support of the development of nAChR ligands for a variety of illnesses that affect humans. However, to date, no new nAChR ligand has been approved for any condition other than nicotine dependence. As discussed in this review, the studies conducted to date provide the impetus for continuing efforts to develop new nAChR strategies (ie, beyond simple agonist and partial agonist approaches) as well as to refine current behavioral strategies and create new animal models to address translational gaps in the drug discovery process.

Introduction

Interest in the prototypic tobacco alkaloid nicotine as a potential therapeutic agent began nearly more than 30 years ago when it was discovered that tobacco smoking could result in enhancements in the performance of certain cognitive tasks. A limitation to these

early observations was that at least some of the task improvements were only reversals of withdrawal-induced performance deficits in tobacco-dependent smokers. However, with additional studies and more extensive reviews of the literature, a general consensus developed that smoking or other means of nicotine administration could

have a modest pro-cognitive effect in nonsmokers as well as nondeprived or minimally deprived smokers.^{1–3} There is now a large body of evidence to support the premise that nicotine can enhance information processing and cognitive function in experimental animals as well as in human nonsmokers.⁴ A small representative list of examples includes positive effects on sensorimotor gating and prepulse inhibition,⁵ sustained attention,⁶ working memory,⁷ and recognition memory⁸ in rats; working and/or short-term memory^{9,10} and distractibility¹¹ in monkeys; and prepulse inhibition,¹² visual information processing, attention, and short-term memory in humans.^{13–16} In addition to its pro-cognitive effects, nicotine has also been shown to have neuroprotective activity in multiple disease models (ie, both in vitro and in vivo), and this has bolstered the argument that nicotine might have both symptomatic and disease-modifying effects in neurodegenerative illnesses such as Alzheimer's disease (AD) and Parkinson's disease.^{17,18}

Concerns about its relatively short half-life, cardiovascular effects, and abuse potential have (at least to some extent), however, limited enthusiasm for the use of nicotine as a therapeutic agent for neurologic and psychiatric conditions to date with the exception of nicotine dependence. It should be noted, however, that nicotine delivered via a transdermal patch for smoking cessation showed no increased risk of cardiovascular events compared with placebo in patients with cardiovascular disease.¹⁹ Moreover, nicotine delivered via a transdermal patch to patients with mild cognitive impairment improved cognition without signs of significant side effects.²⁰ For smoking cessation, nicotine is now delivered safely as a replacement therapy via multiple formulations including patches, tablets, gums, lozenges, nasal sprays, and inhalers to relieve withdrawal symptoms and cravings without the harmful effects of tobacco smoke.²¹ Collectively, this information supports the argument that nicotine should continue to be evaluated as a potential therapeutic agent in conditions beyond nicotine dependence.

Central Nervous System Targets of Nicotinic Acetylcholine Receptor Ligands

In an attempt to address the limitations of nicotine noted earlier, and to enhance pro-cognitive actions and other desirable therapeutic properties, a wide range of ligands targeting specific subtypes of nicotinic acetylcholine receptors (nAChRs) have been developed in the last 20 years, and there is now evidence to support their use in multiple types of diseases that affect humans. (See Table 1 for a list of these potential indications and representative references cited for review.) As therapeutic targets, nAChRs belong to the large superfamily of pentameric, ligand-gated ion channel receptors that

include serotonin 5-HT₃, type-A γ -aminobutyric acid, and glycine receptors. By modulating the flux of cations such as Na⁺, K⁺, and Ca²⁺ across cell membranes, nAChRs regulate neuronal excitability and neurotransmitter release to influence multiple physiologic processes including behavior.²² Neuronal nAChRs are formed through a combination of α and β subunits composing heteromeric receptors, or through homomeric α configurations. Although there have been nine α ($\alpha 2$ – $\alpha 10$) and three β ($\beta 2$ – $\beta 4$) subunits identified to date that can form many different combinations, for the purposes of this review, the heteromeric $\alpha 4\beta 2^*$ and homomeric $\alpha 7$ nAChR subtypes are primarily discussed. These two nAChR receptors are the most predominant subtypes found in the mammalian brain and the most commonly targeted receptor subtypes in drug discovery programs to date, especially for disorders of cognitive function. Compounds selective for the $\alpha 4\beta 2^*$ subtype were the first to be developed in drug discovery programs for cognition enhancement. This may (at least in part) be related to the high density of $\alpha 4\beta 2$ nAChRs in the brain (relative to other nAChR subtypes), their high affinity for nicotine, and the large body of literature available on their role in reward, self-administration, and dependence.²² During the characterization of $\alpha 4\beta 2^*$ nAChR agonists, it was discovered that $\alpha 4$ and $\beta 2$ subunits assembled to form two stoichiometries with different pharmacological sensitivities toward acetylcholine (ACh) activation. The $\alpha 4(2):\beta 2(3)$ subunit possesses high sensitivity (EC₅₀ = 1 μ M) to ACh whereas the $\alpha 4(3):\beta 2(2)$ subunit possesses low sensitivity (EC₅₀ = 100 μ M) to ACh.^{23,24} Moreover, it was demonstrated that several $\alpha 4\beta 2^*$ nAChR ligands (eg, nicotine, NS9283, LY2087101, and varenicline) showed distinct preferences for the high- and low-sensitivity $\alpha 4\beta 2$ subunit populations. Although the therapeutic relevance has yet to be determined, the ability to target one $\alpha 4\beta 2$ nAChR subtype over another will likely rejuvenate future $\alpha 4\beta 2$ nAChR drug development efforts.

In addition to $\alpha 4\beta 2^*$ nAChR ligands, considerable attention has also been given toward the development of homomeric $\alpha 7$ nAChR ligands for the treatment of cognitive disorders. Neuronal $\alpha 7$ nAChRs are abundant in brain regions (eg, prefrontal cortex and hippocampus) that are important for cognition, they influence the phosphorylation of ERK and CREB (signaling pathways linked to long-term potentiation and memory formation), they are often reduced in AD and schizophrenia, and they can promote neuroprotection in model systems.²²

The majority of nAChR ligands developed to date are agonists or partial agonists targeted to orthosteric $\alpha 4\beta 2^*$ and $\alpha 7$ nAChR binding sites, although more recently, several positive allosteric modulators (PAMs) at nAChRs have been developed.²² PAMs avoid direct receptor activation by binding at sites that are distinct from the orthosteric sites targeted by traditional $\alpha 4\beta 2^*$ and $\alpha 7$ nAChR agonists and thus may offer some pharmaceutical advantages. For example, in contrast to traditional nAChR agonists, PAMs would be expected to reduce the likelihood of agonist-induced nAChR desensitization (and potential tolerance) following continuous administration to maintain cholinergic activity. PAMs require endogenous ligand (ie, ACh or choline) to elicit a nAChR response and are, therefore, thought to maintain a more natural (temporal phasic) stimulation pattern of the cholinergic receptor than agonists. However, unlike nAChR agonists (and a potential caveat), there is the requirement that PAMs have sufficient levels of endogenous cholinergic ligand present to activate the nAChR, which may not be the case in all types of cognitive disorders (eg, late stage AD). Despite the functional differences between the nAChR ligands (ie, full vs. partial

Table 1. Therapeutic Potential of Nicotinic Acetylcholine Receptor Ligands

Human condition/disease	Representative literature reviews
Nicotine dependence	25,26
Alcohol use disorder	27
Depression	28,29
Alzheimer's disease	22,30
Schizophrenia	31,32
Attention-deficit/hyperactivity disorder	33,34
Parkinson's disease	35,36
Pain and inflammation	37,38

agonist vs. PAM) and the question of which might be more beneficial as a therapeutic agent, results from early stage clinical trials indicate that, at therapeutically relevant doses, pharmacological tolerance and loss of clinical efficacy (cognitive improvement) do not occur following stabilized daily dosing with either full or partial nAChR agonists.^{16,20,22,31} Unfortunately, too few clinical investigations exist for nAChR PAMs to determine their “therapeutic-use” potential or advantages over nAChR agonists.^{22,39}

In the nAChR field, most of the attention on PAMs to date has focused on the $\alpha 7$ nAChR, though a few reports have identified selective $\alpha 4\beta 2^*$ nAChR PAMs,⁴⁰ with compounds divided into two classes based on their functional properties: type I and type II PAMs. Type I PAMs are defined as molecules that predominately affect the apparent peak current, agonist sensitivity, and Hill coefficient, but not the receptor desensitization profile, and type II PAMs are defined as compounds that possess the aforementioned properties described for type I PAMs, as well as the ability to modify the desensitization profile of agonist responses. It has been argued that type II PAMs are less prone to induce tolerance, which may occur after the chronic administration of nAChR agonists, whereas, type I PAMs may have advantages over type II PAMs as they can minimize potential calcium-induced cytotoxicity. See the following reviews to support the information in the earlier paragraphs related to the physiology and pharmacology of nAChRs and their role as therapeutic targets.^{22,41}

Animal Models and Animal Behavior Tasks: Translational Validity

With the long list of recent clinical trial failures of novel neurologic and psychiatric medications (including nAChR ligands) has come increasing scrutiny of both the animal models and the behavioral test procedures used in preclinical drug discovery research. The subject of “translational validity” in preclinical animal studies has become a hotly debated topic. Regarding the animal models used (eg, transgenic mice, brain lesion models, and pharmacologic impairment models), the degree of phenomenological similarity between the animal model and the human condition it is meant to simulate is known as “face validity”. Here, for example, an animal model of AD would be expected to exhibit deficits in attention, learning, memory, recall, etc. The level of homology in pathological mechanisms (ie, between the animal model and the human illness) that underlie disease symptoms is known as “construct validity”. An optimal AD model would thus be expected to demonstrate amyloid plaques, neurofibrillary tangles, synaptic loss, etc. in brain regions that are affected in human AD (eg, cortex and hippocampus). In drug discovery research, “predictive validity” is primarily defined by the degree to which the model can be used to predict efficacy of a new therapeutic agent in humans. In an animal model of AD, this might be demonstrated by its response to a positive control compound (eg, donepezil). However, in AD (as well as most neurologic and psychiatric conditions), demonstrating construct and predictive validity in animal models is very challenging because the neurobiology of AD remains poorly understood and because the currently marketed prescription medications (ie, the positive controls) are only marginally effective.

As the case for animal models, the same terms used for translational validity are often applied to the behavioral procedures that are used, although it should be noted that the task requires an animal model, thus the terms often overlap and become more difficult to clearly define. For example, a working memory task that shows a

delay-dependent decrement in accuracy by an animal would demonstrate both face validity and at least some components of construct validity (relative to human working memory tasks). In the context of drug discovery research, construct validity of the tasks that assess specific domains of cognition is the most common focus. Here, it is important to note that a clear definition of the specific cognitive domain (construct) must be formulated. The concept of etiological validity is closely related to construct validity and focuses on the anatomical substrates that have been determined to be essential to a specific cognitive domain as reflected in performance on a particular task. As an example, the dependence of a spatial learning task such as the Morris water maze on an intact hippocampus can be used as an evidence of the construct (etiological) validity of the task since damage to the hippocampus impairs spatial learning in humans. For a comprehensive review related to animal behavior task validity, see reference.⁴²

In the text provided later, an overview of the domains of cognition commonly targeted in nAChR drug discovery programs is provided. The review focuses on several rodent behavioral tasks that have been developed and validated (using the criteria discussed earlier). These tasks also map well onto the domains of cognition that are often evaluated in clinical trials in humans for dementia and other neuropsychiatric disorders such as schizophrenia where cognitive deficits are a primary debilitating feature. Important developments in areas more closely related to fear memories and anxiety disorders are beyond the scope of this review. Nicotine has been extensively characterized (ie, acute, chronic, and withdrawal effects) in the fear conditioning model,^{43,44} see also reviews by Kaplan and Moore⁴⁵ and Kutlu and Gould.⁴⁶ In Table 2, an overview of representative ligands at $\alpha 4\beta 2^*$ and $\alpha 7$ nAChRs that have been developed to date to treat cognitive disorders is provided. Although not all-inclusive, the table includes nAChR agonists, partial agonists, and PAMs that have been evaluated in the specific behavioral tasks that are discussed. Table 2 also provides information on additional properties of the compounds beyond memory enhancement (eg, neuroprotective effects and effects on sensorimotor gating) that might be useful for treating conditions such as AD and schizophrenia.

Domains of Cognition Commonly Targeted in Preclinical nAChR Drug Discovery Programs

Please see Figure 1 for an illustration of several domains of cognition that are commonly targeted in rodent models in nAChR drug discovery programs.

Attention

A simple definition of attention is the concentration of nervous system resources on specific (environmentally relevant) stimuli while ignoring other (nonrelevant or less relevant) stimuli. The ability to focus one's attention is inextricably linked to perception, learning and memory, and executive function, and this ability is often impaired in a variety of neuropsychiatric disorders, including attention-deficit hyperactivity disorder (ADHD), depression, schizophrenia, and AD. Accordingly, attention is considered as a major therapeutic target in these disorders.⁸⁶ In drug discovery research, the most commonly used attention-related tasks are the five-choice serial reaction time task (5-CSRTT) and the five-choice continuous performance task (5C-CPT) followed by the signal detection task (SDT). Each of these behavioral tasks has features that are similar to the continuous performance task (CPT) that has been used successfully to detect attention deficits in the aforementioned human neuropsychiatric disorders.⁸⁶

Table 2. In vivo Pharmacological Effects of Nicotinic Acetylcholine Receptor Ligands

Compound name	Receptor function	Cognitive domain enhanced	Additional properties	References
ABT-089	$\alpha 4\beta 2$ and $\alpha 6\beta 2$ partial agonist	Attention, working memory, spatial reference memory	Neuroprotective (glutamate excitotoxicity)	47–49
ABT-594	$\alpha 4\beta 2$ Full agonist	Attention, working memory	Analgesic	50–52
AZD-3480 (TC-1734)	$\alpha 4\beta 2$ Partial agonist	Attention, object recognition, working memory, executive function	Neuroprotective (glutamate), antidepressant	53–55
Sazetidine-A	$\alpha 4\beta 2$ Full agonist (silent desensitizer)	Attention	Analgesic, antidepressant	56–58
Varenicline	$\alpha 4\beta 2$ Partial agonist and full $\alpha 7$ agonist	Attention, object recognition, working memory	Auditory and sensorimotor gating	59–61
NS9283	$(\alpha 4)_3(\beta 2)_2$ PAM	Attention, social recognition, spatial reference memory	Sensorimotor gating	23
ABBF	$\alpha 7$ Full agonist and 5-HT ₃ antagonist	Object and social recognition, working memory		62
ABT-107	$\alpha 7$ Full agonist	Attention, working memory, social recognition	Auditory gating neuroprotective (6-OHDA lesion model),	63–65
AQ051	$\alpha 7$ Partial agonist	Object and social recognition, spatial reference memory		66
BMS-933043	$\alpha 7$ Partial agonist	Object recognition, working memory, executive function	Auditory gating	67
BMS-902483	$\alpha 7$ Partial agonist and 5-HT ₃ antagonist	Object recognition, executive function	Auditory gating	68
EVP-6124	$\alpha 7$ Partial agonist and 5-HT ₃ antagonist	Object recognition		69
EVP-5141	$\alpha 7$ Full agonist and 5-HT ₃ antagonist	Object and social recognition, working memory		70
SEN-12333	$\alpha 7$ Full agonist and histamine H3 antagonist	Object recognition	Neuroprotective (quisqualate lesion model), sensorimotor gating	71
RG 3487	$\alpha 7$ Partial agonist and 5-HT ₃ antagonist	Attention, object recognition, spatial reference memory, executive function	Sensorimotor gating	72
TC-5619	$\alpha 7$ Full agonist	Object recognition	Sensorimotor gating	73
Tropisetron	$\alpha 7$ Partial agonist and 5-HT ₃ antagonist	Attention, object recognition, working memory, spatial reference memory	Sensorimotor gating	74–76
AVL-3288 (Compound 6, CCMI or XY4083)	$\alpha 7$ Type I PAM	Object recognition, working memory, executive function	Auditory gating	77,78
Compound 7z	$\alpha 7$ Type I PAM	Object recognition		79
JNJ-39393406	$\alpha 7$ Type I, type II PAM	Object recognition, executive function	Auditory gating	39
Lu AF58801	$\alpha 7$ Type I PAM	Object recognition		80
NS1738	$\alpha 7$ Type I PAM	Object and social recognition, spatial reference memory		81,82
PAM-2	$\alpha 7$ Type II PAM	Object recognition, executive function	Social interaction model	83
PNU 120596	$\alpha 7$ Type II PAM	Recognition memory, spatial learning, working memory, executive function	Auditory gating	84,85

PAM = positive allosteric modulator.

The 5-CSRTT (Figure 2A), a task of sustained attention, assesses the subject's ability to spatially divide its attention across multiple signal locations to select the correct target stimulus (light in a single aperture and nose-poke location) that, in turn, produces a food reward. In addition to measuring attention (choice accuracy), the 5-CSRTT can assess a number of other cognitive domains such as impulsivity (premature responses), cognitive inflexibility and/or compulsivity (perseverative and timeout responses), and processing speed (response latency). Like the 5-CSRTT, the 5C-CPT assesses the subject's ability to spatially divide its attention across five signal locations and when illumination of a single aperture (correct

target) occurs the subject must choose that hole to receive a reward. However, the 5C-CPT adds a nontarget component in which all five apertures are illuminated, thus requiring the subject to inhibit choice selection. The addition of nontarget trials (false alarms) with correct target trials increases task similarity to the human CPT version⁴² and (along with the other standard 5-CSRTT measurements) allows the assessment of response inhibition, which is an important component of executive functioning.

The SDT was designed to emphasize temporal components of attention by presenting a target stimulus repeatedly in a single spatial location and varying the timing of its presentation. The SDT

Figure 1. Illustration of the several domains of cognition often targeted in rodent models by nicotinic acetylcholine receptor ligands in drug discovery programs for neuropsychiatric disorders.

thus differs from the 5-CSRTT and 5C-CPT in that the SDT requires the detection of a single centrally presented visual signal instead of detection of a target stimulus across multiple choice locations. Briefly, the SDT requires the subject to monitor a central panel for the presence (signal trial) or absence (nonsignal or blank trial) of a visual light stimulus that varies in time and intensity. Following the trial (signal or nonsignal) presentation and a short variable delay, two response levers are extended into the operant chamber and the subject must press the appropriately designated lever, based on trial type presented, to receive a reward. The SDT (similar to the human CPT) thus incorporates the use of a rule about the properties of a single stimulus. The method also allows the investigator to alter stimulus duration, intensity, and modality, which can place greater cognitive load on the subject to better assess sensory function and attentiveness. Distraction (eg, flashing house light) can be added to the protocol to further increase task demands. For a more comprehensive review of attention and rodent models designed to study attentional processes see Callahan and Terry.⁸⁶

Results with nicotine and $\alpha 4\beta 2^*$ nAChR agonists (eg, ABT-418, A-82695, AZD 3480, sazetidine, and varenicline) on improving sustained attention and inhibitory control have been mixed (Table 2).⁸⁶ This drug class has routinely been shown to reverse the pharmacologic-induced impairments (ie, scopolamine and MK-801) of sustained attention, whereas their ability to consistently act as “stand-alone” pro-attentional drugs varies across laboratories.⁸⁶ Fewer studies exist for $\alpha 4\beta 2^*$ nAChR PAMs. Timmermann et al.²³ assessed the low-sensitivity $\alpha 4(3)\beta 2(2)$ nAChR PAM NS 9283 on sustained attention using the 5-CSRTT. Preliminary results indicated that NS 9283 produced small improvements in accuracy and latency to response with decreases in trial omissions and premature responding. In a follow-up study, positive effects on trial omissions were replicated, whereas the effects on accuracy, response latency, and premature responding were not reproduced, indicating that additional investigations on attentional processes are warranted. Mixed results on sustained attention with $\alpha 7$ nAChR agonists and

$\alpha 7$ nAChR PAMs have also been reported.⁸⁶ The $\alpha 7$ nAChR partial agonist/5-HT₃ antagonist RG 3487 was shown to exert beneficial effects on sustained attention comparable to that of nicotine in the rat SDT whereas, the $\alpha 7$ nAChR agonists (ie, AR-R17779 and PNU 282987) and PAMs (ie, CCMI and PNU 120596) did not improve attentional performance in the 5-CSRTT.^{84,86} Nicotinic $\alpha 4\beta 2^*$ and $\alpha 7$ nAChR agonist administration has also resulted in mixed outcomes in clinical trials of ADHD despite being well tolerated. Agonists ABT-089, AZD-3480, and ABT-894 were reported to improve ADHD symptoms as measured by the Conner’s Adult ADHD Rating Scale-Investigator Version (CAARS-INV) whereas the $\alpha 4\beta 2$ agonist AZD 1446 and the $\alpha 7$ nAChR agonist TC-5619 failed to significantly improve ADHD symptoms compared with placebo. Interestingly, the $\alpha 7$ nAChR partial agonist/5-HT₃ antagonist tropisetron was shown to improve sustained visual attention (ie, rapid visual information processing) in patients with schizophrenia (for review see Terry et al.⁸⁷). Because RG 3487 and tropisetron are also potent 5-HT₃ receptor antagonists and blockade of this receptor site also increases the release of ACh, the dual action at both $\alpha 7$ nAChR and 5-HT₃ receptors may account for the observed behavioral and clinical differences on attentional processing.

In summary, there is considerable preclinical (and some clinical) data to support the premise that ligands at both $\alpha 4\beta 2$ and $\alpha 7$ nAChRs have the potential to enhance attention and to improve other behavioral domains related to attention (impulsivity). However, not all studies have reached this conclusion, warranting further investigation. There is a variety of potential reasons for the mixed results including the nature of the specific attention task used and its parameters (eg, stimulus types and durations), and routes of drug administration. Interestingly, Hahn et al.⁸⁸ recently observed distinct rat strain-related differences in the attention-enhancing effects of nicotine in the 5-CSRTT, which would indicate that genetic predispositions may predict variability in the efficacy of nAChR compounds for enhancing attention.

Figure 2. Diagrams of representative rodent tasks often used in drug discovery programs that were developed to model specific domains of cognition. (A) Sustained attention: the five-choice serial reaction time task (5-CSRTT). In the 5-CSRTT, subjects are required to scan five apertures in an operant chamber for the appearance of a brief light stimulus and to make a nose-poke response in the correct spatial location (ie, the aperture where the light stimulus was presented) to receive a food reward. (B) Spatial learning and reference memory: the Morris water maze (MWM). The MWM task assesses the ability of rodents to learn and remember the spatial location of visual cues in a room to find and escape onto a hidden platform located in a pool of water. In probe trials (reference memory) conducted after hidden platform tests, the platform is removed from the pool and the time spent and distance traveled in the quadrant that previously contained the platform is measured. (C) Spatial working memory: the radial arm maze (RAM). In The RAM, subjects move down adjacent arms in a radial maze to retrieve food pellets without reentering empty (ie, unbaited) arms. By remembering which arms were previously entered (ie, by using spatial cues in the room on each trial) the rats use “spatial working memory” to successfully complete the task. (D) Recognition memory: spontaneous novel object recognition (NOR). During the sample trial (A/A), the subject is presented with two identical objects and allowed to explore the objects for a predetermined length of time. After a delay interval, a choice test trial (A/B) is performed in which the subject is presented with one of the previously observed “familiar” objects and a “novel”, unfamiliar object. Recognition memory is demonstrated in the NOR task when the subject explores the novel object more than a previously observed familiar one. (E) Executive function: -attentional set-shifting task (AST). In the food cup-based version of the AST, rodents are trained to retrieve food rewards buried in containers that contain fillers of different textures and (typically) different scents (olfactory cues). To obtain rewards the subjects have to be able to discriminate between the different textures and odors and then to switch between different textures or between odors (intradimensional set shift) or to shift from a specific texture to an odor (extradimensional set shifts) or vice versa. They are also required to perform reversals either within or between dimensions. SD = simple discrimination; CD = complex discrimination; ID = intradimensional set shift; ED = extradimensional set shift.

Spatial Learning and Reference Memory

For more than 35 years, the Morris water maze has been used extensively by behavioral physiologists and pharmacologists for evaluating the effects of aging, experimental lesions, and drugs on learning and memory in rodents. The most commonly used version of the task (the fixed, hidden platform task; Figure 2B) is a test of spatial learning where the test subject relies on distal cues to navigate from start locations around the perimeter of an open swimming pool to locate a submerged platform to escape the water. Spatial learning ability is assessed across repeated (hidden platform) trials and reference memory (recall) is determined in probe trials by the preference of the subject for the platform area when the platform has been removed from the pool. The task requires many of the same processes known to be important components of human cognition including information acquisition and processing, consolidation, retention, and retrieval.

Moreover, with a computer-automated video tracking system a number of nonmnemonic processes such as thigmotaxis, motor function (swim speeds), or motivational responses can be evaluated (for a more comprehensive review of water maze procedures used in drug discovery, see Terry⁸⁹).

Nicotine, $\alpha 4\beta 2^*$, and $\alpha 7$ nAChR agonists have been shown to consistently improve Morris water maze spatial reference memory in aged (24-month-old) rodents, in rodents with chemical and electrolytic lesions, as well as in rodents with pharmacologic-induced impairments (Table 2).^{47,66,70,72} The $\alpha 4(3)\beta 2(2)$ nAChR PAM NS 9283 was also shown to reverse the impairing effects of subchronic phencyclidine administration on spatial reference memory in rats.⁵⁹ The $\alpha 7$ nAChR type I PAM NS 1738 has been shown to reverse scopolamine-induced water maze deficits in young rats⁸¹ whereas the $\alpha 7$ nAChR type II PAM PNU 120596 failed to reverse the spatial reference memory deficits of

aged, cognitively impaired F344 rats.⁹⁰ Interestingly, increasing cholinergic tone via administration of a subthreshold dose of donepezil in combination with PNU 120596 improved spatial reference memory performance in aged, cognitively impaired animals.⁹⁰

In summary, most of the data collected to date across a variety of animal models support the argument that both $\alpha 4\beta 2^*$ and $\alpha 7$ nAChR agonists have the potential to enhance spatial learning and reference memory, whereas additional data are needed before this conclusion can be made for types I and II nAChR PAMs.

Working Memory

The term working memory has been defined in multiple ways, but in short, it refers to a brain system that enables the acquisition, temporary storage, and manipulation of information that is necessary to solve complex cognitive tasks such as language comprehension, learning, reasoning, and problem solving. Working memory is limited in capacity and may contain information about rules, recent stimulus events, expected goals, and planned actions that are maintained and manipulated in real time to affect ongoing behavior. Behavioral tasks in laboratory animals typically assess their ability to use this system to encode, temporarily maintain, and use sensory stimulus information to make a decision (sensory discrimination of some type) to receive a reward. These include the so-called delayed response tasks where the stimuli are either visuospatial or object oriented (eg, spatial delayed alternation or response, delayed match or nonmatch to position, and delayed match or nonmatch to sample).⁹¹ The tasks can be computer-automated in Skinner-type operant chambers with levers or nose-poke devices for response selection. One of the oldest and simplest but best validated methods of assessing working memory in rodents is the win-shift task in the eight-arm radial arm maze (RAM). In brief, the RAM typically uses an octagonal central chamber with eight attached arms (Figure 2C). Each arm is baited with a food reward and the animal is required to enter each arm to retrieve the reward. To complete the task most efficiently, the animal must not reenter a previously visited arm and adopt a win-shift foraging strategy. Spatial working memory performance of the animal is often measured by the number of baited arms entered before reentering a previously visited arm. Successful performance of the task is known to depend on the hippocampus and other brain regions such as the prefrontal cortex that are vital to human mnemonic processes. Performance of the RAM procedure is thought to reflect normal foraging strategies used by rodents in their natural environments, thus it is an ethologically relevant, positive reinforcing (ie, appetitively motivated) paradigm.⁹²

Nicotine, $\alpha 4\beta 2^*$, and $\alpha 7$ nAChR agonists have been shown to significantly improve spatial working memory in rodents (eg, in the RAM) when administered alone and in pharmacologic-impairment models.^{7,53,93} To date, $\alpha 4\beta 2^*$ nAChR PAMs have not been assessed in the RAM task whereas the results of $\alpha 7$ nAChR PAM (ie, CCMI and PNU 120596) evaluations have been mixed.^{77,94} When given alone, the type I PAM CCMI was shown to improve trial accuracy⁷⁷ whereas the type II PAM PNU 120596 was without effects on working and reference memory errors.⁹⁴ PNU 120596 did, however, counteract scopolamine-induced working and reference memory errors.⁹⁴ Very little published clinical evidence exists describing the specific effects of nAChR ligands on spatial reference and working memory. The $\alpha 4\beta 2^*$ nAChR agonist ispronicline was shown to improve working memory (immediate and delayed word recall) in elderly volunteers tested with the Cognitive Drug Research test battery,⁹⁵ whereas AZD 1446 tested in adults with ADHD failed to show improvements in working memory or the spatial problem-solving test components of

the CAARS-INV.⁹⁶ Varenicline also failed to enhance spatial working memory in patients with schizophrenia.⁹⁷ Similarly, $\alpha 7$ nAChR agonists (eg, ABT-126, GTS-21, and RG 3487) have produced inconsistent effects on spatial and working memory during clinical assessment.^{22,87,98} Clinical trials with $\alpha 4\beta 2^*$ and $\alpha 7$ nAChR PAMs have either not been conducted or the effects on spatial and working memory were not published.

In summary, as in the case of spatial learning and reference memory, most of the preclinical data collected to date across a variety of animal models support the argument that both $\alpha 4\beta 2^*$ and $\alpha 7$ nAChR agonists have the potential to enhance spatial working memory, whereas additional data are needed before this conclusion can be made for types I and II nAChR PAMs. The nature of the equivocal results in human clinical trials is yet to be determined.

Recognition Memory

Recognition memory refers to the ability to recognize previously encountered stimuli, (eg, objects, smells, people, conspecifics, and events). This form of memory is believed to consist of a recollective (episodic) and a familiarity component and it is one of the domains of cognition that often deteriorates with age in humans. Moreover, it is commonly impaired in AD^{99–101} and schizophrenia.^{102,103}

Novel Object Recognition

The rodent spontaneous novel object recognition (NOR) task¹⁰⁴ has become a particularly popular method for evaluating compounds (including nAChR ligands) for potential as pro-cognitive agents in preclinical drug discovery programs for a variety of reasons. From a practical standpoint, the task is quite efficient because of the short training times required, and it is not aversive to the test subject as it does not require food restriction, water immersion, foot shock, or other nociceptive stimulus. The NOR task (see diagram and general task procedure in Figure 2D) has ethological relevance because it exploits the natural preference of rodents for novelty and translational relevance because it uses brain regions (eg, perirhinal cortex and hippocampus) in the rodent that support recognition memory processes in humans.^{42,105,106} A variation of this procedure is the spatial object recognition task where subjects explore and prefer novel spatial configurations of objects over previously experienced spatial configurations. This procedure has been used on occasion to evaluate nicotine for effects on spatial recognition memory (see next).

Social Recognition

Social recognition tasks in rodents retain the recognition memory component of NOR, but they also incorporate features of social behavior, thus providing an additional translational component. Although much less complex than human social behavior, social recognition tasks in rodents do use behaviors that are relevant to humans. These include (1) sociability, ie, the preference for interacting with social (conspecifics) as compared with nonsocial objects or spending time alone in an empty chamber; (2) social preference and bias toward novelty, ie, the preference of one individual (usually an unfamiliar individual) over another; and (3) social recognition, ie, the ability to remember an individual and discriminate between individuals.¹⁰⁷ The most typical version of the task is conducted similarly to NOR, with the objects replaced with novel and familiar conspecifics in the test phase.

Nicotine, $\alpha 4\beta 2^*$, and $\alpha 7$ nAChR agonists as well as $\alpha 4\beta 2^*$ and $\alpha 7$ nAChR PAMs have been shown to exert robust pro-cognitive effects on object recognition and social recognition across a variety

of experimental conditions. These include “stand-alone effects” and in pharmacologic-induced impairment models (eg, scopolamine and acute and subchronic administration of *N*-methyl-D-aspartate receptor antagonists). Moreover, the pro-cognitive effects elicited by these agents were attenuated by $\alpha 4\beta 2^*$ or $\alpha 7$ nAChR antagonists, indicating the receptor selectivity of the pro-cognitive effect (Table 2).^{8,59,67,74,79,84} In addition, improvements in episodic memory (ie, word and picture recognition tests) have been observed with these compounds during early phase clinical trials in normal human volunteers, patients with mild-to-moderate AD, and patients with schizophrenia. Unfortunately, in later (larger-scale) clinical studies, the effects were not replicated.^{22,32,87,98,108}

Although receptor subtype-selective nAChR ligands have not been systematically evaluated in the spatial object recognition task, nicotine has been observed to improve performance of the task when administered acutely.¹⁰⁹ Acute administration of the nicotine metabolite cotinine has also been shown to attenuate impairments of a spatial object recognition task in a mouse model of Fragile X syndrome.¹¹⁰ In the case of nicotine, the effects can shift from enhancement of performance to impairments as administration changes from acute to chronic and during withdrawal, however.¹⁰⁹ These effects were specific for spatial object recognition (as opposed to NOR), which led the authors to hypothesize that this could be because of differing underlying neural substrates involved performance of the two tasks. Melichercik et al.,¹¹¹ however, demonstrated that nAChR activation of either the perirhinal cortex or the hippocampus via intracranial infusions of nicotine facilitated performance on both object recognition and object-location memory tasks. These findings were interpreted as further supporting the putative interactive relationship between the hippocampus and perirhinal cortex in object information processing and highlight the potential therapeutic value of nAChR activation in amnesic disorders.

Collectively, the preclinical data described previously support the argument that activation of both $\alpha 4\beta 2^*$ and $\alpha 7$ nAChRs is a rational strategy for improving various forms of recognition memory including object recognition, social recognition, and object location memory.

Executive Function—Attentional Set Shifting Tasks

Executive function is an integrative component of normal cognition that is essential for the adaptive abilities and complex human behaviors. Optimal executive function requires attention, working memory, the planning, organization and initiation of tasks, cognitive flexibility (task or rule switching), as well as the inhibition of inappropriate responses.^{112,113} Impairments in executive function are among the most robust cognitive deficits known to be associated with schizophrenia and other neuropsychiatric disorders, especially where there is damage to the prefrontal cortex.¹¹⁴ Over the last several years, new behavioral tasks have been developed specifically for rodents to evaluate executive function. For example, intradimensional and extradimensional set shifting tasks¹¹⁵ are considered rodent analogs of procedures such as the Wisconsin Card Sorting Tasks, which are commonly performed in humans to assess executive function (see Figure 2E and the legend for a diagram and brief, general overview of this task). More recently, computer-automated (operant) tasks for rodents have been developed that retain many of the features of the Birrell and Brown (manual food cup-based tasks), but are somewhat less labor intensive and also allow multiple animals to be tested per day.⁴⁰ In the manual, food cup-based subjects are typically required to discriminate between the different

textures and odors to acquire a food reward. The cognitive flexibility component of the tasks is assessed when the subjects are required switch within dimensions (eg, between different textures) or between dimensions (eg, from texture to odor) and to perform reversals either within or between dimensions. In the computer-automated (operant) tasks, the dimensions (or rule strategies) commonly include spatial location (left vs. right lever) and visual stimuli (light presentation).

Similar to the previously reported pro-cognitive effects across multiple domains, nicotine, $\alpha 4\beta 2^*$ (ie, 5IA-85380), $\alpha 7$ nAChR agonists (eg, A-582941, RG 3487, and SSR 180711), and $\alpha 7$ nAChR PAMs (eg, CCMI, PAM-2, and PNU 120596) have been shown to improve executive function in the attentional set-shift task whether given alone or after pharmacologic-induced impairment with either acute or subchronic administration of *N*-methyl-D-aspartate receptor antagonists (Table 2).^{67,72,78,83,116} A literature search indicated that $\alpha 4\beta 2^*$ PAMs have not been assessed in the attentional set-shifting task to date. Clinically, improvements in executive function following $\alpha 4\beta 2^*$ and $\alpha 7$ nAChR agonists during early phase clinical trials have been reported, although as observed for the other cognitive domains discussed earlier, results have not been replicated.^{22,32,87,98,108}

In summary, even though the evaluation of novel nAChR ligands in animal tasks of executive function is in the relatively early stages, the data published to date suggest that $\alpha 4\beta 2^*$ agonists, $\alpha 7$ nAChR agonists, and $\alpha 7$ nAChR PAMs have significant potential as therapeutic agents.

Some Topics of Debate Regarding the Development of Optimal Therapeutic Strategies Using nAChR Ligands

As noted earlier, although the preclinical literature continues to grow in support of the development of nAChR ligands for a variety of conditions that affect humans, no new nAChR ligand has been approved to date for any condition other than nicotine dependence. It has been argued that one major limitation of the rodent models used in drug discovery research to date (particularly mice) is their limited genetic diversity. This is a major consideration as the two most common causes of early withdrawal from clinical trials, lack of drug efficacy and unanticipated side effects, can be strongly influenced by the genetic heterogeneity of the clinical trial subjects. The recent development of the Collaborative Cross and Diversity Outbred mouse initiatives is providing new tools capable of approaching the genetic diversity found in human populations.

There are also several unresolved topics of debate regarding the development of optimal therapeutic strategies using nAChR ligands to improve cognition that should be discussed. Such topics include receptor subtype selectivity and the desired net pharmacologic effect of the ligand (eg, receptor stimulation, desensitization, and antagonism). In each of these scenarios, in vivo conditions can be very complex and difficult to interpret. As noted earlier regarding nAChR selectivity, the field has primarily concentrated on developing ligands selective for heteromeric $\alpha 4\beta 2$ and homomeric $\alpha 7$ nAChRs to date because these receptor subtypes are thought to be the most prevalent in memory-related brain regions (eg, cortex and hippocampus) and because other subtypes (eg, $\alpha 3\beta 4$ nAChRs) have been associated with undesired size effects (eg, abuse potential and cardiovascular side effects). In the text at the end of this section, the potential value of $\alpha 4\beta 2^*$ -selective nAChR agonists to promote phasic neurotransmission is also discussed. However, the administration of a single pharmacologic agent with selectivity at $\alpha 4\beta 2$ or $\alpha 7$ nAChRs may

not be adequate or the most rational therapeutic due to the complex anatomical distributions of nAChRs as well as the nature and/or extent of the specific disease pathology. As just one example of the anatomical complexity, differing combinations of $\alpha 7$, $\alpha 4\beta 2$, and $\alpha 3\beta 4$ nAChRs found at specific locations on interneurons in the hippocampus affect both inhibitory and excitatory tones to collectively drive theta wave synchronization and long-term potentiation, the most widely proposed mechanism of memory storage, see Rogers et al.¹¹⁷. Differing physical properties of these receptor subtypes (eg, rates of activation and desensitization, and variable permeability to calcium vs. sodium) also make it unclear if pure selectivity at one of these specific nAChR subtypes is optimal. Moreover, as has been reviewed previously,⁸⁷ given the complexity of both the pathophysiology and symptom profile of conditions such as AD and schizophrenia, it would appear rational to attempt to modulate several therapeutic targets simultaneously.

The classical view that receptor stimulation is the key action responsible for nAChR-induced cognitive enhancement can also be challenged by the observation that nAChRs are easily desensitized by nicotine and other nAChR agonists and that in some cases nAChR antagonists can exert pro-cognitive effects. As an example, although an extensive literature exists on the amnesic effects of the nonselective nAChR antagonist mecamylamine, pro-cognitive effects of this compound have been reported in working memory tasks in both rodents and monkeys as well as a recognition memory task in humans with ADHD. For details of these studies, additional examples of nAChR antagonist-related pro-cognitive effects, and a discussion of the provocative subject of “nAChR desensitization of as a strategy for pro-cognitive drug development,” see Refs. ^{22,118}.

Another topic of debate related to the best cholinergic-based approaches to improving cognitive function is the type of neurotransmission or neuromodulation that should be targeted (eg, volume vs. phasic transmission). The diffuse projections of basal forebrain cholinergic neurons to the cortex and the locations of cholinergic receptors at multiple sites on neurons (presynaptic, postsynaptic, extrasynaptic) as well as on nonneuronal sites in the central nervous system have fueled the argument that they primarily facilitate paracrine (also referred to as diffuse, nonjunctional, or volume) transmission. In this scenario, ACh slowly reaches the extrasynaptic space as opposed to remaining confined to the synaptic cleft (wired neurotransmission) and mediates different arousal states to influence cognition, especially attentional performance. This model would appear to fit well with the most commonly prescribed treatments for AD, the acetylcholinesterase inhibitors that, by blocking the hydrolysis of ACh, significantly elevate basal extracellular levels of ACh to improve cognition. However, the modest clinical efficacy of acetylcholinesterase inhibitors, and the observation that they often fail to significantly improve the performance of attention-based tasks in animals, argues that this may not be the most optimal approach to improving attention. Recent studies demonstrated that phasic release of ACh, at the scale of seconds, mediates precisely defined cognitive operations. Moreover, an increasing body of evidence suggests that nAChR agonists, especially $\alpha 4\beta 2^*$ -selective nAChR agonists, generate phasic cholinergic signals to robustly enhance cognitive function (reviewed in McGonigle¹¹⁹). This type of observation would support the future development of selective nAChR agonists as opposed to additional efforts to enhance “volume transmission” in cholinergic pathways. Alternatively, adjunctive strategies where a selective nAChR agonist or PAM (to enhance phasic cholinergic signals) is combined with an acetylcholinesterase inhibitor (which

promotes volume transmission) may be promising. Notably, in previous studies in our laboratory, we have observed robust cognitive enhancements in young and aged rats as well as aged monkeys when we combined the $\alpha 7$ -nAChR-selective PAM, PNU 120596 with the acetylcholinesterase inhibitor donepezil.⁹⁰

Conclusions and Future Directions

The high rate of clinical trial failures for novel compounds developed to treat neuropsychiatric disorders has fueled the argument that there is a significant translational gap between the preclinical animal studies and the human clinical trials.^{120,121} The clinical failures include novel nAChR ligands at both $\alpha 4\beta 2$ (eg, ispronnicline) and $\alpha 7$ nAChRs (encenicline) for AD and/or schizophrenia where the compounds did not show statistically significant efficacy as pro-cognitive agents or they were discontinued prematurely due to unanticipated side effects.^{121,122} Both study failures occurred despite a large body of encouraging preclinical data and early clinical results. There may be a variety of reasons for these failures, at both the preclinical and clinical trial design levels.²² However, the studies do provide the impetus for continuing efforts to develop new nAChR strategies beyond simple agonist and partial agonist approaches (to include PAMs) as well as to refine current behavioral strategies and create new animal models to address translational gaps in the drug discovery process. The preclinical literature continues to grow in support of the development of new nAChR ligands for a variety of conditions that affect humans. Moreover, there is some literature to support the repurposing of nAChR ligands that are approved and prescribed for other therapeutic purposes (eg, tropisetron) for disorders of cognition (eg, AD, Parkinson disease, and schizophrenia)^{74,87} and nicotine withdrawal-induced cognitive impairments.⁹⁷

Supplementary Material

Supplementary data are available at *Nicotine & Tobacco Research* online

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Declaration of Interests

None declared.

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