COGNITION

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1. INTRODUCTION

The aging process and traumatic insults to the brain frequently result in a decline in cognitive performance with an associated decrease in the individual quality of life. At the tissue level, this decline involves neuronal/glial dysfunction and/or overt neurodegeneration with marked cell loss, disruption of discrete synaptic pathways, and altered neural network function [1].

Drugs to treat cognitive dysfunction are a high priority in biomedical research as the elderly population grows, showing an increased incidence of what has been termed “benign senescent forgetfulness” that progresses to mild cognitive impairment (MCI) and Alzheimer’s disease (AD). Aspects of cognitive dysfunction also occur in association with Parkinson’s disease, AIDS-associated dementia, stroke, schizophrenia, stress, sleep deprivation, depression, anxiety, recreational, prescription drug usage, and surgical procedures.

The degree of cognitive deficit and its underlying causality differs both anatomically and, to the extent known, mechanistically in this broad spectrum of CNS disorders, making it somewhat naive to expect that new chemical entities acting via a single mechanism will have efficacy in all CNS disorders that involve some aspect of cognitive dysfunction. The molecular and anatomical substrates of cognition and associated behaviors that include attention and memory are complex and diffuse and suggest that drugs addressing this area will necessarily be polypharmic [2]. As an example, given the relative ease of diagnosis of AIDS and its relatively short progression, AIDS dementia was for a period of time viewed as a surrogate disease state for testing compounds that might have potential utility in the treatment of AD. However, equating the cognitive dysfunction associated with the slow progressive timeline of AD neurodegeneration with the cerebral atrophy occurring due to opportunistic viral, fungal, protozoal, and bacterial infections in AIDS to a common mechanistic pathway is, based on present knowledge, an optimistic stretch.

1.1. Cognition

Cognition is defined by the Merriam-Webster Online Dictionary as “to become acquainted with, know.” Cognition can also be defined as the ability to process contemporary information in the context of existing knowledge to appropriately respond—in terms of decision making—to a given situation. In either definition, cognition involves several distinct behavioral domains. These include attention, perception, emotion, memory, action, and problem solving [2].

Therapeutic approaches to ameliorating cognitive dysfunction can be simplistically viewed as involving two ends of a continuum—at one end the prophylactic enhancement of cognitive function and at the other end the restoration of function and/or arrest of the decline occurring to the aging or traumatized brain. Cognition enhancers include psychostimulants such as caffeine, the most widely ingested drug in the world in the form of coffee and soft drinks [3], amphetamines [4], nootropics such as piracetam 1, and the antioxidant, idebenone 2 [5]. Drugs currently approved for use in the treatment of neurodegenerative disorders such as AD include the cholinesterase inhibitors, tacrine 3, donepezil 4, rivastigmine 5, and galanthamine 6 [6] and memantine 7, a partial agonist at glutamate receptors [7]. None of these drugs works especially well in treating the symptoms of AD [8] probably reflecting the advanced stage of the disease when it is diagnosed than the intrinsic efficacy of these drugs. To restore function to a dead cell, a “Lazarus-like” effect, is a phenomenon that has yet to be achieved outside the realm of fiction.

A variety of new chemical entities (NCEs) are currently being explored to identify new cognitive enhancers. Some have discrete mechanistic targets against that they are/were optimized. Others involve a heuristic
postrationalization of a target based on a phenotypic behavioral response while yet others have their origins in ayurvedic medicine or folklore and currently lack a robust mechanism of action (MoA), for example, *Ginkgo biloba* and the nootropics [5].

In neurodegeneration-associated cognitive deficit, significant efforts have been focused on the β-amyloid [9] and tau hyperphosphorylation [10] hypotheses of AD that reflect the involvement of amyloid plaques and neurofibrillary tangles (NFTs), respectively, in disease pathophysiology. Both are found in postmortem AD brain although whether they are causative, or a result, of the disease remains to be determined [11]. Prevention of β-amyloid (Aβ) formation by altering cleavage products via inhibition/alteration of secretase enzyme processing [12], prevention of Aβ deposition or enhancing its removal by the use of metal chelators or vaccination will eventually provide information on the causative role of this peptide fragment in AD. However, the path forward is far from certain as initial positive results have, more often than not, been confounded by results from subsequent studies, the more important of which have been Phases II and III clinical trials where approaches to reducing brain amyloid have failed to show efficacy in altering disease progression [11]. Approaches to altering the hyperphosphorylation state of tau as a target for AD treatment are similarly complex. There are some 80 serine and threonine residues on tau that are potential substrates for kinase activity [10]. With only 30 of these functioning as phosphorylation sites under normal physiological conditions and 25 of these being identified as sites of “abnormal phosphorylation” there is significant redundancy in the ability to alter tau phosphorylation. Candidate kinases for tau phosphorylation are the proline-directed kinases, GSK3β, CDK5, and ERK2 that can phosphorylate 13 residues in tau associated with proline residues. Microtubule-affinity-regulating kinase (MARK) and cAMP-dependent protein kinase A (PKA) are nonproline-directed kinases that may also affect tau hyperphosphorylation. Inhibitors of “tau kinase,” dual GSK3/CDK5 inhibitors such as the indirubicins—while effective in altering phosphorylation of key serine and threonine
sites on tau in cellular and transgenic animal systems—have yet to demonstrate robust biochemical or phenotypic effects in native systems.

One inevitable outcome from research into cognition enhancers for the potential treatment of disease-associated cognitive dysfunction is that of nootropics or “smart drugs.” [14] In the same way that caffeine and amphetamines are viewed as improving normal function via their stimulant actions, newer generations of cognition enhancers are anticipated to improve performance [14].

1.1.1. Domains of Cognition and Memory

Historically, the understanding of cognitive processes is based on the empirical school of psychology known as behaviorism [15]. Behaviorist theories, championed most notably by James Watson and B.F. Skinner, evolved from the early learning and memory studies of Pavlov and Thorndike. These behaviorists were primarily concerned with defining stimulus–response relationships and focused on precise observation and mathematical formalism that viewed the intervening processing of stimuli as irrelevant as only directly observable behaviors were believed to be amenable to scientific investigation. While this approach was successful in rigorously characterizing certain behaviors, it was soon apparent that an understanding of mental processing, the realm of cognitive psychology [16], was essential for interpreting the full spectrum of behavior, and of human behavior in particular.

A useful framework for understanding cognitive processes is provided by the information processing models of cognitive psychology. These models range in complexity from the black-box model of the behaviorists (Fig. 1a) to extremely complex connectionist models [17]. However, common themes are apparent that allow the derivation of a simplified model (Fig. 1b). Importantly, this framework allows for the deconstruction of the cognitive process into experimentally addressable domains that can be studied both preclinically and clinically.

Perception A detailed description of the processes underlying perception is outside of the scope of cognition. However, deficits in preattentive processing and attention play significant roles in defining the scope and consequences of the cognitive deficits observed in a number of CNS disorders including schizophrenia [18]. Since these early processing events are critical for subsequent cognitive processing, a brief treatment is necessary.

Preattentive processing refers to the non-conscious events that occur at the earliest stage of information processing. Information

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**Figure 1.** Schematic representation of information processing models of cognitive function. (a) The black-box model employed in behaviorist theory. Stimulus and response are quantifiable while knowledge of the inner workings of the cognitive process is considered intractable to experimental methods. (b) The more complex model if information processing based on probing cognitive function in both animal and human using the tools of psychology and neurobiology. See text for explanation of labels.
enters the CNS as a sensory stream (e.g., visual or auditory input from the eyes or ears, respectively) representing a massive amount of data that would quickly saturate downstream systems. A large number of distractors exist in the environment that must be quickly filtered out of the sensory stream to pass along information that is behaviorally and contextually relevant. This is accomplished by a system of filters that determine the saliency of stimuli based on temporal–spatial frequency and importance [19]. The preattentive processing and filtering of the sensory stream occurs rapidly, within 100 ms of the appearance of a stimulus, and has been demonstrated by simple visual search experiments in which a subject is asked to identify a unique target in a field of distractors [20]. For simple targets and dissimilar distractors, the time required for target identification is independent of the number of distractors, indicating that a conscious search through the field is unnecessary and therefore the process is preattentive.

Once preattentive processing filters the sensory stream to a manageable number of stimuli that then enter into consciousness, it is necessary that the system attends and responds to the relevant stimulus. Attention is the cognitive process of selectively focusing on a relevant stimulus while ignoring other stimuli in the environment. This focusing of consciousness may be an overt process in which attention is directed at a particular stimulus by focusing the sensory apparatus on that stimulus, or covert, where attention is mentally focused independently of sensation. In addition, attention may be focused on cognitive processing independent of sensory input. This executive attention or executive function is discussed in greater detail below.

Attention is far from a simple linear selection process as it is subject to a number of complex modulatory interactions [21]. Notably, significant top–down processing occurs during when information in working memory, conscious determination of the importance of stimuli, and motor control of the sensory stream, all play a role in directing and maintaining attention.

Memory The processes of information acquisition (learning) and recall (memory) form the core of cognitive processing. These processes are significantly impaired in the aged and/or traumatized brain. Memories from years past appear facile to recall, while events that are more recent cannot be recalled. Whether the problem is in the acquisition of recent memories or the ability to recall these is unknown and has considerable impact on studying the problem of cognitive dysfunction from a drug discovery perspective. If a memory is not acquired, searching for drugs to recall that memory at a point distal to its acquisition is an exercise in futility.

Memory is divided into two main categories: working, or short-term memory, and long-term memory.

Working Memory Working memory is classically described as consisting of three subcomponents: a central executive, a verbal or phonological rehearsal loop, and a visuospatial sketchpad [22]. The central executive function, which maintains control over voluntary activities, is described below. Both verbal rehearsal and visuospatial sketchpad are methods of temporarily holding information arising from the perceptive process or which is recalled from long-term memory. For example, keeping a phone number in mind by verbally rehearsing until the number is dialed, or imagining the landmarks on a street corner when relaying directions. These working storage elements provide a dynamic representation of both the inner and the outer world that constitutes the substrates for further cognitive processing. The key characteristics of working memory are a limited duration of only a few seconds, and a relatively small capacity known to range between 4 and 10 items [23,24]. Because of its dynamic nature, working memory is subject to rapid change and can be easily disrupted. Therefore, information can only be maintained through consolidation into long-term memory.

The process of memory consolidation in which an item in working memory is transferred into long-term memory is essential to all forms of learning. Memories are consolidated over time, and with repetition or rehearsal, in a process that is facilitated by sleep [25]. Much is being uncovered regarding the molecular substrates of memory consolidation discussed further below.
Long-Term Memory  In contrast to working memory, long-term memory is static, long lasting, and resistant to disruption and can be divided into declarative and nondeclarative memory [26]. Nondeclarative or nonconscious memory includes items learned by nonassociative means such as habituation or sensitization, innate motor and cognitive skills, and dispositions. While it is debatable as to whether these items are learned without conscious input, they all clearly involve unconscious processing. Declarative or conscious memory is the type of memory more obviously associated with cognitive processes and can be further divided into semantic and episodic memories.

Semantic memories are factual recollections. They involve facts about the world that are independent of a particular place or time. Semantic memories include facts about the individual, others, or shared knowledge. For example, one’s height, a friend’s birthday, or the capital of a particular country all are items of semantic memory. Semantic memories are distinguished from episodic memories in that the former are things that are “known” rather than reexperienced or “remembered.”

Episodic memories hold specific relevance in time and space and are often autobiographical and involve life experience. As such, these memories are self referential and frequently context dependent involving relationships to times, places, other individuals and other events. Episodic memories are typically organized around particular periods of time, and are recalled in a manner in which the individual mentally recreates the events.

It should be obvious that there is a close relationship between semantic and episodic memories. Both types of memories may contribute to a given recall event [27] and, over time episodic memories are believed to be transformed into semantic memories. For example, a memory of an event that occurred several years ago may not be recalled as a mental re-creation of the event but rather a knowledge of attendance at the event and a list of related occurrences.

Processing The box labeled processing in Fig. 1 is the cornerstone of cognitive function. It represents a generalization of a number of complex, and, to date, poorly understood processes that take the simple inputs and storage devices described above to create conscious cognition. These are the processes that behaviorists considered intractable in terms of the scientific method, and while they have begun to yield to well-designed experimentation, there is still much to be learnt.

As mentioned above, the concept of a central executive is a necessary component of working memory and consolidation required to oversee verbal rehearsal and the visuospatial sketchpad. Furthermore, there is implicit in the concept of attention the need for a process that directs the focusing of consciousness. This observation led Broadbent [28] to distinguish between automatic and controlled processes ultimately leading to the concept of the central executive as a type of orchestra conductor or CEO that oversees cognitive processes (for review, see Ref. [29]).

The central executive oversees a variety of functions often termed “higher order” processes. These include process such as planning, goal setting, and the initiation of actions. Furthermore, the central executive is responsible for planning, motivation, problem solving, language processing, and a variety of other higher order functions.

From this brief overview of the processes involved in cognition, it is obvious that drugs to potentially improve cognitive function will target particular domains of cognition. As the disruption of cognitive domains in disease states will be heterogeneous with respect to the underlying pathologies, it is important to understand the different indications for which cognitive enhancing drugs are being developed.

1.1.2. Indications and Diagnostic Criteria The Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revised (DSM-IV-TR, 2004) includes cognitive dysfunction under the general heading “Delirium, Dementia, and Amnestic Disorders.” [30] This is a broad spectrum of CNS disorders that includes delirium (Diagnostic code 293), AD (294.1), vascular dementia (290), (Delirium) and amnestic disorders/Other Cognitive Disorders (294). Delirium, also known as acute confusional state or reversible madness is a behavioral response to
widespread disturbances in cerebral metabolism that can be caused among other events by: substance intoxication or withdrawal, head trauma, dehydration, congestive heart failure, sleep deprivation, endocrine dysfunction, etc. Acetylcholine (ACh) is the primary neurotransmitter involved in delirium with the primary neuroanatomical site being the reticular formation. The clinical abnormalities associated with delirium occur in the domains of arousal, language and cognition, perception, orientation, mood, sleep and wakefulness, and neurological functioning. Dementia involves multiple cognitive deficits that are differentiated on the basis of etiology [30] and include dementia due to AD, Parkinson’s disease (PD), Pick’s disease, Huntington’s disease, HIV disease, head trauma, vascular disease, substance-induced persisting, other medical conditions, and medications. Quite clearly, the ability to diagnose the precise form of dementia, for example, cognitive dysfunction, will dictate both defining the potential molecular lesion and treatment which has a major impact on identifying viable drug discovery approaches and effective patient treatments. Evaluation of medical history, physical examination, routine and specialized laboratory tests and brain scans [30] are all pertinent to diagnosis underlying a major need for definitive biomarkers for cognitive dysfunction [31–33].

**Cognition in Alzheimer’s Disease** AD and its variants are neurodegenerative diseases where cell death plays a major role in the cognitive dysfunction phenotype. Memory impairment, difficulty in solving problems and decreases in spontaneity, reaction speed and accuracy are early signs of AD. The inability to remember names, misplacing items, forgetting what one was about to be done and difficulty in word finding (anomia) are early signs of AD that are usually dismissed as signs of “getting older” or “needing more sleep” that can lead to depression, aggression, confusion, and wandering that can further exacerbate cognitive function. The memory loss in early stage AD is most obvious with newly acquired memories that also lead the individual to avoiding new, unfamiliar situations. AD can progress to overt memory loss, for example, dementia with accompanying physical traits that lead to the AD patient being bedridden and ultimately to death. AD is a major healthcare challenge with current estimates of 25–34 MM individuals being currently affected worldwide [11] with projections of triple this number by 2050. Since diagnosis of AD is exclusionary, a major challenge in finding effective treatments for AD is the ability to diagnose the disease at a stage early enough when drugs may act to arrest and potentially reverse the disease process, for example, before the cells are dead and the cellular substrates for drug action are no longer present [34]. Current biomarkers, including plasma and CSF amyloid levels and brain imaging [31] are insufficiently robust to be useful in disease diagnosis [11]. In October 2008, the FDA’s Peripheral and Central Nervous System Drugs Advisory Committee in reviewing the use of radionucleotide imaging for the detection of cerebral amyloid to assist in AD diagnosis “felt that too many questions remain about the relationship between cerebral amyloid and AD to diagnose AD or predict risk of the disease based on a positive test for the marker.” [35] Given that the currently approved treatments for AD have questionable efficacy [8] and the major research focus on the amyloid hypothesis is also in question [11], there is considerable effort ongoing to identify drug candidates that restore brain function via effects in enhancing neurotransmitter release, reducing brain inflammation and oxidative stress and in enhancing mitochondrial function [11]. These approaches are discussed in detail below.

**Schizophrenia** Cognitive impairment in schizophrenia occurs before the manifestation of overt psychotic symptoms, remaining severe through the course of the disease. Schizophrenia-associated cognitive dysfunction involves multiple domains including executive function, attention, processing, vigilance, verbal learning and memory, verbal and spatial working memory, semantic memory, and social cognition [30,36]. There is a major unmet medical need in finding effective treatments to treat the cognitive domain of schizophrenia (CDS) since it is considered to be of equal or greater importance than positive or negative symptoms in predicting the functional consequences of schizophrenia, especially in regard
to work status and quality of life [37]. The US federal initiative, Measurement and Treatment Research to Improve Cognition in Schizophrenia (MATRICS) is discussed further below.

**Parkinson’s Disease** While the major clinical features of PD reflect tremor, rigidity and bradykinesia that result from the degeneration of dopaminergic neurons in the locus coeruleus and substantia nigra, dementia occurs in approximately 40% of PD patients older than 70 [38]. Like AD, the cerebral cortex of PD patients contains amyloid plaques, NFTs, and also eosinophilic Lewy bodies. The latter also occur in Lewy body dementia (LBD), a degenerative brain disease that is a leading cause of dementia in the elderly population and accounts for up to 20% of all dementia cases. PD is a form of LBD. Treatment approaches to PD-associated dementia and LBD, such as those for AD are currently focused on plaque removal with additional initiatives in enhancing dopamine formation and release, reducing brain inflammation and oxidative stress and enhancing mitochondrial function.

**HIV-Associated Dementia** Dementia associated with the HIV infection in AIDS results from the decline in immune competence associated with viral load. In approximately 10% of HIV-positive individuals, the neurological symptoms that lead to dementia reflect the first sign of AIDS [30]. Cognitive disorders in AIDS dementia include memory impairment, lack of concentration and confusion. These are accompanied by apathy, depression, anosognosia, disorientation, delusions, and hallucinations as well as motor dysfunction that can lead to PD-like symptoms. Neuropathological abnormalities occur in 90% of brains of individuals that reflect opportunistic viral, fungal, protozoal, and bacterial infections that lead to cerebral atrophy and nonspecific white matter loss [39]. Additionally, several of the antiviral agents used to treat AIDS have their own effect on CNS function leading to anxiety, depression, and confusion. In addition to antiviral therapy to reduce the cause of the neuropathological abnormalities in AIDS, treatment approaches to PD-associated dementia and LBD, such as those for AD are currently focused on plaque removal with additional initiatives in enhancing dopamine formation and release, reducing brain inflammation and oxidative stress and enhancing mitochondrial function.

**Stroke/Vascular Dementia** Vascular dementia is the second most common form of dementia after AD and is caused by a major or multiple cerebrovascular accidents (CVAs), now being more commonly known as stroke or “brain attack.” A stroke is defined by the WHO as a “neurological deficit of cerebrovascular cause that persists beyond 24 hours or is interrupted by death within 24 hours” and occurs when blood flow to the brain is attenuated by the ischemia resulting from thrombosis or an embolism or from hemorrhage. Some four million cases of stroke occur each year highlighting the need for effective therapies [40]. Memory impairment following a stroke is accompanied by aphasia, apraxia, agnosia, and deficits in executive function, spasticity, ataxia, hemiparesis, and white matter lesions [30]. While t-PA is effective as antithrombolytic therapy, the search for drugs to treat the consequences of the excitotoxic insult associated with stroke has been frustrating due to a lack of predictive animal models and compounds advanced to clinical trials that lacked efficacy or had side effects that limited their use or made the consequences of the stroke worse [41,42]. As with AIDS and PD, there are expectations that medications effective in treating the cognitive dysfunction associated with AD will have utility in the treatment of the cognitive decline following stroke.

**Life Style-Associated Cognitive Dysfunction** In addition to stress, sleep deprivation, depression, and anxiety, life style choices associated with impaired cognitive function include recreational and prescription drug usage and severe alcohol dependence which is the third leading cause of dementia. The latter occurs late in life following 15–20 years of heavy drinking [30]. Additionally, the use of sedatives, hypnotics, and/or anxiolytics can lead to dementia.

### 1.1.3. Disease Diagnosis ADAS-COG

The most commonly used rating instrument in trials of cognition enhancers is the cognitive subscale of the AD Assessment Scale (ADAS-cog) [43,44]. This scale was designed to reliably identify and rate the major characteristics of
AD across a range of dysfunction from mild to severe dementia. The ADAS-cog is a relatively brief test taking approximately 30 min to complete. The test consists of 11 parts that primarily rate memory, language, and praxis (performance of an action). Notably, nearly half of the items in the ADAS-cog relate to memory making this scale a very sensitive measure of memory dysfunction ideally suited for the assessment of AD patients [44].

**CANTAB** While the ADAS-cog is a useful instrument for the study of AD, it is limited in the domains of cognitive function assessed, and is subject to rater errors that can be compounded during long disease progression trials where the raters may turnover several times during the course of the study [45]. The Cambridge Neurophysiological Test Automated Battery (CANTAB) was developed as a computer-based tool that has high precision, speed, and reliability and can produce objective feedback [46]. The battery is administered at a computer terminal and relies entirely on nonverbal stimuli and responses. CANTAB measures different aspects of cognitive function including visual memory, attention, and planning. As such, CANTAB can measure certain aspects of executive function that are not addressed in detail in the ADAS-cog. The CANTAB instrument was designed as a battery of tests that mimic behavioral paradigms used in preclinical studies that have helped to establish the neuronal substrates of cognitive function. Therefore, the battery has the unique potential to not only identify cognitive dysfunction but also indicate possible sites of disease action in the brain. The CANTAB has proven to be a sensitive measure effectively detecting cognitive dysfunction in a number of disorders including AD, PD, schizophrenia, and mood disorders, as well as the cognitive decline associated with normal aging.

**MATRICS** Schizophrenia is often thought of in terms of the positive symptoms of the disorder including hallucinations and paranoia. However, it is now understood that the cognitive dysfunction associated with schizophrenia is both severe and poorly treated by current therapies. Cognitive impairment in schizophrenia begins before the onset of the psychosis and can worsen throughout the course of the illness. Schizophrenia is associated with widespread, multifaceted impairments in cognitive function, including executive function, attention, processing, vigilance, verbal learning and memory, verbal and spatial working memory, semantic memory, and social cognition. Cognitive impairment in schizophrenia may be of equal or greater importance than positive or negative symptoms in predicting functional outcomes and quality of life [37].

The recognition of the prevalence of cognitive dysfunction in schizophrenia together with the realization that there was a lack of a consensus as to how cognition in schizophrenia should be measured, led the National Institute of Mental Health to sponsor the Measurement and Treatment Research to Improve Cognition in Schizophrenia (MATRICS) initiative that has produced the MATRICS Consensus Cognitive Battery (MCCB) [47]. The MCCB employs 10 tests that assess performance in 7 cognitive domains: speed of processing, attention, working memory, verbal learning, visual learning, reasoning and problem solving, and social cognition. The battery is intended to be a comprehensive assessment of cognitive dysfunction in schizophrenia, and therefore requires more than 1 h for administration. The MCCB relies on neuropsychological tests that are standardized and widely used making it somewhat user friendly for the clinician. However, it lacks the translational power of CANTAB in that many of the tests that have no preclinical analog. In an attempt to remedy this, efforts are ongoing to match domains of clinical efficacy with appropriately predictive animal models [48].

### 1.2. Substrates of Cognition

**1.2.1. Neuroanatomical** Our understanding of the neuroanatomical substrates of cognition began in the 1920s with relatively crude lesion studies pioneered by Karl Lashley during his search for the elusive memory trace termed the engram. While Lashley’s work was influential and set the stage for all future research on the neural basis of cognition, his focus on the reductionist approach led him to famously conclude after 30 years of research that he discovered “nothing directly of the real nature of the engram” and that he sometimes felt in
reviewing the evidence that “the necessary conclusion is that learning just is not possible.” [49]. The development of functional imaging methods along with the ability to produce discrete anatomical, pharmacological, and molecular lesions has greatly advanced the field now known as cognitive neuroscience.

**Neuroanatomical Substrates of Memory** As first identified in the work of Penfield [50], focal electrical stimulation delivered during brain surgery to the cortex, the temporal lobes in particular, can evoke strong conscious memories. These studies suggested that memories are somehow stored in the cortex, possibly in a distributed network formed by altering synaptic connections and weights that can be selectively activated by electrical stimulation. The medial temporal lobe that includes the hippocampus, entorhinal, and perirhinal cortex is the key brain complex responsible for processing information from the neocortical and limbic regions and integrating the information into a memory that encodes for various aspects of an event [51]. The medial temporal lobe interconnects with a number of brain regions including sensory cortical regions, for example, the superior temporal gyrus and insular cortex, regions that encode for fear and emotion responses, including the amygdale and cingulated cortex, and executive regions of the neocortex. The medial temporal lobe is therefore ideally situated to receive information regarding the multiple components of an experience (sensory, emotional, and cognitive) and integrating these. The “bound” memory is then consolidated into long-term memory in the neocortex [52].

Perhaps the most dramatic proof of the role of the medial temporal lobe in the formation of new memories comes from the classic case of the patient known as HM [53] who died in 2008. HM suffered from severe intractable epilepsy that was treated by bilateral removal of the medial temporal lobes [54]. This procedure was performed prior to an understanding of the role of this brain structure, and produced a surprising and remarkable change in HM’s ability to encode memory in that he developed complete anterograde amnesia. While appearing otherwise normal, in terms of problem solving ability, IQ, and language comprehension, HM was unable to incorporate any new memories. Interestingly, HM and other patients with damage to the medial temporal lobes also experienced a temporally graded retrograde amnesia with more recently encoded memories of events prior to the damage being lost, but older memories being maintained. This has led to the suggestion that the process of memory consolidation is a slow multicomponent process with the initial encoding of long-term memories residing in the medial temporal complex followed by a slow transfer to the neocortex over time [52].

**Neuroanatomical Substrates of Executive Function** As described above, the central executive function is a necessary yet poorly defined function that oversees the bulk of what is considered conscious processing. While the complexity of the overall role of the central executive makes it difficult to accurately define, it is clear that it resides in prefrontal cortex [55]. The prefrontal cortex is connected to all functional units within the central nervous system [56] implying that it exerts a broad controlling influence on behavior. Of particular interest when considering central executive function is that the prefrontal cortex has extensive interconnections with the dorsomedial thalamic nucleus, a key integrator of information from the thalamus a region encoding emotional states that includes the amygdale and cingulate cortex and the key memory areas in the medial temporal lobe.

The prefrontal cortex, and the dorsolateral prefrontal cortex in particular, is involved in the processing of goals, actions, and planning, executive functions that operate with working memory [57]. These functions include both the ability to guide behavior by internal representations and the ability to adapt to changes that require subsequent alterations in behavior. This cognitive flexibility, or the ability to shift cognitive set, is a key aspect of central executive function that is disrupted in number of neuropsychiatric disorders involving impairments in prefrontal cortical function [58].

Damage to the prefrontal cortex induced by stroke or traumatic brain injury can produce a variety of syndromes that underscore the importance of this structure in central executive function. Resultant symptoms
include perseverative behaviors, flat affect or dramatic changes in personality, a loss of ability to follow internal plans or maintain attention, and a loss of cognitive flexibility. These same behaviors are observed in neurological and neuropsychiatric disorders associated with prefrontal dysfunction as discussed below.

1.2.2. Molecular Substrates of Memory

As discussed above, memory is believed to exist in a distributed network created by altering synaptic connections and weights. The concept that neuronal activity can lead to changes in synaptic coupling efficiency was first proposed in Hebb’s postulate [59]. Hebb proposed that when a given neuron excites a second neuron repeatedly and persistently, some change occurs such that the efficiency of coupling between the two cells is increased. This activity-dependent synaptic plasticity has been the focus of considerable research in an attempt to identify the molecular substrates of memory.

**Long-Term Potentiation**

The finding that repetitive activation of glutamatergic synapses in the hippocampus led to a long-lasting increase in synaptic strength provided evidence for the first potential physiological substrate for memory in mammals [60]. This phenomenon, long-term potentiation (LTP) has since been observed at a number synapses and has been the subject of intensive research efforts aimed at understanding the molecular events underlying synaptic plasticity. The best studied of these synapses remains the Schaffer collateral-CA1 synapse of the hippocampal formation.

There are two major types of ionotropic glutamate receptors that contribute to fast postsynaptic response at glutamatergic synapses [61] named for their selective agonists, AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) and NMDA (N-methyl-D-aspartate). AMPA receptors families are monovalent cation channels that are gated by glutamate and produce rapid excitatory responses. NMDA receptors are permeant to monovalent cations and to calcium. A critical feature of the NMDA receptor channel is that it is blocked by extracellular magnesium in a voltage-dependent fashion. This magnesium block essentially renders the NMDA receptor inactive at normal membrane potential, but allows it to open at more depolarized potentials. Induction of LTP at the Schaffer collateral-CA1 synapse requires activation of NMDA receptors [62]. Strong synaptic stimulation, such as those produced by a high-frequency (100 Hz) electrical stimulation of the Schaffer collateral fibers, leads to sufficient AMPA receptor-mediated depolarization of the CA1 pyramidal neurons to relieve the magnesium block of the NMDA receptor. This allows an influx of calcium through the NMDA receptor that triggers the biochemical cascades that lead to a persistent change in synaptic efficacy. While a large number of putative signaling molecules have been implicated in this process, the exact biochemical pathway underlying LTP remains unknown [63]. However, there are several key events that have been identified, which provide an understanding of molecular substrates. The calcium/calcmodulin-dependent kinase, CAMKII appears central to LTP induction. CAMKII is activated by calcium influx and autophosphorylates with LTP induction [64]. Knockout of CAMKII or replacement with an autophosphorylation-deficient variant prevents LTP induction [65,66]. Other kinases have also been implicated in LTP induction including cAMP-dependent protein kinase [67], extracellular signal-related kinase [68], Src kinase [69], and protein kinase C [70]. The biochemical steps linking CAMKII activation and autophosphorylation to the expression of LTP are not fully understood. Enhanced synaptic efficacy appears to involve an increase in postsynaptic AMPA receptors produced by a modulation of receptor trafficking [71]. Intracellular AMPA receptors are trafficked from recycling endosomes to the plasma membrane by a process that requires Rab11a, a small GTP binding protein [72]. The AMPA receptors interact with a family of transmembrane AMPA receptor regulatory proteins (TARPs) that provide an interaction with postsynaptic density proteins (PSDs) [73] directing the AMPA receptors to their appropriate location within the synaptic membrane. CAMKII phosphorylation of TARPs appears to be important for LTP expression [74,75] providing a plausible link between CAMKII activation, an increase in postsynaptic AMPA...
receptor density, and resultant enhanced synaptic efficacy.

**Long-Term Depression** The finding that the Schaffer collateral-CA1 synapse and other synapses can also express long-term depression (LTD) suggests that bidirectional control of synaptic strength is possible [76]. LTD can be induced by a prolonged low-frequency (1 Hz) stimulation. Interestingly, this form of LTD, like LTP is NMDA receptor-dependent and relies on calcium influx suggesting that the temporal and spatial dynamics of spike timing and calcium influx into the postsynaptic dendritic spine are critical in determining the form of plasticity expressed [77]. NMDA receptor-dependent LTD appears to involve the activation of the protein phosphatases, PP1 and PP2B [78]. Subsequent calcium-dependent dephosphorylation of AMPA receptors and associated proteins lead to a dissociation of the receptors from the postsynaptic complex and a triggering of clathrin and dynamin-dependent endocytosis [79].

1.2.3. **Other Forms of Synaptic Plasticity** This brief overview of the molecular basis of plasticity has focused on NMDA-dependent plasticity in the hippocampal CA1 region. While this represents the best-studied form of synaptic modulation, plasticity occurs at many other synapses within and outside of the hippocampus. The events may be NMDA-independent [80], have a presynaptic locus of action [81], be mediated and expressed by GPCRs [82], or involve retrograde synaptic transmission [83]. Furthermore, plasticity is not solely a property of excitatory synapses also occurring at inhibitory GABAergic synapses [84]. Therefore, memory is very likely encoded in a distributed fashion across both excitatory and inhibitory synapses expressing multiple forms of plasticity. This suggests that the identification of a single molecular substrate of memory, or even of a particular type of memory may be as elusive as Lashley’s engram.

1.3. **Preclinical Behavioral Assessment of Cognition**

The facts that most, if not all, of the disorders discussed above are uniquely human represent a significant challenge for drug discovery efforts to identify therapeutically relevant cognitive enhancing agents. While it is relatively simple to ask a human subject if they recall a particular word or number, rodents and nonhuman primates are typically less cooperative. In addition, preclinical species do not have the capability for certain aspects of cognitive function, especially those involving language. The importance of assay and model selection and careful interpretation of results is underscored by a large increase in preclinical studies reporting cognitive enhancement in animal models that is not reflected in the paucity of new therapeutics emerging from clinical trials, a relationship that may be in part due to an underappreciation of the complexity of measuring cognitive function in preclinical species [32]. Despite these hurdles, a number of assays have been developed that allow a reliable and informative assessment of cognition in both rodents and nonhuman primates.

1.3.1. **Disease Models** The validity of an animal model for any disorder can be rated on three scales; predictive, construct, and face validity. *Predictive validity* focuses on how well results produced in the animal model are born out in the clinic. More often, animal models are back-validated using clinical benchmarks to provide a basis for arguing for future predictive validity. This is particularly problematic in the field of cognition because there are few approved drugs, and those that are approved have modest efficacy [85]. *Construct validity* concerns the theoretical rationale underlying the model. A model with a high degree of construct validity would disrupt the same neurotransmitter systems and engage the same neuronal circuitry as the human disorder. Unfortunately, understanding of the underlying pathophysiology of cognitive dysfunction is far from complete, suggesting that construct validity is difficult to ascertain. *Face validity* is a measure of how accurately the model reproduces the symptoms of the human disorder. As noted, face validity can be a challenge because there are certain aspects of cognitive function that are not expressed in preclinical species.
Because of the problems with model validity, and construct validity in particular, animal models of cognitive dysfunction typically model some aspect of a disorder rather than recapitulate the human syndrome. For example, attempts at generating an AD model by genetically recreating alterations in the amyloid system have recapitulated certain aspects of the disease including cognitive dysfunction and altered synaptic plasticity [86], the approach has failed to produce a mouse that faithfully produces plaque deposition, tangle formation, neurodegeneration, and cognitive dysfunction and recent data have questioned the amyloid hypothesis [11].

A large number of genetic and pharmacological models of cognitive dysfunction exist. Some commonly used models include scopolamine-induced impairments to mimic cholinergic dysfunction in AD [87] and phencyclidine (PCP) 8-induced impairments thought to replicate NMDA-hypofunction associated with schizophrenia [88]. A major caveat to the use of these models in drug discovery is the particular pharmacological or genetic insult used to disrupt function may not be involved in the clinical pathology, and even if it is, may not be the sole cause of cognitive dysfunction. Therefore, a risk exists that an NCE will be developed with utility restricted to treatment of the animal model. Perhaps the best path for developing novel cognitive enhancers with broad therapeutic potential is lies in testing NCEs for cognitive enhancing effects in normal animals, or in aged animals exhibiting age-associated cognitive impairment. The use of such models requires careful attention to verifying both the presence and the function of the target in the disease state.

1.3.2. Preclinical Cognitive Assays As mentioned above, there are aspects of human cognition such as verbal memory and fluency that cannot be modeled in preclinical species. Furthermore, due to the complexity and variability inherent in animal studies, the assessment of long-term memory is typically limited to a simple increase in intertrial time that occurs on a time scale on the order of hours to days rather than weeks or years. Below some of the assays more commonly used in drug discovery research are described. While the focus is on a basic overview of the tasks, it is important to keep in mind that these assays exist in multiple forms and often are conducted with significant procedural differences between laboratories. Most of the assays described can be used with short or long intertrial intervals to assess both short- and long-term memories, and can be employed to assess function in genetically or pharmacologically impaired models. Furthermore, by varying the timing of treatment, it is often possible to tease out the aspect of memory formation impacted by the treatment such as acquisition or consolidation.

**The 5-Choice Serial Reaction Time Task as a Measure of Attention and Impulsivity** In the clinic, attention, and impulsivity are typically measured using the continuous performance task (CPT) in which the subject is asked to attend to a stream of stimuli and respond to an infrequent target (e.g., the number 9) while withholding response to irrelevant distractors (e.g., the numbers 0–8). The 5-choice serial reaction time task (5-CSRTT) has been developed as a preclinical analog of the CPT that allows the assessment of attention and impulsivity in rodents [89]. In its simplest form, the task requires the animal to attend to five small openings in the wall of a test chamber and wait for one of the openings to become illuminated. The animal responds to the illumination with a nose poke into the appropriate opening and receives a food reward. The openings are illuminated in a random fashion, and the animal is required to respond within approximately 1 s. Errors may be measured as incorrect responses in which the animal pokes the wrong opening, misses in which the animal fails to respond, or premature responses in which the animal responds before the illumination. An error produces a short period of darkness (time-out) that serves as a negative reinforcement. Treatments that enhance attention would be expected to decrease the number of errors, with premature response rate serving as an indication of impulsivity.

The 5-CSRTT has been used to assess the proattentive properties of a number of clinical and preclinical compounds including the psychostimulant methylphenidate 9, the
selective norepinephrine uptake (NET) inhibitor atomoxetine 10 [90], the H₃ histamine receptor inverse agonist ciproxifan 11 [91], and the α₄β₂ nicotinic agonist ABT-418 12 [92].

**Social Recognition and Novel Object Recognition as Measures of Working Memory** Recognition memory has become an increasingly popular way of assessing the effect of cognitive enhancing agents in rodents. These methods rely on the animal’s natural curiosity regarding a novel stimulus. The social recognition assay is based on the finding that social recognition memory of adult rats for juvenile rats decreases as the time interval between presentations of the same juvenile rats to adult rats is increased [93]. The assay is typically performed by exposing an adult rat to a juvenile and measuring the time the adult spends investigating the juvenile during a short (3–5 min) trial. Reexposure to the same juvenile after a short intertrial interval (5–15 min) will result in significantly decreased investigation time suggesting that the adult rat remembers and recognizes the juvenile. Longer (>2 h) intertrial intervals produce investigation times similar to those observed on the initial presentation suggesting a lack of memory for the juvenile. Administration of a test compound that improves working memory would be expected to decrease the investigation time observed after a long intertrial interval.

In the novel object recognition paradigm, the animal is exposed to two objects and exploration is assessed as in the social recognition assay. After an appropriate intertrial interval, the animal is reexposed to one of the original objects plus a novel object. The animal’s memory of the familiar object is reflected in an increased duration of exploration of the novel object [94]. As with social recognition memory, longer intertrial intervals lead to a loss of memory that can be rescued with the administration of cognitive enhancing agents.

A large number of preclinical and clinical compounds produce promnesic effects in recognition memory models including the histamine H₃ receptor inverse agonist GSK189254 13 [95], the cholinesterase inhibitor tacrine 3 [96], and the ampakine CX-546 14 [97].

**The Morris Water Maze as a Measure of Spatial Memory Working Memory** One of the most commonly used memory tasks in preclinical drug discovery research is the Morris water maze (MWM) [98]. The assay employs a large circular pool filled with opaque water in which a small escape platform is hidden. A rat or mouse placed in the pool for the first time will swim and randomly encounter the escape platform. In the simplest form of the assay,
the animal learns over successive trials to employ spatial cues to find the platform resulting in shorter escape latencies. Treatments that enhance spatial memory are expected to accelerate the learning process such that the escape latency decreases significantly with fewer trials. Importantly, swim speed should be measured and should not vary with treatment to avoid assigning cognitive enhancing properties to an agent that simply produces hyperlocomotion. Similar information on spatial learning and memory can be obtained using other maze-based assays, for example, the radial arm maze [99], however, these methods are more complex involving the use of food reward while the MWM relies on the animals native escape response. A large literature exists describing the effects of pharmacological treatment on learning in the MWM. A number of clinical and preclinical compounds are effective in this model including the cholinesterase inhibitors donepezil 4 and rivastigmine 5 [100], the 5-HT₆ receptor antagonist SB-271046 15 [101], and the α₇ nicotinic receptor positive allosteric modulator, NS1738 16 [102].

**Conditioned Fear as a Measure of Contextual Memory** A number of conditioned fear assays have been employed in drug discovery research as a test for cognitive enhancement. The most common variants of these methods are the passive avoidance task [103] and contextual freezing [104]. In the passive avoidance task, the animal is placed in a chamber that is divided between an open illuminated side and an enclosed dark side. Rodents have a natural preference for dark spaces, so the will quickly move into the dark side of the chamber. Entry into the dark side of the chamber is accompanied by a foot shock. On subsequent trials, the latency to enter the dark chamber increases as the animal learns to associate the shock with the context of the dark compartment. Contextual freezing is measured by placing the animal in a box with obvious contextual cues, and administering a foot shock. The animal is later placed in the same context and the amount of time the animal remains immobile (freezes) is recorded. This freezing behavior is a natural response in expectation of receiving a foot shock. Therefore, the time spent in the frozen posture is related to the memory for the context in which the shock occurred. Conditioned fear assays are relatively facile, and therefore in common use in drug discovery research. However, it must be noted that these assays are primarily measuring fear and emotional memory processed through the amygdala that may not represent other forms of memory. Pharmacological manipulations improve conditioned fear memory including the cholinesterase inhibitor physostigmine 17 [105], the 5-HT₁A antagonist, WAY-100635 18 [106], and the PDE4 inhibitor, rolipram 19 [107].

**Set shifting as a Measure of Executive Function** A commonly used clinical test of executive function is the Wisconsin card sorting test...
The test employs a series of cards containing shapes (stars, circles, triangles, etc.) present in different number and in different colors. The subject is asked to sort the cards and is rewarded for discovering the appropriate sorting rule (e.g., sort by shape). The experimenter then changes the rule without telling the subject. The ability to shift set to the new rule is considered a measure of cognitive flexibility. Patients with deficits in prefrontal executive function, for example, those with schizophrenia, will exhibit difficulty shifting set, and will make numerous perseverative errors [109]. The rodent analog of the WCST is termed attentional set shifting [110]. In this assay, rodents are trained to dig in one of the two containers to obtain a hidden food reward. The reward may be paired with a particular odor (e.g., lavender), or a particular digging medium (e.g., sawdust). Once an animal learns to correctly identify the rewarded container, the rules are changed, and the animal’s performance is measured. The change may be an intradimensional shift (e.g., the correct container was associated with lavender and is now associated with a clove odor), or extradimensional (e.g., the correct container was associated with lavender and is now associated with shredded paper). The experimenter can then measure the number of trials needed for the animal to learn the new rule. Compounds active in improving attentional set shifting include modafinil 20 [111], the 5-HT6 receptor antagonist, SB-271046 15 [112], and the nonselective PDE10 inhibitor, papaverine 21 [113].

2. SMALL-MOLECULE APPROACHES TO COGNITIVE ENHANCEMENT

2.1. Historical

Amyloid plaques, neurofibrillary tangles, and neuronal loss characterize AD. This loss of neurons produces insufficiencies in several neurotransmitter systems; one of the more prominent is the loss of cholinergic neurons in the basal forebrain, which project into the hippocampus and cortex, brain regions that play an important role in memory and cognitive function. Loss of cholinergic neurons results in up to 90% reduction in the activity of choline acetyltransferase (ChAT) needed for synthesis of ACh. These findings led to the cholinergic hypothesis of AD, some 50 years old, that the dementia resulted from cholinergic dysfunction and/or loss [114,115]. This hypothesis was supported with animal studies of cholinergic dysfunction that impaired learning and memory tasks. Although the exact relationship between the cholinergic neuron loss and dementia is not well understood, it is accepted that the cholinergic system is involved in cognitive (attention and memory)
and noncognitive (apathy, depression, psychosis, sleep disturbances, aggression) processes of AD. Patients with MCI have increased ChAT in frontal cortex and hippocampus at autopsy, suggesting it may be a compensatory mechanism to slow disease progression [116]. Early treatment strategies have therefore focused on cholinergic replacement therapy with the development of cholinesterase inhibitors (ChEIs) and cholinomimetic agents.

### 2.1.1. Cholinesterase Inhibitors

Cholinesterase (ChE) enzymes function to hydrolyze and terminate the action of ACh at postsynaptic sites. There are two forms of the enzyme, acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE, also known as pseudocholinesterase), with different CNS and PNS localization. Both forms are found in the brain, although AChE represents approximately 95% of the ChE activity in the normal human brain and colocalizes with cholinergic synapses. BuChE is in lower abundance in brain, primarily in glial or satellite cells, and is virtually absent in neurons. BuChE is synthesized in the liver and highly distributed in the plasma, hematopoetic cells, intestine, liver, heart, and lung. Although AChE selectively hydrolyses ACh, BuChE hydrolyses ACh, in addition to multiple xenobiotic esters. Two forms of AChE exist in the brain, a tetrameric extracellular membrane-anchored G4 isoform, and a monomeric, intracellular cytoplasmic G1 isoform [117]. During AD progression, levels of AChE decrease to approximately 10–15% with preferential loss of the G4 isoform, while BuChE increases to approximately 120%. With the preferential loss of the G4 isoform of AChE, an ideal AChEI for treating AD may require high specificity for the G1 form. Inhibition of AChE results in accumulation of ACh in the synapse, thus producing effects equivalent to enhanced stimulation of cholinergic receptors. The AChEIs currently approved for treatment of mild to moderate AD include donepezil 4, rivastigmine 5, and galantamine 6 (Table 1). The initially approved AChEI, tacrine 3, has been removed from the market and replaced by the newer compounds due to modest efficacy, significant side effects and hepatotoxicity.

**Tacrine** Tacrine 3 was the first generation AChEI being approved in 1993 for treatment of mild to moderate AD. It is a reversible, noncompetitive inhibitor with similar potency for AChE and BuChE and the G1 and G4 isoforms. Tacrine also displays multiple biochemical activities, including interaction with potassium channels, inhibition of histamine N-methyltransferase, and weak blockade of muscarinic receptors. The use of tacrine was limited by poor oral bioavailability, poor pharmacokinetics (qid dosing), and limiting adverse drug reactions (nausea, diarrhea, urinary incontinence, hepatotoxicity) such that few patients could tolerate therapeutic doses, which led its withdrawal from the market [118].

<table>
<thead>
<tr>
<th>Chemotype</th>
<th>Donepezil</th>
<th>Rivastigmine</th>
<th>Galantamine</th>
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<tbody>
<tr>
<td>Inhibition</td>
<td>Piperidine</td>
<td>Carbamate</td>
<td>Alkaloid</td>
</tr>
<tr>
<td></td>
<td>Reversible, noncompetitive; AChE &gt; BuChe</td>
<td>Pseudo-irreversible, noncompetitive; AChE &gt; BuChe</td>
<td>Reversible competitive; AChE &gt; BuChe; nAChR</td>
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<tr>
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<td>5–10</td>
<td>6–12</td>
<td>16–32</td>
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<tr>
<td>Dosage interval</td>
<td>qd</td>
<td>bid</td>
<td>bid</td>
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<tr>
<td>Plasma half-life (h)</td>
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<td>1–1.5</td>
<td>40</td>
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<tr>
<td>Plasma protein binding (%)</td>
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<td>10–20</td>
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<tr>
<td>Metabolism</td>
<td>CYP2D6, CYP3A4</td>
<td>Nonhepatic esterases</td>
<td>CYP2D6, CYP3A4</td>
</tr>
<tr>
<td>Route of elimination</td>
<td>Parent and metabolites in urine</td>
<td>Metabolites in urine</td>
<td>Unchanged parent in urine</td>
</tr>
<tr>
<td>Improvement in ADAS-cog scale</td>
<td>2.9</td>
<td>4.9</td>
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</table>
**Donepezil**
Donepezil 4 is a benzyl piperidine-based reversible, noncompetitive inhibitor approved in 1996 for treatment of mild to moderate AD and dementia. It had greater than 570-fold selectivity for human AChE over BuChE and greater potency for brain over peripheral AChE with no inhibition in cardiac and smooth muscle. It had a long elimination half-life (70 h), excellent brain permeability, and oral bioavailability (%F = 100) with no hepatotoxicity. Donepezil is metabolized in the liver by CYP2D6 and CYP3A4 and thus has potential for drug–drug interactions with drugs such as fluoxetine and paroxetine. In clinical efficacy and safety studies evaluating both short- and long-term effects, donepezil showed significant improvement in ADAS-cog measures (2.49 at 5 mg; 2.88 at 10 mg) and the CIBIC-plus rating compared to placebo [119]. The double blind study was followed by a blind washout phase that found, on all measures, a decline to values not different from placebo, indicating the treatment was symptomatic in nature. The most serious issues noted were insomnia, agitation, nausea, and leg cramps with GI disturbances. In addition to increasing ACh levels, donepezil may also affect cellular and molecular processes involved in early stage AD pathogenesis [120].

**Rivastigmine**
Rivastigmine 5 is a pseudo-irreversible carbamate type AChEI approved in 2000 for the treatment of mildly to moderately severe AD. It is also approved for the treatment of Parkinson’s dementia. Rivastigmine is a centrally and brain region selective inhibitor that facilitates cholinergic activity in the cortex and hippocampus [121]. Increased levels of ACh were seen with rivastigmine without changes in levels of NE, 5-HT, DA, and their metabolites. Rivastigmine showed equal activity for AChE and BuChE, and displayed greater potency for the G1 versus the G4 isoform of the enzyme. Since rivastigmine is metabolized by esterases rather than liver cytochrome P450 enzymes, there is minimal potential for drug–drug interactions. It had a short plasma half-life (1–1.5 h), although the pharmacodynamic half-life (10 h) was much longer due to the pseudo-irreversible, slow dissociation from the enzyme. Pharmacokinetic/pharmacodynamic results showed a 6 mg dose reduced AChE activity by 50% for up to 7 h postdosing [122]. Preclinical studies also showed that rivastigmine had selectivity for central versus peripheral AChE. In the clinic, rivastigmine (6–12 mg/day) was efficacious as assessed by the ADAS-cog, CIBIC-plus and activities of daily living scale compared to placebo. No blood pressure changes were observed, and the most serious adverse events reported were cholinergic side effects, agitation, nausea, anorexia, and GI disturbances.

**Galantamine**
Galantamine 6 is a tertiary amine alkaloid from the bulbs of the Caucasian snowdrop, *Galanthis woronowi*, approved in 2001 for treatment of mild to moderate AD. Galantamine has a unique dual mechanism of action combining reversible, competitive inhibition of AChE, with positive, albeit weak, allosteric modulation of the nicotinic acetylcholine receptor (nAChR) [123]. It is the only actively marketed drug approved for AD that has demonstrated both mechanisms of action. Galantamine has been proposed to increase ACh levels and to facilitate glutamate, 5-HT and norepinephrine release in key brain regions involved in cognition [124]. In healthy volunteers, galantamine was rapidly absorbed after oral dosing and showed high oral bioavailability (%F = 100) and a plasma half-life of 7 h. The plasma protein binding was low (10–20%) with no accumulation of drug observed after 2–6 months of dosing. Galantamine is metabolized by CYP2D6 and CYP3A4, and thus has the potential for drug–drug interactions. In double-blinded placebo-controlled clinical studies, galantamine showed improvements in ADAS-cog, the disability assessment for dementia (DAD), and the AD cooperative study/activities of daily living (ADCS/ADL) [124].

**Miscellaneous AChEIs**
Only huperzine A 22, an alkaloid isolated from the Chinese moss *Huperzia serrata* that has been used for centuries in Chinese folk medicine, is currently in clinical trials [125]. It is a reversible AChEI with additional weak NMDA antagonist activity. Phenserine 23, a dual AChE/β-amyloid (Aβ) protein inhibitor, had been in phase III trials to evaluate its potential to lower levels of β-amyloid precursor protein (β-APP) and Aβ levels in patients with mild to moderate
Alzheimer’s disease [126]. Clinical development of this compound was halted when results from a phase III trial showed no benefit over placebo in the primary efficacy endpoints. As noted retrospective meta-analyses of clinical trials has indicated that none of the ACHEIs works especially well in treating the symptoms of AD [8].

AChEIs in Schizophrenia Current marketed AChEIs have modest benefit on cognition and global functioning but do not alter the course of the disease, raising concerns about their cost-effectiveness [127,128]. AChEIs have been evaluated for cognitive enhancing activities in schizophrenia patients in trials to broaden clinical indications [129,130]. In double blind placebo-controlled clinical trials, donepezil 4 and rivastigmine 5, failed to demonstrate a benefit in treating cognitive deficits, while galantamine 6 did exert a benefit for processing speed and verbal memory, but failed to show a significant difference in global composite score [131]. Galantamine 6 differs from donepezil 4 and rivastigmine 5 in its dual mechanism; it acts also as a positive allosteric modulator of both α7 and α4/β2 nAChRs [123]. The allosteric nicotinic properties of galantamine 6 could lead to increased release of ACh or indirectly affect cognition through effects on glutamate and DA. Galantamine enhanced DA release in prefrontal cortex and hippocampus via allosteric modulation of nAChRs [132].

2.1.2. Muscarinics The muscarinic receptor family is composed of five GPCRs, M1–M5. M1, M3, and M5 receptors are coupled with Gq linked to phospholipase C and are associated with an increase in intracellular Ca2+. M2 and M4 subtypes are associated with Gi subunits coupled to adenyl cyclase. The M1 and M2 receptor subtypes are involved in cognition. M1 receptors are postsynaptic and abundant in cortex and hippocampus and M2 receptors are presynaptic inhibiting ACh release. At least 12 putative M1 receptor selective agonists have advanced to the clinic. However, due to the broad tissue distribution of M1 receptors and dubious selectivity and efficacy, a plethora of undesirable cholinergic side effects have limited the utility of these NCEs. Xanomeline 24, the most widely studied M1 agonist had good M1/M4 potency but modest functional selectivity over M2 and M3 receptors [133]. The M1 receptor was associated with the procognitive effects of xanomeline, while the M4 subtype may be responsible for its antipsychotic-like actions [134]. Clinical data with xanomeline reported cognitive improvements, but with a high incidence of cholinergic side effects that led to considerable patient dropout and discontinuation of the clinical program [135]. Cevimeline (AF-102B) 25 is an M1/M3 agonist originally studied for the treatment of AD. It later gained approval for symptoms of dry mouth in patients with Sjögren’s syndrome, an autoimmune rheumatic disease [136]. Alvameline (LU-25-109T) 26 had higher affinity and a unique M1 agonist/M2 antagonist profile, but was discontinued due to adverse events including salivation, dizziness, GI disturbances, and cardiovascular side effects [137]. AF267B (NGX267) 27 improved cognitive symptoms, cholinergic markers, and tau hyperphosphorylation in vivo and reportedly advanced into phase I for cognitive impairment in schizophrenia and for the treatment of AD dementia. It is currently in Phase II for treatment of xerostomia. In vitro, M1 agonists including AF267B increase secreted APP and decrease Aβ levels and tau hyperphosphorylation [138]. Norclozapine (ACP-104) 28, the desmethyl
metabolite of clozapine 29, a partial agonist at M₁ and M₅ receptors failed in Phase II clinical trials for cognitive deficits in schizophrenia [139]. Due to the high sequence conservation in the M₁–M₅ orthosteric binding site, recent interest has focused on allosteric M₁ agonist to achieve improved selectivity and side effect profiles [140,141]. The positive allosteric modulators, VU0090157 30 and VU0029767 31 were identified using a functional screen and function by potentiation of agonist activation at the M₁ orthosteric site [142].

2.1.3. NMDA Modulators Memantine Glutamate is the main excitatory neurotransmitter in the human brain and exerts its effects via a number of receptors, including the NMDA subtype. Under normal conditions, NMDA receptor activation results in long-term potentiation of neuronal activity, a process believed to be the basis of learning and memory [60]. In neurodegeneration of AD, an increase in extracellular glutamate leads to excessive activation of NMDA receptors with consequent deficits in cognitive function and ultimately, neuronal death. Memantine 7, an orally active, weak uncompetitive NMDA receptor antagonist, was approved in 2002 for treatment of moderate to severe AD [7]. Memantine binds to the open channel state of the NMDA receptor and blocks tonic pathological activation induced by excessive glutamate concentrations. Preclinically, it was shown to permit physiological activation of the NMDA receptor while protecting against cytotoxicity under conditions of chronic glutaminergic stimulation [143]. The clinical efficacy of memantine has been questioned [8] and it is increasingly being used as an adjunct to ChEI therapy rather than replacement.
2.1.4. Nootropics  The term nootropic was coined by Giurgea in 1972 from the Greek noos (mind) and tropos (turn) to describe the properties of the first substance, piracetam \(1\), which had positive effects in the treatment of memory loss, age related memory decline and lack of concentration. Piracetam, which has no known mechanism of action, stimulated the design and synthesis of a large number of structural “acetam” analogs that had a similar pharmacological profile with modest clinical benefit. Nootropics have been proposed to work through modulation of AMPA, NMDA, and cholinergic signaling and the preclinical \textit{in vivo} effects are normally associated with conditions of impaired brain function such as aging, hypoxia, glucose deprivation, injury, or neurodegeneration, for example, normal healthy animals show little benefit from treatment with nootropics such as piracetam [5]. Studies have suggested effects on membrane fluidity and mitochondrial dysfunction could explain the effects of piracetam [144]. Aniracetam \(32\) was reported as more potent than piracetam, with essentially no side effects. In a 10-patient clinical trial, it demonstrated enhanced vigilance based on EEG analysis similar to piracetam (2 g p.o.). Oxiracetam \(33\), an analog of piracetam reversed scopolamine-induced deficits in the radial arm maze in rats, but failed to show benefit in AD patients and produced insomnia, agitation, headaches, and occasional GI upsets in the clinic [5]. Nebracetam (WEB-1881 FU) \(34\) blocked scopolamine-induced disruption of spatial cognition at 10 mg/kg p.o. and enhanced oxotremorine-induced tremors, indicating a cholinergic enhancing mechanism. It also decreased the \(\Delta^8\)-tetrahydrocannabinol-induced disruption of spatial cognition, suggesting additional limbic and hippocampal noradrenergic mechanisms in its cognition enhancing profile [145]. It was abandoned in Phase III. Levetiracetam \(35\), the \(S\)-enantiomer of etiracetam, an antiepileptic approved in the European Union, significantly improved cognitive function and QoL in patients with refractory partial seizures based on performance time on the WCST and scores on cognitive and social function [146,147].

2.2. Emerging Targets

2.2.1. Neuronal Nicotinic Agonists  Neuronal nicotinic acetylcholine receptors (nAChRs) are pentameric ligand-gated cationic channels, highly expressed in the CNS. Twelve subunit genes, designated \(\alpha_2-\alpha_{10}\) and \(\beta_2-\beta_4\) have been identified, with five additional subunits expressed in the peripheral nervous system (\(\alpha_1, \beta_1, \gamma, \delta, \) and \(\epsilon\)). Various subunits can combine to provide a diversity of receptor subtypes with unique brain and neuron-specific distribution [148,149]. The \(\alpha_7\) and \(\alpha_4/\beta_2\) subtypes are involved in cognition and attention, and are highly expressed in brain regions involved in learning and memory, including the hippocampus, thalamus, and cortex [150,151]. nAChRs are involved directly and indirectly in release of neurotransmitters including ACh, dopamine (DA), glutamate, and norepinephrine (NE), and in AD and schizophrenia, levels of cortical nAChRs are decreased [152]. In schizophrenia, a link between loss of nAChRs and sensory gating deficits has been proposed as a self-medicating phenomenon for the higher incidence of cigarette smoking in schizophrenia patients. One neurophysiological abnormality in schizophrenia patients, P50 auditory gating deficit, indicates an impaired information processing
and a diminished ability to filter unimportant information or repetitive sensory information. These deficits can be normalized by nicotine and studies indicate that the \( \alpha_7 \) subtype is involved in the cognition [153]. Nicotine is not selective and its clinical utility was limited by side effects including seizures, irregular heartbeat, hypertension, and GI effects including nausea. DMXB-A (GTS-21) is a weak \( \alpha_7 \) partial agonist, had preclinical efficacy in rodent models of auditory gating [154]. An initial proof-of-concept trial in schizophrenia involving single-day administration showed positive cognitive effects on attention [155]. A second clinical trial with 4-week administration (75 and 150 mg bid) showed significant improvement on the negative symptoms but not in the cognitive battery assessment [156]. Adverse events included mild tremor and nausea. DMXB-A generates several active metabolites in humans that may contribute to a mixed pharmacological profile. A-582941 is a pyridazine \( \alpha_7 \) partial agonist with good psychopharmacological and pharmacokinetic properties, including CNS penetration. It had good efficacy in several rodent and primate cognition models thought to reflect memory and learning [157]. SSR-180711 is a partial \( \alpha_7 \) agonist that advanced to Phase II, displayed high affinity for rat and human \( \alpha_7 \) (22 and 14 nM, respectively) with 330-fold selectivity over other \( nAChRs \). In vivo it was active in object recognition memory with no tolerance, in the MWM, and reversed MK-801 deficits on short-term memory and novelty learning [158]. TC-5619 is a moderately potent \( \alpha_7 \) agonist, is one of the four additional NCEs where the structure is known reported to be in Phase I for treatment of CDS. The selective \( \alpha_7 \) partial agonist JN403 showed rapid CNS penetration after oral administration in mice and rats and was active in the social recognition test over a broad dose range [159]. The urea analog, NS-1738, is a positive allosteric modulator of the \( \alpha_7 nAChR \), has shown cognition-enhancing activity [160]. NS-1738 was unable to activate \( \alpha_7 nAChR \) alone. It effectively enhanced ACh-evoked currents in cells transfected with human \( \alpha_7 nAChR \) as well as in rat hippocampal neurons. NS-1738 showed little effect on desensitization kinetics of \( \alpha_7 nAChRs \). In rats, NS-1738 (10 and 30 mg/kg i.p.) improved cognitive performance in the social recognition test and reversed the scopolamine-induced performance impairment in the MWM (MED = 30 mg/kg i.p.).

Varenicline is a partial agonist/\( \alpha_7 \) full agonist launched for treatment of smoking cessation. It reversed nicotine withdrawal-induced deficits in learning and memory preclinically, and improved mood and cognition during smoking abstinence in the clinic [161,162]. Varenicline is being evaluated in Phase III for CDS. The full \( \alpha_7 \beta_2 \) agonist ABT-418 showed efficacy in acute studies in AD patients, but failed to show efficacy in a double blind, placebo-controlled AD trial. Ispronicline (TC-1734; AZD-3480) showed preclinical activity in several models of cognition (step through passive avoidance, object recognition, radial arm maze) and was well tolerated up to 320 mg in Phase I, but similarly failed to show benefit in Phase IIb [164]. The pyridyl ether ABT-089 and ABT-894 are \( \alpha_7 \beta_2 \) agonists that advanced to Phase II for cognitive impairment in AD and ADHD, respectively. ABT-089 was effective in preclinical models of impaired cognitive function, including aging, septal lesion, and scopolamine-induced deficits in the MWM. Clinically, it showed positive signs of cognitive activity in a reaction time test [165]. ABT-894 was efficacious in an adult ADHD Phase II trial, comparable with atomoxetine. The primary endpoint was the total score of the Connors Adult ADHD Rating Scale (CAARS). Overall, progress in this field continues to be slow despite years of research, due mainly with the lack of efficacy and selectivity-related side effects including emesis, motor dysfunction, and hallucinations. Current drug discovery directions are focused on positive allosteric modulators with the goal of improved selectivity and avoiding agonist-induced receptor desensitization [166].

### 2.2.2. Histamine

The histamine GPCR family consists of four members: \( H_1 – H_4 \). \( H_3 \)Rs are expressed predominately in the brain, localized to the cerebral cortex, amygdala, hippocampus, striatum, thalamus and hypothalamus, where they are expressed on presynaptic terminals and function as inhibitory auto- and heteroreceptors [167]. \( H_3 \) antagonists increase
the release of various neurotransmitters, including histamine, ACh, NE, 5-HT, and DA, and have potential utility in treating cognitive deficits associated with various dementias and schizophrenia [168]. H₃R knockout mice and H₃R inhibitors demonstrate enhanced learning and memory in various animal models of cognitive function. A nuance of the H₃R and its ligands is the high degree of constitutive activity in vitro and in vivo [169].

The search for H₃ antagonists with drug-like properties has focused exclusively on amine-based compounds as NCEs with reduced side effect liabilities have been identified and advanced into clinical evaluation.

ABT-239 46 is currently the most widely studied H₃R inhibitor [168]. It is a potent H₃R inverse agonist and effective at low doses (0.1 mg/kg sc) in a repeat trial inhibitory avoidance task, the rat social recognition model of short-term memory and in a water maze model [170]. Although ABT-239 had an impressive in vivo profile for cognition enhancement, its development was halted due to cardiovascular liabilities [168]. GSK189254 13 had high affinity for recombinant human H₃R (Kᵢ = 0.9 nM) and for rat H₃R blockade in vivo (ID₅₀ = 0.05 mg/kg p.o.), with greater than 10,000-fold selectivity for H₃ versus other receptors [95]. GSK189254, currently in
Phase II, was efficacious preclinically across a panel of models designed to test different cognitive domains in rodent at 0.3–3 mg/kg p.o., reversing scopolamine-induced deficits in passive avoidance tasks, improving performance of aged rats in a water maze model and improving memory in an object recognition task. GSK239512 (undisclosed structure) is in Phase II for the treatment of AD. BF2.649 47, despite its unusual pharmacokinetics and safety profile is in late stage clinical trials for various indications, including cognitive enhancement [168]. Quinazolone 48 is a compound of further interest from Merck [171]. H3 antagonists continue to be an area of intense interest for drug development as the field awaits clinical data [168].

2.2.3. Serotonin The serotonin (5-hydroxytryptamine; 5-HT) 49 system originates from the raphe nucleus in the mid- and hindbrain regions and projects to virtually all brain regions, including the cortex, hippocampus, amygdala, hypothalamus, and thalamic nuclei. There are presently 14 5-HT receptor subtypes, some of which exist as multiple splice variants that are classified into 7 families according to their primary structures, signal transduction coupling, and pharmacology [172,173]. Except for the 5-HT3 subtype, which is a ligand-gated cation channel, 5-HT receptors are GPCRs. While all subtypes have been linked to learning and memory, the 5-HT1A, 5-HT2A, 5-HT4, 5-HT6, and 5-HT7 subtypes are current targets of interest for drug discovery and have resulted in several clinical candidates for cognition [173,174].

5-HT1A 5-HT1 receptors are grouped into five major subtypes and are negatively coupled to adenylyl cyclase via Goi [172,176]. Evidence suggests that 5-HT1A antagonists may reverse the cognitive deficits seen in AD [176]. 5-HT1A receptors are most highly concentrated in cortical and hippocampal pyramidal neurons and provides inhibitory tone to cholinergic and glutamatergic neurons. 5-HT1A antagonists facilitate glutamate and cholinergic transmission [172–175]. Serotonergic neurons also provide inhibitory tone to the cortical pyramidal pathway via 5-HT1A receptors. Thus, 5-HT1A antagonists may reverse AD-associated cognitive deficits both by enhancing excitatory cholinergic and glutamate neurotransmission and by blocking direct inhibitory 5-HT input [176]. 5-HT1A antagonists facilitate glutamatergic activation and signal transduction by blocking the hyperpolarization and Ca2+ flux induced by inhibitory 5-HT tone. Except for clozapine 29, antipsychotics have mixed results on CDS [177,178]. The atypical antipsychotics aripiprazole, clozapine, olanzapine, and quetiapine are partial 5-HT1A agonists while risperidone 50 and sertindole 51 are full antagonists [174]. WAY-100635 18 was the first well-characterized, selective 5-HT1A antagonist [179]. The first clinical compound from this series, lecozotan 52, was a potent full antagonist with greater than
100-fold selectivity against 50 other receptors except the D4 DA receptor, and was active in several models of learning and memory [180,181]. It completed a Phase II/III study in combination with donepezil in patients with mild to moderate AD. The ability to distinguish full 5-HT$_{1A}$ antagonists from partial agonists in the assay systems has been problematic and remains a key issue in developing antagonists.  

5-HT$_{2A}$ Postsynaptic 5-HT$_{2A}$ receptors are highly localized in cortical pyramidal neurons and may play a role in integrating cognitive and perceptual information [174]. The 5-HT$_{2A}$ receptor is colocalized with the NR1 subunit of the NMDA receptor, suggesting that an antagonist may potentially be beneficial in treating cognition in schizophrenia by normalizing NMDA-receptor function. Limited clinical data on 5-HT$_{2A}$ antagonists are available. The 5-HT$_{2A}$ agonist, psilocybin 53 produced a deficit in a continuous performance test in healthy volunteers. The antagonist, mianserin 54 showed improvement in the ANAM but not the WCST test of cognition in patients stabilized with antipsychotic therapy. M100907 (volinanserin) 55, active in preclinical models of schizophrenia and cognition, and showed fewer errors in the WCST, was discontinued in Phase III.

5-HT$_{4}$ 5-HT$_{4}$ receptors are enriched in the nigrostriatal and mesolimbic regions. They are positively coupled to adenyl cyclase and modulate the release of ACh, DA, GABA, and serotonin. The partial agonist, RS17017 56, improved performance in rodent tests of social, olfactory-associated learning, and spatial memory, and also improved delayed matching-to-sample responses in young and old primates [175]. SL650155 (capeserod) 57, a partial agonist with high affinity for 5-HT$_{4}$ ($K_i = 0.4$ nM) had greater than 100-fold selectivity over other 5-HT receptor subtypes. In vivo, SL650155 improved performance in rodent models of learning and memory including novel object recognition (0.001–0.1 mg/kg)
i.p. or p.o.), the linear maze task in aged rats (0.01 and 0.1 mg/kg i.p.), and the MWM in mice, where it reversed scopolamine-induced deficits at 0.1 and 0.3 mg/kg i.p. SL650155 had a greater than additive effect on cognitive performance in the Y-maze in combination with rivastigmine \(^5\) \([182]\). SL650155 (0.1 mg/kg s.c.) improved performance in the 5-CSRT task \([183]\) and was advanced to Phase II before being terminated. Nonetheless, 5-HT\(_4\) receptors remain an active area in drug discovery, largely due to actions on amyloid deposition, although GI side effects of agonists may ultimately limit their use.

**5-HT\(_6\)** The 5-HT\(_6\) receptor is positively coupled to adenylyl cyclase via Gs with expression almost exclusively in the CNS in the olfactory tubercles, cerebral cortex, nucleus accumbens, and hippocampus. Blockade of 5-HT\(_6\) receptor function increases cholinergic and glutamnergic transmission and \textit{in vivo} cognitive efficacy in rodent behavior models \([174,175]\). Atypical antipsychotics, such as clozapine \(^29\) and olanzapine \(^58\), bind with high affinity as inverse agonists at 5-HT\(_6\) receptors, which coupled with its distribution in key brain areas involved in learning and memory has enhanced interest in identifying clinical candidates for this target \([174,175]\). Early 5-HT\(_6\) receptor antagonists had high affinity and good selectivity, but were very hydrophilic and had poor brain penetration that limited their utility. The preclinical \textit{in vivo} data on SB-271046 \(^{15}\) led to its being the first NCE in clinical trials, but it was discontinued after Phase I due to poor brain partitioning \([184]\). Further efforts led PRX-07034 \(^{59}\), SB-742457 \(^{60}\), and SAM-315 (undisclosed structure) advancing into the clinic. SB-742457 showed clinical proof-of-concept with results from a Phase II trial demonstrating that treatment of patients with 35 mg of SB-742457 for 24 weeks resulted in a significant improvement in global function compared to placebo. A second Phase II trial comparing SB-742457 and donepezil \(^4\) to placebo demonstrated that patients receiving SB-742457 had similar improvements in global function and cognitive function compared to donepezil-treated patients \([185]\). Dimebon \(^{61}\), an orally active NCE approved in Russia as an antihistaminic that has emerged as a novel treatment for AD binds with nanomolar potency to the 5-HT\(_6\) receptor \([186]\). Additionally, it also interacts with 5-HT\(_7\) receptors, butyryl- and acetlycholinesterase, \(\alpha\)-type calcium channels, the mitochondrial permeability transition pore, AMPA and NMDA receptors \([187]\). In a cohort of 183 patients with mild-to-moderate AD, dimebon demonstrated a significant improvement in ADAS-cog and CIBIC-plus scores \([188]\). If these initial findings are substantiated in the second pivotal trial, this compound will represent a major milestone in AD treatment.

**5-HT\(_7\)** The 5-HT\(_7\) receptor is the newest member of the 5-HT GPCR family. In addition to depression, schizophrenia, and migraine, antagonists of the 5-HT\(_7\) receptor may find utility in sleep disorders and cognitive dysfunction, although the role of the 5-HT\(_7\) receptor in learning and memory processes is still under investigation \([189]\). Interest in 5-HT\(_7\) receptors for cognition is based on localization in the brain (thalamus, hypothalamus, and hippocampus, with lower levels in cortex and amygdala) and behavioral pharmacology \([189,190]\). A lack of selective 5-HT\(_7\) ligands has slowed progress with this target. The sulfonamide, SB-258719 \(^{62}\) was one of the first 5-HT\(_7\) antagonists reported. Lead optimization produced SB-269970A \(^{63}\) (\(K_i = 1\) nM), a compound with improved affinity and selectivity. SB-269970A enhanced working and reference memory in a radial arm maze task but had poor pharmacokinetic properties with low oral bioavailability \([191]\). SB-656104A \(^{64}\), a potent and selective 5-HT\(_7\) receptor antagonist had a \(K_i\) value of 2 nM with low affinity for \(\alpha_{1B}\) adrenoceptors (\(K_i = 220\) nM) and greater than 100-fold selectivity over other 5-HT receptor subtypes \([192]\). The 5-HT\(_7\) receptor remains a target of interest in drug discovery for cognition and also as an antipsychotic agent to address positive symptoms of schizophrenia. However, better NCEs are needed for clinical proof of concept.

**2.2.4. Dopamine** Interest in the role of dopamine in cognitive function has focused on its ability to modulate executive function, including working memory, planning, and attention \([193]\). In schizophrenia, positive symptoms are hypothesized to be due to
subcortical hyperdopaminergic function, while cognitive deficits result from hypodopaminergic activity in the prefrontal cortex [194]. Consistent with this view are clinical reports that D1 receptors in prefrontal cortex are upregulated in schizophrenia due to a localized decrease in DA activity, and that D1 antagonists worsen psychotic symptoms.
In monkeys, DA levels in prefrontal cortex increase during working memory in a delayed alternation task and inhibition of prefrontal DA decreases working memory performance [196]. Five distinct DA receptor subtypes are known: D1-like (D1 and D5) and D2-like (D2, D3, and D4). The D1 receptors interact with the Gs complex to activate adenyl cyclase, while D2 receptors interact via Gi to inhibit cAMP production [197]. D1 agonists are of interest as targets for cognition, while D4 agonists have cognitive efficacy in animal models [195,196]. The isochroman, A-68930, and the benzazepine, SKF-81297, the first full D1 agonists reported had procognitive effects in animal models [196]. Dihydrexidine, under evaluation for cocaine dependency, improved cognitive performance in rodents and primates and was under development for treatment of CDS. It is a potent full D1 agonist ($K_i = 5.5$ nM) with modest 11-fold selectivity over D2 receptors and 23-fold over $\alpha_2$ receptors [198]. Although safe and well tolerated in man, poor oral bioavailability and short half-life hindered the advancement of this NCE. Adrogolide (ABT-431/DAS-431), a prodrug of the di-phenol A-86929, reversed working memory deficits associated with chronic antipsychotic drug therapy in primates but was inactive in the MWM. Adrogolide failed in clinical trials for PD and is still being studies for cocaine dependence. The DA D4 agonist, A-412997 was efficacious in a social recognition test of short-term memory and in a 5-trial repeated acquisition inhibitory avoidance model, while the nonselective agonist, PD168077 was active only in short term memory [199]. A-412997 increased ACh and DA levels in the rat medial prefrontal cortex but not in the dorsal hippocampus. A major issue that has plagued advancement of full D1 agonists is receptor desensitization.

2.2.5. Norepinephrine Noradrenergic neurotransmission in the prefrontal cortex plays a key role in attention and cognitive processing [200–202]. Moderate increases in NE levels can enhance cognitive function through activation of postsynaptic $\alpha_2A$ receptors [203]. The $\alpha_2A$ agonists clonidine and guanfacine improved cognitive function in humans [202]. Selective NE reuptake inhibitors (SNRIs) are an additional approach to elevate extracellular levels of NE in the brain. These include reboxetine, approved for treatment of depression in Europe and purportedly under evaluation for treatment of CDS in the United States [204], and atomoxetine, the first nonstimulant approved for use in ADHD. In a phase II study, adjunctive atomoxetine treatment to second-generation antipsychotics showed no improvements on prefrontal cognitive ability and function in schizophrenics [205]. Nicergoline, an ergot alkaloid, had been used to treat symptoms of cognitive decline in elderly patients with cerebrovascular insufficiencies. It has a broad spectrum of activity, including $\alpha_1$-adrenoceptor antagonism, vasodilation and increased arterial blood flow, and enhancement of cholinergic and catecholaminergic neurotransmitter function [206].

2.2.6. Glutamate The excitatory neurotransmitter glutamate mediates its effects via both ionotropic and metabotropic receptors. Ionotropic glutamate receptors (iGluRs) include NMDA, AMPA, and kainate subtypes. The NMDA receptor is a ligand-gated ion channel composed of a combination of two NR1 and two NR2 subunits, and requires concomitant binding of glutamate at the NR2 subunit and glycine or a glycine site coagonist for activation. AMPA receptors mediate fast excitatory transmission in the CNS and exist as hetero- and homotetrameric receptors composed of GluA1-GluA4 subunits, with each subunit comprised of one of the two splice variants. NMDA and AMPA receptors operate in an independent, complementary fashion in controlling excitatory neurotransmission. The AMPA receptor conducts primarily Na$^+$ ions, while NMDA receptors are high conductance, slow activating nonselective cationic channels that are permeable to calcium [61,207,208]. At normal membrane potentials, the NMDA receptor channel is subject to voltage-dependant Mg$^{2+}$ blockade and its opening requires membrane depolarization by AMPA receptors. Thus, increasing AMPA receptor activity can increase NMDA receptor function. As discussed above, NMDA receptor activation is involved in membrane trafficking of AMPA receptors, a
process believed to underlie the basis of neuroplasticity (LTP and LTD) [61].

**Metabotropic Glutamate Receptors (mGluRs)**

Metabotropic glutamate receptors are GPCRs comprised of eight receptor subtypes grouped into three families: Group I (mGluR1, mGluR5), Group II (mGluR2, mGluR3), and Group III (mGluR4, mGluR6–mGluR8). mGluRs have important roles in synaptic activity in the CNS and are targets of current interest in treating schizophrenia and cognitive dysfunction. Group I receptors are linked to Gq and increase phosphatidylinositol turnover via phospholipase C activation to elevate intracellular Ca²⁺. Group II and Group III receptors are located presynaptic, inhibit adenylyl cyclase activity via Gi and modulate glutamate release. The potential for group II
mGluR2/3 receptor agonists to treat positive and negative symptoms of schizophrenia has been established with LY2140023 [75], the orally active prodrug of LY404039 [76] [209]. LY404039 also increased cortical DA turnover in rats, an event predictive of procognitive activity. Conversely, mGluR2/3 antagonists, such as LY341495 [77] reduce memory performance. mGluR5 receptors, in additional to being a potential antipsychotic target, potentiate NMDA receptor currents in a number of brain regions, indicating that activation of this target would result in cognitive enhancement. mGluRs have subtype-specific allosteric sites. Positive allosteric modulation offers several advantages over classically orthosteric competitive agonists, including subtype selectivity and lower risk of toxicity by avoiding agonist overstimulation [210]. The pyrazole, CDPPB [78] was the first sufficiently selective mGluR5 positive allosteric modulator for \textit{in vivo} testing. CDPPB shifted the glutamate-induced Ca\(^{2+}\) increase fourfold with an EC\textsubscript{50} of 20 nM, and reducedamphetamine-induced locomotor activity and normalizedamphetamine-induced disruption of prepulse inhibition. The oxadiazole, ADX-47273 [79] increased novel object recognition and reduced impulsivity in the 5-CSRT test at 1 and 10 mg/kg i.p., respectively [211]. The selective mGluR5 positive allosteric modula-

\textbf{AMPA Potentiators} Positive modulation of AMPA receptors may have therapeutic potential in the treatment of cognitive deficits and potentially avoid many of the issues of direct AMPA receptor activation, for example, seizures, excitotoxicity, and loss of efficacy due to desensitization [207,208]. Ampakines are a drug class that enhance attention, alertness, and facilitate learning and memory by allosteric activation of the AMPA receptor [212]. AMPA receptor potentiators include the pyrrolidone nootropics, for example, piracetam 1 and aniracetam 32, benzothiazides (cyclothiazide 80), benzylpiperidines (CX-516 81, CX-546 14 and CX-691 82), and biarylpropylsulfonamides (LY404187 83 and LY503430 84). These compounds enhance cognitive function in rodents, which appears to correlate with increased hippocampal activity. In addition to directly enhancing glutamatergic synaptic transmission, AMPA receptor activation can increase neurotrophin expression \textit{in vitro} and \textit{in vivo}, which may contribute to the functional and neuroprotective effects of LY404187 and LY503430 [207,208,212]. CX-516 had been in numerous Phase II trials for the treatment of autism, schizophrenia and AD dementia but was discontinued for the treatment of MCI due

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to lack of efficacy. The toxicity of CX-516 limited the ability to achieve dose levels comparable to the efficacious doses in animal studies. CX-691/ORG-24448 (faramptator) is being evaluated as an adjunct therapy for CDS [213]. It improved short-term memory, but appeared to impair episodic memory in a group of 16 healthy elderly volunteers. Side effects included headache, somnolence, and nausea.

**GlyT1** Glycine is a major inhibitory neurotransmitter in the cerebellum, brainstem, and spinal cord, acting via ligand-gated strychnine-sensitive glycine-A receptors. It also acts as a required positive allosteric modulator of glutamate by binding to the glycine-B site on the NMDA receptor, which facilitates glutamate binding to the NR2 subunit of the NMDA complex enhancing excitatory glutamatergic transmission in cortex and hippocampus [214]. The NMDA receptor antagonist PCP mimics the positive, negative, and cognitive symptoms of schizophrenia in man [215]. Glycine was efficacious in improving negative symptoms and some aspects of cognitive dysfunction as an add-on therapy in schizophrenia [216,217]. Furthermore, NMDA and glycine agonists such as d-serine and d-cycloserine improved negative symptoms in schizophrenics undergoing conventional antipsychotic therapy, with apparent decreased EPS/tardive dyskinesia [215]. These studies demonstrated improvements in negative or cognitive symptoms, but not in positive symptoms, possibly due to the presence of the antipsychotic agent.

Blockade of the Type 1 glycine transporter (GlyT1), a member of the sodium/chloride-dependent transporter family is responsible for regulation of synaptic glycine levels. Its distribution mirrors that of NMDA receptor expression, suggesting colocalization with the NMDA receptor. The GlyT2 transporter colocalizes with inhibitory strychnine-sensitive glycine-A receptors. Inhibitors of GlyT1 are either substrate-based (sarcosine series) or nonsubstrate-based compounds [218]. Sarcosine was efficacious as an add-on therapy against positive, negative and cognitive systems of schizophrenia in two trials, but had weak GlyT1 inhibitory activity (IC$_{50}$ = 38 μM) with poor pharmacokinetic properties and brain penetration, thus limiting its clinical utility as a drug candidate. Newer sarcosine analogs include NFPS (ALX-5407), ORG4461, and ORG-25935. Preclinically, ORG-25935 elevated glycine levels and reversed PCP-induced deficits in novel object recognition. It is reportedly in Phase II for the treatment of psychosis. While these potent and selective inhibitors have been instrumental in studying the role of GlyT1 in schizophrenia and cognition, a variety of serious side effects such as ataxia, hypoactivity, and decreased respiration have been observed with sarcosine-based inhibitors in rodents [219]. SSR504734, a selective and reversible nonsarcosine GlyT1 inhibitor had
a human GlyT1 IC\textsubscript{50} value of 18 nM [220]. SSR103800, an analog of SSR504734 (undisclosed structure) is more potent (human GlyT1 IC\textsubscript{50} = 1.9 nM) and reportedly has advanced into the clinic. SSR103800 and SSR504734 were active in the social recognition model and potentiated MK-801- and amphetamine-induced disruption of latent inhibition, models believed to be predictive of positive, negative, and cognitive aspects of schizophrenia, respectively [221]. Two newer nonsarcosine-based NCEs are DCCCyB 93 and GSK1018921 (undisclosed structure) that have advanced into early clinical development.

2.2.7. GABA\textsubscript{A} Receptor \( \gamma \)-Aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the CNS. GABA\textsubscript{A} receptors are pentameric GABA-gated chloride channels, composed of four transmembrane subunits (\( \alpha 1-6, \beta 1-3, \gamma 1-3, \delta, \varepsilon, \theta, \) and \( \pi \)) [222]. Nineteen GABA\textsubscript{A} receptor subunits have been identified, with the majority of receptors in the brain comprising \( \alpha-, \beta-, \) and \( \gamma- \) subunits in a 2:2:1 stoichiometry. Binding of GABA to the GABA\textsubscript{A} receptor can modulate simultaneous binding of various modulators to allosteric sites on the ion channel complex; the most studied is the benzodiazepine (BZ) site. Positive allosteric modulators, for example, diazepam 94, of the BZ site enhance the action of GABA on GABA\textsubscript{A} receptors. Negative allosteric modulators or inverse agonists reduce GABA effects on GABA\textsubscript{A} receptors, whereas agents that block the actions of both positive and negative allosteric modulators are categorized as neutralizing allosteric modulators, for example, the BZ antagonist flumazenil (Ro-151788) 95. BZ site agonists produce their anxiolytic, sedative, anticonvulsant, and cognitive-impairing effects via GABA\textsubscript{A} receptors containing \( \beta- \) and \( \gamma- \) and \( \alpha 1-3 \) or \( \alpha 5 \) subunits. Diazepam 94, which has been used as an anxiolytic, hypnotic, and muscle relaxant for nearly 50 years enhanced the inhibitory effects of GABA and impair learning and memory in man [223]. DMCM 96, a full nonselective inverse agonist
not only enhanced cognitive performance in rats but also produced anxiogenic and proconvulsant activity and altered attentional processing [224]. Pharmacological and genetic research suggests that the α5-subunit-selective inverse agonists may enhance cognition. Mice lacking the α5 gene show improved performance in the MWM, whereas performance in nonhippocampal-dependent learning and anxiety tasks was unaltered compared to wild type [225]. The α5 inverse agonist, L-655708 97 enhanced spatial learning in the MWM [226]. The clinical candidate α-5IA 98 robustly enhanced LTP in mouse hippocampal slices and performed in a rat hippocampal-dependent test of learning and memory. In humans, α-5IA was toxic due to the formation of the hydroxymethyl isoxazole metabolite that precluded its use in long-term studies. In healthy volunteers, α-5IA reversed the memory-impairing effects of alcohol [227]. Pyrazolotriazine 99 had a better preclinical efficacy and safety profile compared to α-5IA, but questions remain regarding its overall BZ subtype selectivity profile. While the field awaits clinical efficacy data to validate the GABA_A subtype selective inverse agonist approach to cognitive impairment, concerns remain regarding sedation, and the potential for proconvulsant activity of α5-inverse agonists.

2.2.8. Other Approaches

Adenosine The neuromodulator adenosine plays a major role in the regulation of synaptic transmission and neuronal excitability in the CNS. Four adenosine GPCRs (A1, A2A, A2B, and A3) are known [228]. A1 and A3 receptors are coupled to the inhibitory G-proteins Gi and Go, and A2A and A2B receptors are coupled to stimulatory Gs proteins. A1 receptors are highly expressed throughout the CNS, including the cortex, hippocampus, and cerebellum—important areas for cognitive function. A2A receptors are localized in the striatum where they are coexpressed with dopamine D2 receptors in GABAergic striatopallidal neurons and play important roles in DA neuromodulation. In contrast, A2B and A3 receptors have low abundance in the brain.

Preclinical pharmacological and genetic studies support the involvement of adenosine receptors in learning and memory [3,229]. Adenosine modulates cognition primarily through A1 receptors, but there is now emerging evidence for a role of A2A receptors. Administration of selective A1 receptor agonists disrupt learning and memory in rodents,
while nonselective antagonists such as caffeine 100 or theophylline 101, or selective A1 (DPCPX 102) or A2A antagonists (ZM241835 103) facilitate rodent learning and memory in diverse behavioral tasks [230]. Clinical results on the cognitive effects of caffeine in nondemented humans were inconclusive [229]. Axaphylline 104, a selective and potent A1 antagonist (Ki = 5 nM) that had 100-fold selectivity over A2A, reversed scopolamine-induced behavioral deficits in rats, increased vigilance and enhanced ACh release in cats. Axaphylline advanced to Phase II for treatment of AD but was discontinued due to its short half-life and the extensive formation of CNS active metabolites.

A2A receptor antagonists such as istradefylline (KW-6002) 105 have been assessed for the treatment of PD acting as indirect DA agonists [230]. They have also shown neuroprotective and cognitive enhancing activity [230]. A2A receptor knockout mice were resistant to motor impairment and MPTP-induced neurotoxicity and had improved spatial recognition memory, while overexpression of A2A receptors resulted in working memory deficits in rats [231]. While istradefylline was not approved for the treatment of PD due to inconclusive Phase III trials, the selective A2A antagonists SCH-420814 106, BIIB014 107, and SYN-115 (undisclosed structure) are in clinical trials for PD and have shown beneficial effects on cognitive-related functions including motivation, attention and reward-related behavior [230]. The dual A1/A2A antagonist ASP5854 108 is under investigation to treat both motor disabilities and cognitive deficits in PD and AD [232]. As a class, the main issues with development of A1 antagonists for CNS diseases have been poor water solubility, pharmaceutical properties, poor brain penetration, and cardiovascular side effects.

Neurotrophic Agents Neurotrophic factors are polypeptides that support the growth, differentiation, and survival of neurons in development and sustain neurons in the mature adult nervous system. Nerve growth factor (NGF) has selective, survival promoting properties for cholinergic neurons in the CNS as well as neurite outgrowth promoting properties on sympathetic and sensory neurons of the dorsal root ganglia [233,234]. A large body of evidence indicates that NGF promotes survival of basal forebrain cholinergic neurons [235]. NGF reversed reductions in ChAT and AChE activity in nucleus basalis magnocellularis (NBM) lesioned rats, promoted survival of septal cholinergic neurons and improved learning after fimbria–fornix transection, supporting its rationale for evaluation in the clinic [235]. In the clinic NGF was infused intraventricularly in one patient over 3 months resulting in an increase in uptake and binding of [11C]nicotine in the frontal and temporal cortex and improved verbal episodic memory [236]. In an alternate strategy to enhance delivery of NGF to the brain by ex vivo gene transfer, a Phase 1 trial in six patients with mild AD revealed the rate of cognitive decline slowed by 36–51% based on ADAS-cog and MMSE assessments, with no reported adverse effects; however, the study did not include a placebo group [237]. Although showing encouraging results, the therapeutic potential of polypeptides remains limited due to their size and pharmacokinetic characteristics, which prevent their systemic administration for treatment of CNS diseases.

Brain-derived neurotrophic factor (BDNF) and its receptor tyrosine kinase TrkB are highly expressed in the hippocampus, cortex, and basal forebrain and are targets for the treatment of AD [238]. BDNF supports survival and differentiated function of ACh and DA neurons, and improved learning and memory in animals via activation of TrkB and the low affinity NGF receptor p75 [239]. Low plasma levels of BDNF mRNA have been suggested as a marker for therapeutic monitoring in AD [240]. Insulin-like growth factor I (IGF-I) deficiency has also been implicated in cognitive deficits seen in AD. IGF-I levels were investigated for associated cognitive performance and decline, and were related to information processing speed, memory, and MMSE score [241].

Cannabinoids The endocannabinoid system consists of two GPCRs, CB-1 and CB-2. CB-1 receptors are abundant in brain regions associated with memory and learning, while CB-2 receptors are confined to cells of the immune system. CB-1 receptor antagonists may have therapeutic utility for the treatment
of cognitive deficits associated with AD or schizophrenia [242]. The CB-1 antagonist rimonabant (SR141716) improved olfactory short-term memory assessed by the social recognition test and enhanced spatial memory in the radial-arm maze task in rodents. In addition, rimonabant reversed amnesia induced by i.c.v injections of β-amyloid fragments in mice. In vivo rimonabant selectively increased NE, DA, and ACh efflux in the prefrontal cortex, suggesting a potential role for CB-1 antagonists in treatment of attention and ADHD [242,243]. The future of CB-1 receptor antagonists as drugs is confounded by the psychiatric side effects associated with Rimonabant use that has led to its non-approval as an antiobesity agent in the United States and its withdrawal from the market in the European Union for the same indication [244].

Neuropeptides Neuropeptides and their receptors have represented novel targets for CNS disorders for more than half a century, with minimal success as evidenced by the inability to find improvements on morphine as analgesic acting via the opioid receptor
family and the failure of neurokinin-1 (NK-1) antagonists as analgesics and antidepressants. Nonetheless, several neuropeptides or their inhibitors including neurokinin B, angiotensin IV, galanin (GAL), adrenocorticotropic hormone (ACTH), oxytocin (OT), arginine vasopressin (AVP), and thyrotrophin-releasing hormone (TRH) have shown efficacy in cognition models.

The neurokinins (NKs) are a family of three neuropeptides, substance P (SP), neurokinin A (NKA), and neurokinin B (NKB), that mediate their biological effects via activation of NK1, NK2, and NK3 GPCRs, respectively [245]. NK3 receptors are expressed in brain regions involved in emotion and cognition, and stimulation of NK3 receptors can enhance DA, NE, 5-HT, GABA, and ACh release. Antagonists may have therapeutic value in treating psychosis and CDS. NK3 knockout mice displayed deficits compared to wild-type mice in several cognition tests, including passive avoidance, acquisition of conditioned avoidance responding and MWM [246]. Two NK3 antagonists, osanetant 110 and talnetant 111 displayed antipsychotic activity the clinic; however, both compounds suffer from poor pharmacokinetics and were abandoned [247].

GAL and its receptors (GALR1–GALR3) are distributed in basal forebrain, cortex, hippocampus, and amygdala, where it modulates ACh, NE, and 5-HT pathways [248]. GAL inhibits ACh release in vitro and in vivo, and impairs cognitive performance in models of spatial learning and memory [249]. Mice over expressing GAL have selective search and spatial navigation deficits with impaired learning and memory. The GALR1 antagonist, RWJ-57408 112, reversed the GAL-inhibited release of ACh in vitro [250] and the selective peptide antagonists, M35 and M40, reversed GAL-induced deficits in various models of learning and memory in rats [248]. Taken together, the data suggest GALR1 as a promising target for cognitive deficits in AD. However, to date, high-throughput screening and drug discovery efforts have failed to identify potent, drug-like NCEs for this target.

ACTH modulates cognition and attention in humans. ACTH_{4-10} is a potent modulator of attention in humans. It may also have neurotrophic or neuroprotective properties, and was studied for the treatment of memory...
disturbances in AD [251]. ORG2766 (H-Met (O2)-Glu-His-Phe-d-Ly-Phe-OH) enhanced recovery in behavioral models of forebrain lesioned animals and ebratide, an analog of ACTH4–9, increased ChAT and AChE activities in aged rats, suggesting potential therapy in AD [252].

Angiotensin IV (Ang IV), initially thought to be an inactive product of Ang II degradation, enhanced learning and memory in normal rodents and reverse memory deficits in models of amnesia. The CNS effects are mediated by the AT4 receptor, found in high levels in brain regions involved in cognition. The AT4 receptor was identified as the transmembrane enzyme, insulin-regulated membrane aminopeptidase (IRAP). Inhibition of IRAP via the AT4 receptor may inhibit degradation of neuropeptides involved in cognitive enhancement [253].

There is growing body of evidence that the neuropeptides OT and AVP modulate complex social behavior and social cognition [254,255]. OT knockouts display social amnesia in the social recognition test, despite a normal ability to recognize familiar nonsocial scents. The deficit in social recognition can be completely reversed with OT infusion [256]. AVT increased glutamate release and intracellular Ca2+ concentration in hippocampal and cortical astrocytes and blocked Aβ-induced impairment of LTP in rat hippocampus in vivo [257].

In addition to its role in regulating thyroid function, TRH also modulates ACh activity in the CNS. TRH administration (0.3 mg/kg i.v.) in 10 AD patients showed statistically significant increases in arousal and improvement in affect, with modest improvement in semantic memory [258].

The successful transition of peptide hormones to small-molecule mimetics or antagonists remains a major challenge in CNS drug discovery perhaps due to the fact that as labile peptides, these neuromodulators have very short half-lives whereas small molecules may be too long lasting in their effects to be efficacious and side effect free. Similarly, antagonists may provide prolonged blockade of autocrine signaling that is deleterious to tissue function. As examples, NK1 receptor antagonists MK-869 (Aprepitant) 113 and CP-122721 114 despite good drug-like properties and copious preclinical efficacy, data have singularly failed in the clinic. Likewise the considerable efforts to improve on morphine as an analgesic have yet to yield viable NCEs that provide convincing evidence that opioid receptor subtype selectivity will avoid the side effects of morphine and its congeners including addiction, respiratory depression, constipation, and euphoria.

Phosphodiesterase Inhibitors Phosphodiesterases (PDEs) function to hydrolyze the phosphodiester bond and degrade the key second messengers, cyclic adenosine monophosphate (cAMP), and cyclic guanosine monophosphate (cGMP) to control their intracellular levels [259]. At least 11 distinct PDE families (PDE1–PDE11) are known that are classified by their substrate specificity. PDE3, PDE4, PDE7, and PDE8 are specific for cAMP; PDE5, PDE6, and PDE9 are cGMP selective enzymes; and PDE1, PDE2, PDE10, and PDE11 function as dual-substrate PDEs [259].
Essentially all PDEs are expressed in the CNS with evidence suggesting that PDE2, PDE4, PDE5, PDE9, and PDE10 may have therapeutic potential for cognitive disorders. A substantial body of genetic and pharmacological evidence demonstrates that the cAMP response element binding (CREB) protein is a required process in formation of long-term memory [260,261]; lower levels of CREB result in memory impairment, while higher levels facilitate memory formation [262,263]. cAMP signaling through PDE4 inhibition has been associated with consolidation and retention of LTP. The prototype PDE4 inhibitor rolipram facilitated LTP in the hippocampus and increased phosphorylation of CREB and expression of the cAMP-dependent, memory-related protein, Arc [264]; changes linked to retention of long-term memory. In vivo, rolipram reversed scopolamine deficits in object recognition and radial arm maze tasks [265]. PDE4 inhibition and activation of cAMP/CREB signaling cascade appear to specifically facilitate long-term, but not short-term memory formation [264]. HT-0712 [115], MK-0952 [116], and MEM1414 (undisclosed structure) are PDE4 inhibitors that reportedly advanced to the clinic. The primary obstacle with the development of PDE4 inhibitors has been dose-limiting side effects of emesis and nausea. The four PDE4 subtypes (PDE4A–PDE4D) are differentially expressed in the CNS [266]. PDE4 knockout and behavioral studies hypothesized that the PDE4D isozyme was specifically involved in emesis [267] and PDE4B implicated in the regulation of LTP in the hippocampus [268].

The PDE2A expression pattern in CNS stimulated interest in the study of PDE2 inhibitors for treatment of cognitive disorders. BAY 60-7550 [117] (PDE2A IC_{50} = 4.7 nM) had selectivity versus PDE3B, PDE7B, PDE8A, PDE9A, PDE11A, and PDE1, increased cGMP levels in culture and enhanced LTP in hippocampal slices [269]. BAY 60-7550 improved performance in social and object recognition memory tasks, and reversed MK-801-induced deficits in T-maze spatial alteration. Although PDE5 expression in the brain is low the PDE5 inhibitor, sildenafil [118] facilitated memory consolidation in rodent novel object recognition tasks and reversed scopolamine-induced deficits in performance in a T-maze task [270]. PDE9A is a cGMP-specific PDE widely distributed in the CNS. The selective PDE9 inhibitors, BAY 73-6691 [119] enhanced long-term memory in social recognition and object recognition tasks and attenuated scopolamine-induced deficit in passive avoidance and MK-801-induced deficit in a T-maze alteration task [271]. BAY 73-6691 is reportedly under development for AD [265]. PDE10A is widely expressed in brain regions associated with DA and glutamate function. Increasing interest in the discovery of PDE10A inhibitors stems from reports of the antipsychotic activity of papaverine [21] and the potential for PDE10A inhibitors to treat CDS [265,272]. Papaverine enhanced CREB phosphorylation, reversed PCP-induced deficits in the EDID-set shift assay, a test of executive function, and was efficacious in the novel object recognition assay [273,274]. PF-02545920 [120], a selective, picomolar PDE10A inhibitor, reportedly entered Phase II clinical trials for schizophrenia [275].

### 3. SMART DRUGS

As noted in the introduction [14], a natural extension of the use of drugs intended to treat cognitive impairment in disease states such as AD, PD, schizophrenia, and ADHD and in situations following brain trauma and stroke, is the use of such agents to improve cognitive performance and quality of life in healthy individuals. Stimulant compounds such as caffeine [100] (in coffee and soft drinks), amphetamine [121], methylphenidate [9], and modafinil [20] are frequently used by students to improve examination performance, by shift workers (including those in the medical professions), airline crews, and by active military personnel [14]. Their potential use in airport-security screeners has also been suggested. This has led to an ethical debate on the use of cognition enhancers to improve “brain energy” and overcome the effects of sleep deprivation in “an overworked 24/7 society pushed to the limits of human endurance.” [14] In the United States, the nonprescription use of the
stimulants amphetamine and methylphenidate is a crime [276] creating a black market in their sale. Yet questions have been raised as to whether the responsible use of cognition enhancers in the healthy is actually a “good health habit” and how, in these circumstances, taking a prescription psychostimulant differs from consuming a double espresso. Indeed, the increased consumption of coffee may be related to the fact that an 8 oz cup of Starbucks coffee contains nearly three times the caffeine as a regular coffee [277]. Modern-day society is excessively sleep deprived, stressed, and overloaded with information via the Internet such that quality of life is at a premium. Accordingly, there have been calls [276,278] for further research to generate an evidence base on the risk and benefits associated with the use of cognition enhancers in the healthy with the argument that these modalities may in time be viewed in the same prophylactic manner as vitamins or vaccines.

4. SUMMARY AND FUTURE CHALLENGES

The need for drugs to treat cognitive dysfunction in all its forms has been clearly established and represents a major challenge in drug discovery given the many failures of compounds robustly active in animal models of attention and memory in the clinic. It may be argued that nearly every class of NCE active on CNS targets will, at one point or another, be examined for effects on cognitive function. Similarly, the lack of clinical success may be argued as reflecting either the inadequacy of the animal models used to triage CNS
compounds for advancement to the clinic [32,279] or the inability to diagnose patients at a sufficiently early stage in their disease progression to show benefit [11].

With an understanding of these limitations, a better temporal and informational interface between preclinical and clinical research activities [280] and the use of appropriate biomarkers, it may be anticipated that newer compounds advanced to the clinic may show improved efficacy and patient benefit and add significantly to the quality of life in patients with currently untreatable neurodegenerative and traumatic disorders of the brain.

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