

Milestones in Drug Therapy

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Cognitive Enhancing Drugs

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MDT

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Cognitive Enhancing Drugs

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Dedication

This book is dedicated to my wife Regina, our children Chris and Marty; to my parents Dominick and Rose, and to my Aunt Angie – with love and appreciation for their constant encouragement and support, without which this book would not be possible.

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Preface

Improving cognitive power – the dream of retaining vast amounts of information and having instant access to that information – the photographic memory, each has been part of the realm of imagination and fiction. This is with good reason. Currently our knowledge pertaining to the neural construct of intelligence and to the make-up of the basic engram is in its infancy. We know that memory involves protein synthesis within neural cells, and possibly the restructuring of the three-dimensional interactions among dendritic processes. We have uncovered the electrophysiological basis of conditioned cellular behavior, e.g., long-term potentiation, and we have determined some of the cellular signaling events involved. Yet this knowledge, prodigious as it is, has not moved us significantly closer to the dream of greater cognitive power. Yet as anyone subject to the advertising media knows, there are drugs and natural product preparations that purport to do just that. Whereas some of these products are often considered harmless nutritionally-based brain boosters, the possibility of achieving significant improvement in cognitive power or of increasing memory and enhancing learning by standard pharmaceutical means in otherwise cognitively normal individuals is sometimes viewed as ethically suspect. Thus, most serious scientific inquiry has been directed at the reversal of cognitive impairment associated with disease syndromes, particularly Alzheimer's disease. There is no doubt that drugs effective in humans with dementia or in animal impairment models are often effective in cognitively normal subjects; and it is likely – if not inevitable – that pharmaceutical products, like their natural product counterparts, will be used to enhance cognitive power in multiple settings.

In developing this book, contributors were sought who have years of experience working with drugs and natural substances that have been suggested or observed to improve aspects of cognition and memory in experimental animals and in human beings. The first two chapters underscore the cholinergic hypothesis of memory and describe the advent and ultimate use of long-acting inhibitors of acetylcholinesterase for the treatment of the symptoms associated with Alzheimer's disease. The subsequent chapters specialize in areas less advanced clinically, but with promise for future applications. One of the key themes that reverberates through the chapters and culminates in the final chapter pertains to the possibility of targeting multiple brain substrates to develop additive or synergistic positive mnemonic actions. Some work has already been accomplished to support this approach. New hybrid molecules are now being tested that were specifically designed with this hypothesis in mind.

This book also is relevant for those interested in how animal models are used in the development of cognition-enhancing agents. Since many of the compounds discussed have binary modes of action that often include both positive mnemonic effects and neuroprotective actions, the methodology described in these chapters includes cell and molecular techniques, tissue culture procedures, various studies in normal and transgenic mouse strains, rat models, and studies in non-human primates.

Perhaps most importantly, this book will present to the reader some of the most well-studied molecular targets for cognition enhancement. Potential targets have been partitioned based on primary neurotransmitter systems, although significant overlap among systems exists. There will be some areas not covered in this book, but the approaches to the development of novel drug entities will be similar to those described here. Ultimately the goal is to prepare the reader for a virtually wide open field with the hope that new discoveries will proceed from our readership in the coming years.

Jerry J. Buccafusco

Augusta, September 2003

The cholinergic hypothesis – past and present

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The past

It is difficult to pinpoint the birth of cognitive pharmacology, i.e., a study of the effect of drugs on the behavioral and neurochemical processes we attribute to higher order thinking, learning, and memory. Moreover, it is not possible here to discuss and critique the various dominant theories of cognition. Rather it would be more relevant and in-keeping with the focus of this book to consider some of the past work, primarily pharmacological, that led to current concepts that will be described in detail in some of the following chapters. One of these concepts, the “cholinergic hypothesis” of learning and memory, has undergone the most vigorous scientific scrutiny relative to other theories. The culmination of the cholinergic hypothesis is represented by the current application of the theory to the treatment of Alzheimer’s disease (AD) in the form of centrally-acting inhibitors of acetylcholinesterase (AChE), the enzyme that degrades synaptically-released acetylcholine. Despite this important application of the hypothesis, the origin of the cholinergic theory likely derives mainly from the effects produced by anticholinergic drugs, chiefly natural products like atropine and scopolamine which are active ingredients of various medicinal and cosmetic preparations of antiquity. Perhaps one of the oldest published studies pertaining to the physiological and behavioral effects of the administration of atropinic drugs in man was the account of Gauss in 1906 pertaining to the use of hyoscine in the induction of “twilight sleep” during labor [1]. Early rigorous experimentation with centrally-acting antimuscarinic agents largely confirmed the clinical observations [2–4]. Over subsequent decades scopolamine was in routine use for induction of sedation and the production of amnesia during labor and delivery and during surgical procedures. Scopolamine and other antimuscarinic agents are particularly effective amnestic drugs in inhibitory avoidance tasks performed by rodents. Scopolamine is particularly effective when administered pre-training i.e., during the acquisition phase of the task (e.g., [5]). The scopolamine-induced amnestic action in rodents is one of most widely utilized models during early stages of new drug development for therapeutic indications that require cognitive enhancement [6]. The antimuscarinic agent as

used in various memory-related behavioral paradigms such as inhibitory avoidance, spatial reference and working memory, fear conditioning, and sensory gating is useful to a degree in the prediction of positive mnemonic efficacy in human beings; i.e., drug candidates that effectively reversed scopolamine-induced task impairment were shown to enhance cognition during clinical trials. In fact, the role of cholinergic neurons in the processes subserving learning and memory had been appreciated years prior to the first studies linking the loss of basal forebrain cholinergic neurons to the symptoms of AD. In fact, the *post mortem* neurochemical findings associated with AD pathology seemed to finally validate the cholinergic hypothesis of AD, as the loss of nigro-striatal dopaminergic neurons validated the dopaminergic hypothesis of Parkinson's disease.

The present

Despite this body of supporting evidence there have been recent challenges to the cholinergic hypothesis of AD. For example, Davis and colleagues [7] recently reported that the activities of AChE and choline acetyltransferase (ChAT) were not reduced in *postmortem* neocortical tissues obtained from individuals recently diagnosed with mild AD. These findings cast doubt as to the role of cholinergic neuron loss in mediating the early symptoms associated with AD. Likewise, DeKosky and co-workers [8] failed to detect a reduction in ChAT activity in cortical regions studied in patients diagnosed with mild cognitive impairment (a pre-AD state?) Finally, little cholinergic cell loss was detected in brain samples derived from individuals suffering near the time of death from mild cognitive impairment or early AD [9]. This impressive body of work was performed by using very rare tissue samples, and the results have had profound implications for the study of AD. However, they do not necessarily spell the death knell for the cholinergic hypothesis of AD. In fact, as early as 1999 Bartus and Emerich [10] pointed out that since neither ChAT nor AChE are rate limiting in the biosynthesis of acetylcholine, they are unlikely to accurately reflect active cholinergic function. And in fact, a variety of systems important for the dynamic function of cholinergic neurons could be compromised well before the degradation or loss of the more static cholinergic markers. Moreover, it should be considered that the collection of *postmortem* human tissues available for analysis often involves delays from a few hours to days as opposed to animals studies in which the *postmortem* intervals often involves a matter of minutes. The unavoidable degradation of tissue viability contributing to variability in the data poses a significant challenge. It may require the development of *in vivo* imaging methods capable of assessing the dynamic function of central cholinergic neurons in living patients suffering from early stages of AD to resolve these issues.

As age currently represents the most potent of the known risk factors for AD, it seems relevant to ask whether the function of central cholinergic neu-

rons is impaired in the aged. Challenges to the cholinergic hypothesis of AD appear to ignore the body of evidence in support of the relationships between aging, cholinergic impairment and cognitive decline. A study of the effect of advanced age on brain cholinergic function began in earnest in the early 1980s when chemical enzymatic methods were developed with the specificity and sensitivity to measure the dynamic aspects of transmitter function. Methods for the rapid stabilization of brain levels of acetylcholine and choline by near freezing or focused microwave irradiation were also introduced for routine use. For example, Gibson and co-workers [11] examined the whole brain synthesis of acetylcholine in aged mice from 3–30 months of age. They reported that the biosynthesis of acetylcholine (measured by injection of a radio-labeled precursor) declined by up to 75% in the 30-month-old animals. Mild hypoxia further decreased acetylcholine synthesis by 90%. Moreover, aged cholinergic neurons were more impaired in their ability to release acetylcholine following potassium stimulation than they were in their ability to synthesize the transmitter [12]. Subsequent *in vivo* microdialysis methods largely confirmed these early findings (e.g., [13]). The concept that aged brain cholinergic neurons function relatively normally until stressed has been supported through experiments that used various methods to increase acetylcholine output [14–17], or which damage cholinergic neurons [18, 19]. It seems reasonable to conclude, therefore, that any sustained insult to forebrain cholinergic or hippocampal cholinergic neurons could interfere with the ability of these cells to provide sufficient transmitter release for normal function. This possibility was tested directly in a longitudinal series of experiments in which chemical lesions of basal forebrain cholinergic neurons were induced in young rats with the aim of producing only limited loss of the cholinergic cells [19]. The rats had been previously well trained in the performance of a sustained attention task. Whereas initially there was a similar degree of task performance in both experimental groups, a significant dissociation between lesioned and control rats in terms of task efficiency did not occur until the animals reached 31 months of age when the lesioned group exhibited significant task impairment. The results of these studies in aged rodents are relevant to the topic of this review considering the observation that most of the age-related changes pertained specifically to dynamic aspects of brain cholinergic neurons. In many of the studies cited above and in many other reports, indirect measures of standard cholinergic markers (as might be determined from autopsied tissues) often do not show such dramatic age-related differences.

As mentioned above, the cholinergic hypothesis has engendered the potential use of cholinergic agonists such as the AChE inhibitors for the treatment of the symptoms of AD. It should be pointed out, however, that this drug class may also improve cognitive performance in younger, non-impaired individuals. Non-human primate data (e.g., [20, 21]) as well as the results obtained in young human subjects [22] certainly support this possibility. AChE inhibitors also may exhibit efficacy in non-AD syndromes in which cognitive impairment is an accompanying symptom, including schizophrenia [23, 24],

Parkinson's disease [25, 26], Lewy-body dementia [27], traumatic brain injury [28], and others [29, 30]. And of course AChE inhibitors are effective in improving memory-related task performance in aged non-human primates, even in animals that are not that impaired. Indeed, it has been our experience that there is little difference in effectiveness in terms of task improvement to AChE inhibitors between young and aged subjects. In fact, as Bartus [21] pointed out, and as we have experienced [31, 32], young animals often provide a less variable and a more dose-dependent model for revealing the cognitive-enhancing actions of new therapeutic agents. In such studies, however, task variables such as retention intervals are optimized for all subjects to allow for drug-induced improvements to become manifest [31]. Drug-induced memory enhancement in young subjects is not relegated to cholinergic drugs, but is apparent for drugs of different pharmacological classes [32]. These observations speak to what is likely generally accepted, but not often discussed: that pharmacological enhancement of cognition can occur in normal individuals, particularly if the individual is stressed to his mnemonic limits. The burgeoning sales in over-the-counter "memory aids" has yet to benefit the prescription drug market. However, it is likely that many physicians who treat AD, as well as many Alzheimer's research centers, have received inquiries (as our Center has) regarding the acquisition of prescription AD therapeutic agents as memory aids for use in young individuals hoping to gain an advantage during scholastic examinations.

Notwithstanding the obvious ethical issues implied in the use of cognition-enhancing agents in non-impaired individuals, the cholinergic hypothesis has provided a framework for the development of drugs that have the potential to impact the most complex of brain functions. Despite the lack of disease specificity for the AChE inhibitors, the cholinergic hypothesis is not diminished. At greater issue is the possibility that central cholinergic neurons play an important role in at least some aspect of learning and memory that may be targeted pharmaceutically. It is also likely that other non-cholinergic compounds that have exhibited effectiveness in improving performance in memory-related tasks exert their effects indirectly via cholinergic pathways. For example certain biogenic amine-mimetics have been demonstrated to increase the release of endogenous acetylcholine. Even drugs acting at nicotinic receptors may owe their positive mnemonic effects to their ability to cause brain acetylcholine release [33]. It may be of interest to consider the fact that many, if not most, of the drugs demonstrated to offer improvement in the performance of memory-related tasks, irrespective of their pharmacological class, were initially examined in a model that used scopolamine to induce task impairment. The less often utilized experimental paradigm would be to ask whether pre-treatment with scopolamine or other cholinergic antagonist (at non-amnestic doses) would block the positive effects of a non-cholinergic cognitive-enhancing agent in, for instance, an aging model. We used this approach to study the mechanism of the nicotine-induced increase in delayed matching-to-sample accuracy in macaques [33]. In this study low non-amnestic doses of scopo-

lamine were shown to block the increased accuracy produced by nicotine. The data were interpreted to suggest that nicotine increased acetylcholine release (as was known from *in vitro* studies) which in turn acted on muscarinic receptors involved in the mnemonic pathway. It would be of interest to know whether this situation would apply to the various non-cholinergic compounds known to improve memory-related task performance, particularly since a wide variety of neurotransmitters and neuropeptides have been shown to directly or indirectly modulate the release of acetylcholine within the CNS. Many of these interactions were shown to have implications for learning and memory (for review, [34–44]).

In 1974, R.D. Myers asked how a transformation arising from a cytoplasmic protein molecule could almost instantaneously retrieve an old memory by way of activating the release of a substance such as acetylcholine (rephrased). He termed this conundrum "...a puzzle to end all puzzles" [45]. During those years there was no mechanism that could account for the millisecond requirement for memory retrieval. More recently, Nancy Woolf and her colleagues [46–48] have put forth a cogent and intriguing argument for the role of cortical cholinoreceptive neural cells in human memory. Her work and that of others cited in her papers indicate that cortical acetylcholine release represents a primary mechanism for the cortical response adaptation associated with behavioral conditioning. Cortical cholinoreceptive cells also are enriched in microtubule-associated protein-2 (MAP-2), a protein whose expression is highly correlated with the consolidation of contextual memory as measured behaviorally in animals. She also describes anatomical modules within the cytoarchitectonically-defined regions of the cerebral cortex, hippocampus, and amygdala that likely contain the mnemonic engrams. Memory storage and retrieval are visualized as dendritic modifications (altered dendrite length and/or arborization) within cholinoreceptive cells induced by the destabilization or degradation of MAP-2 protein. MAP-2 degradation is subsequent to increased neuronal activity (LTP?), neurotropin (e.g., NGF) release, and enhanced acetylcholine release. Subsequent activation of cholinoreceptive cells and their linked G-proteins results in calcium influx and activation of calcium-sensitive proteases that easily degrade MAP-2 protein thus allowing for dendritic modifications. Dr. Woolf points out that in AD, early in the syndrome, degenerative pathology is relegated to distal dendrites of hippocampal pyramidal cells – the potential sites for the storage of recent memories, and in fact, most cells possessing neurofibrillary tangles are cholinoreceptive. She goes on to propose the interesting possibility that the selective vulnerability of cholinergic neurons in AD is related to the repeated restructuring of cholinoreceptive cells due to the continuous encoding of long-term representations. It is possible that enhanced acetylcholine release associated with contextual memory results in the delayed degradation of MAP-2 in cholinoreceptive neurons which must continually increase axonal growth to suitable dendritic segments for memory encoding. Apparently this increasing requirement for plasticity in basal forebrain cholinergic neurons as they are continually challenged by the

need to seek out unmodified cholinceptive dendritic segments leads to vulnerability.

From a pharmacological perspective Dr. Woolf concludes that a more effective treatment modality for AD could be developed by simultaneously inhibiting neuronal growth within AD-damaged areas like the hippocampus while applying cholinergic agonists to selectively enhance memory encoding and retrieval. Alternatively cholinergic function within the neocortex might be requisitioned to replace the damaged hippocampal function. This latter possibility might be achieved by simultaneous inhibition of muscarinic receptor function within the hippocampus and muscarinic receptor stimulation within the neocortex. The potential for such a pharmacologic scenario could reside in new drugs designed to be selective for muscarinic receptor subtypes. Whereas there is not that much difference in the overall expression of M1 receptors between cortex and hippocampus, within the hippocampus, M1 cholinceptive cell bodies are relegated almost exclusively to the CA1 region and the dentate gyrus; and M3 receptor expression may be slightly greater in the cortex [49, 50]. In AD it might be possible to inhibit neural growth in already-damaged hippocampal regions with the judicious use of a selective M1 receptor antagonist, while simultaneously activating M3 receptors which are relegated to more unaffected cortical regions. Another approach might include the combination of an M1 antagonist with an α_2 -adrenergic (or D1 dopaminergic) agonist. Attention and memory may be enhanced by adrenergic agonists, particularly via their actions within the prefrontal cortex ([46] and see Chapter by Edward D. Levin). This latter possibility could be more easily tested because the receptor-selective compounds already exist.

Finally, nicotinic receptor agonists, including nicotine itself, have the potential for inducing the release of a variety of neurotransmitters and neuropeptides. Cortical nicotinic acetylcholine, norepinephrine, and dopamine release along with low M1 muscarinic receptor blockade within damaged hippocampal regions might provide another useful approach. In fact, Buccafusco and Jackson [51] first reported that nicotine can produce a protracted improvement in task accuracy in monkeys trained in the performance of a delayed matching-to-sample task. The protracted feature of nicotine's beneficial mnemonic action was unexpected, particularly in view of the short plasma half-life of these drugs in rhesus monkeys. Levin and colleagues [52] reported similar findings for nicotine in rats. In fact, they demonstrated that this protracted beneficial effect of nicotine on radial arm maze performance was not dependent upon the presence of the drug at the time of behavioral training. In monkeys, the improvement in task efficiency that was measured on the day after nicotine administration generally occurred for trials associated with long delay intervals as they had for the previous day's session. This pattern (the retention interval receiving the greatest improvement on the day of testing was the same retention interval receiving the greatest improvement upon testing 24 hours later) was generally maintained for other compounds evaluated under similar conditions [53]. Nicotinic drugs are by no means the

only class that appears to exhibit protracted improvement in task performance. For example, we have noted similar responses with the α_2 -adrenergic agonist clonidine [54], and with the muscarinic (M1-preferring) receptor agonist WAY-132983 [55]. It is tempting at this point to consider the possibility that these drugs set into motion pharmacodynamic processes linked to the factors discussed by Dr. Woolf (*vide supra*). All of these compounds have the potential to affect either cholinergic or adrenergic processes within the frontal cortex and it would be of interest to determine whether their protracted positive mnemonic actions are related to distal dendritic modifications within the relevant cortical regions.

The future

It seems, therefore, that the cholinergic hypothesis of memory will continue to drive drug discovery and basic experimentation for some time to come. In the pages to follow we hope to provide an overview of the potential neural systems that have, and continue to be, exploited for cognition enhancement. Although there is much to rejoice in the wide variety of potential drug targets, the challenge will continue to be to develop drugs more selective for memory, and which are less apt to affect the other myriad brain functions which often translate to side-effects or toxicity. It is without doubt that the perfect cholinergic drug for cognition does not yet exist; and it is somewhat disappointing to read (in trade journals and reviewers' comments) about the 'limitations' of the current crop of clinically useful AChE inhibitors (as if these compounds represented the potential epitome of the class), and by way of association to downplay the future of cholinergic pharmacology in general. However, as you will read in subsequent chapters, cholinergic drugs have more to offer than mere cognition enhancement. Both muscarinic and nicotinic drugs have the ability to alter amyloid processing to the betterment of the brain. Nicotinic drugs are potent neuroprotective agents; and nicotine itself has been shown to prevent amyloid deposition in the brains of transgenic mice developed to over-express A β 1-42 [56]. As such, cholinergic drugs may exhibit both cognitive-enhancing and disease modifying effects in AD individuals. Even the lowly cholinesterase inhibitors have shown their efficacy in slowing the progress of AD and significantly delaying nursing home placement.

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Drugs that target cholinesterases

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Development of cholinesterase inhibitors in Alzheimer's therapy

Currently, cholinesterase inhibitors (ChEI) represent the treatment of choice for Alzheimer's disease (AD). Following the introduction in the 1980s of a first generation of drugs such as physostigmine and tacrine, a second generation of more suitable compounds was developed in the 1990s. These drugs are clinically more efficacious and produce less severe side-effects at effective doses. Contrary to the discovery of other neurotransmitter-based CNS drugs such as neuroleptics, tricyclic anti-depressants and anxiolytics, the clinical application of ChEI in the treatment of cognitive deficits in AD was neither accidental nor serendipitous. Its rationale was solidly founded on data derived from experimental physiology and behavioral pharmacology of the cholinergic system in animals and humans. Clinical results on the effect of these drugs on cognition (memory, attention and concentration) and more recently on behavioral symptoms in AD (apathy, hallucinations and motor agitation) confirmed predictions of potential clinical efficacy based on laboratory data.

Historically, the first ChEI to be used against AD was physostigmine administered under various modes [1]. Under those conditions, the effect of physostigmine was found to be too short lasting and the drug too toxic, therefore it was followed by oral tacrine [2]. Subsequently, metrifonate [3] and galantamine [4] were also tested orally.

Changes in cholinesterase activity related to Alzheimer disease and the role of butyrylcholinesterase in brain

Table 1 shows the changes in cholinesterase activity in the cortex of AD patients. Acetylcholinesterase (AChE) activity decreases progressively in certain brain regions from mild to severe stages of the disease to reach 10 to 15% of normal values while butyrylcholinesterase (BuChE) activity is unchanged or even increased by 20% [5–7].

In spite of the general reduction in brain AChE activity, the enzyme appears to be increased within and around neuritic plaques. In the plaques, AChE is

Table 1. Variation in cholinergic enzyme activity determined in autopsied human cortex from individuals with late stage Alzheimer's disease relative to normal controls

Enzyme	Localization in brain	Activity (% Control)	Molecular form
AChE	Neuronal	10–15	50–70% decrease, mainly G4
BuChE*	Glia-neuritic plaques	120	20% decrease in G4; 30–60% increase in G1
ChAT**	Neuronal	10–15	

*BuChE = butyrylcholinesterase; **ChAT = Choline acetyltransferase

closely associated with β -amyloid. As examples of regional difference in changes, BuChE/AChE ratio increases from 0.6 to 0.9 in the frontal cortex but from 0.6 to 11 in the entorhinal cortex [8]. This change may reflect a combination of reactive gliosis following severe neuronal damage (glial cells having preponderantly BuChE) and of an accumulation of BuChE in neuritic plaques, which contain both enzymes [9]. As the disease progresses and concentration of synaptic AChE (in particular the membrane-anchored G4 form) decreases [10], ChEI probably increase acetylcholine (ACh) concentrations to levels which may be inhibitory for AChE activity. This increase in substrate concentration may trigger glial BuChE to hydrolyze ACh and could thus represent a compensatory mechanism to the loss of neuronal AChE activity. Given the close spatial relationship between glial cell protoplasm and the synaptic gap, it is likely that extracellularly diffusing ACh may come into contact with glial BuChE and be effectively hydrolyzed, as demonstrated in our experiments in the rat with intracerebral microdialysis (Fig. 1) [11] and by the administration of rivastigmine (an AChE-BuChE-mixed inhibitor) in AD patients [12]. In these patients, CSF BuChE inhibition significantly correlates with cognitive benefit measured with a comprehensive computerized neuropsychological test (CNTB) (Fig. 2) [12].

Molecular forms of acetylcholinesterase in the human brain

Human brain AChE exists in multiple molecular forms as defined by their different sedimentation coefficient. Based on their shapes, collagen-tailed asymmetric forms and globular forms can be separated. Studies on whole brain fractions suggest that 60–90% of the tetrameric (G4) form is extracellular and membrane-located while the monomeric (G1) form is 90% intracellular and cytoplasmic [10, 13]. The different effect of certain inhibitors may be primarily related to the localization of the enzyme and the penetration of the inhibitor rather than to pharmacological or tissue selectivity. Selective loss of the membrane-bound G4 form has been reported in AD, suggesting a pre-synaptic

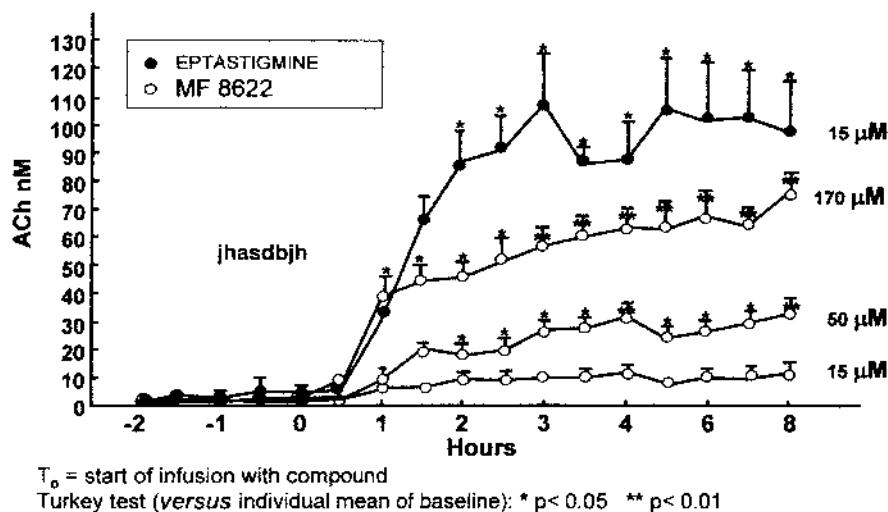


Figure 1. Extracellular levels of ACh measured in the rat cortex with microdialysis without adding a second ChEi. Following intracortical administration of eptastigmine, a non-selective ChE inhibitor, or MF 8622, a selective BuChE inhibitor at two different concentrations, a significant and prolonged increase in ACh levels is seen for both drugs lasting several hours.

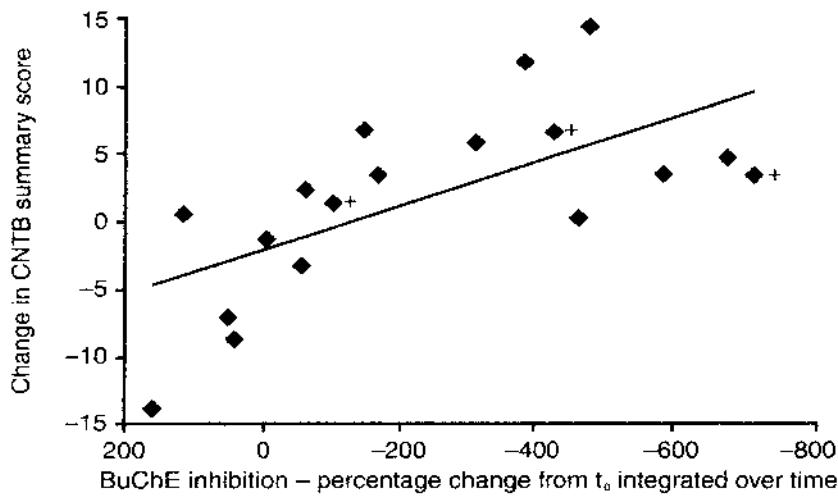


Figure 2. BuChE inhibition in CSF correlates significantly with cognitive improvement in AD patients administered rivastigmine. Cognitive changes have been measured with the Computerized Neuropsychological Test Battery (CNTB). Modif. from [12].

localization (Tab. 1). In severe Alzheimer patients, the membrane-bound G4 form is decreased in the frontal (-71%), the parietal cortex (-45%) and in the

caudate putamen (-47%) in comparison to control levels. The exact function of the G1 form, which is not significantly decreased in AD, has not been elucidated yet. The most effective inhibitor would ideally be one that selectively inhibits brain AChE forms without having any effect on peripheral tissues such as skeletal or cardiac muscle. Rivastigmine, a carbamate compound, inhibits preferentially the G1 form [13], while tacrine and metrifonate, inhibit G4 and G1 forms with similar potency [14].

Decline in cholinergic function with progression of Alzheimer disease: premises for a cholinergic strategy

During a course of 15 to 20 years of AD, a continuous loss of cholinergic neurons (50–87%) is observed in the nucleus basalis Meynert (nbM) as well as a loss of cortical cholinergic synapses [15]. From a total average of 350,000 neurons in young adult controls, a number as low as 72,000 is found in the nbM of AD patients. This profound loss in subcortical nuclei results in a progressive cortical cholinergic denervation [8]. It is still controversial whether or not early decline in cognition in AD is associated with a decrease in cortical choline-acetyltransferase (ChAT) activity or with other changes in cholinergic function, such as selective choline uptake, ACh vesicular storage and release or ACh synthesis (Tab. 1). A cholinomimetic strategy should therefore increase cholinergic activity and consequently improve cognitive capacities of AD patients. The most efficacious intervention so far has been the use of drugs such as ChEI. Research data suggest that doses of ChEI capable of doubling ACh levels in the cortex of mild to moderately severe AD patients could re-establish normal levels of the neurotransmitter. Pre-clinical experimental results in animals and clinical data in humans demonstrate that such a goal can be achieved with most of the second generation ChEI without causing severe or irreversible side-effects [16].

Cholinesterase inhibitors in Alzheimer's therapy

Cholinesterase inhibitors and cognition

Cholinesterase inhibitors tested in clinical trials or in current use in Japan, USA and Europe include approximately ten drugs. Four compounds (tacrine, rivastigmine, donepezil and galantamine) have been registered in USA and in Europe. Tacrine, a ChEI of the first generation, has been withdrawn from the market. Two new ChEI are in clinical Phase II; Huperzine A (a chinese natural product) and phenserine (a carbamate). Galantamine (galantamine hydrobromide), the latest introduced drug, is a reversible inhibitor of AChE and also an allosteric modulator of nicotinic acetylcholine receptors [17]. Because galantamine binds to a site on nicotine acetylcholine receptors which is differ-

ent than the acetylcholine binding site, it has been suggested that this provides galantamine with an additional mechanism of action which may activate non-cholinergic pathways impaired in AD [18]. Further clinical development of two other compounds, eptastigmine and metrifonate, were suspended because of side-effects (bone marrow suppression for eptastigmine and muscular weakness for metrifonate). To replace tacrine, the second generation ChEI (donepezil, rivastigmine, galantamine) had to fulfill specific requirements such as lower toxicity (hepatic) and easier administration besides demonstrable clinical efficacy [15].

There are differences between the tested compounds with regard to efficacy, percentage of treatable patients and responders, drop outs, severity and incidence of side-effects. Table 2 compares the effect of six ChEI using "intention to treat" criteria (ITT) [19–25, 27]. Pharmacologically, these drugs represent either reversible (tacrine, eptastigmine, donepezil and galantamine) or pseudo-irreversible or irreversible (rivastigmine, metrifonate) ChEI. The duration of these Phase III clinical trials varied from 24 to 30 weeks and over 10,000 patients were included in 26 different countries. The six most extensively clinically tested ChEI (tacrine, eptastigmine, donepezil, rivastigmine, metrifonate and galantamine) all produced statistically significant improvement in multiple clinical trials using similar standardized and internationally validated measures of both cognitive and non-cognitive functions. The most frequently used instrument for the evaluation of cognition, the ADAS-Cog, measures memory, orientation, language and praxis with a total score of 70 points. The mean annual change in ADAS-Cog scores in untreated AD patients was estimated to be approximately nine points per year in longitudinal studies. Obviously, there are large variations among patients, as the level of change seems to be dependent on the stage of the illness. The magnitude of cognitive effects measured with the ADAS-Cog scale for all six drugs – either expressed as the difference between drug- and placebo-treated patients or as the difference between drug-treated patients and baseline – is similar under present treatment conditions. This similarity after 26–30 weeks of treatment suggests a "ceiling effect" of approximately five ADAS-Cog points on average for approximately one third of patients in mild to moderate stages of the disease. It should be pointed out that this gain becomes more substantial, both clinically and economically, when evaluated after one year (8–9 points or more). Differences in effect between the drugs may be related partly to the rate of deterioration of the placebo group which can vary from trial to trial. The results obtained with some irreversible compounds suggest that the maximal clinical effect has not been reached yet. On the other hand, cholinergic toxicity related to maximal tolerated doses indicates a limit in safe achievable levels of ChE inhibition. Analysis of results also imply that both very mild and more severe cases need to be studied. Furthermore, the data showed wide variations of effect among patients; in some patients the gain was twice as large as in average. Cholinergic side-effects were transient, reversible and similar for all drugs. The percentage of improved patients varied from 25% to 50% with an average of 34%. This indicates that

Table 2. The effects of cholinesterase inhibitors on the ADAS-COG Test (ITT)

Drug	Dose mg/day	Duration of study weeks	Treatment difference from placebo*/*baseline**	Improved patients (%)	Drop out %	Side effects %
Tacrine	120–160	30	4.0–5.3/0.8–2.8	30–50	55–73	40–58
Donepezil	45	25	4.7/1.8/3	12	35	
Rivastigmine	5–10	24	2.8–4.6/0.7–1	40–58	5–13	6–13
Rivastigmine	6–12	24	1.9–4.9/0.7–1.2	25–37	15–36	15–28
Metrifonate	25–75–80	12–26	2.8–3.1–3.2/0.75–0.5	35–40	2–21–8	2–12
Metrifonate	60–80	26	3.9/2.24	15	7	
Galantamine	24	20–24	3.1–3.9/1.73	10–23	10–13	
Galantamine	32	24	3.8–3.9/1.6	34	32	13–16

ADAS-cog = AD Assessment Scale-cognitive subscale; ITT = Intention to treat; * study end point versus Placebo; ** study end point versus baseline. Modified from [20].

more than one third of treated patients showed a significant clinical response to ChEI. This effect can be maintained for five drugs (tacrine, donepezil, rivastigmine, galantamine and metrifonate) for at least one year representing a high impact value for patients and caregivers. A smaller percentage (about 10–15%) of patients did not improve on the ADAS-Cog with any of the used drugs, while a second group of patients (5% or more) showed a response significantly higher than 5 points. The similarity in clinical efficacy of the tested drugs is underlined by a practically identical effect on global scales such as the Clinicians Interview-Based Impression of Change-plus (CIBIC-plus).

An even more important result of the six or 12 month clinical data is the observation that patients treated with the active compound changed little cognitively and behaviorally from the beginning of the study to the end (Fig. 3). This suggests a stabilization effect of disease-related deterioration, which is clinically more significant than expected symptomatic improvement. Placebo-controlled studies also indicate that this effect can be prolonged for at least one year (Fig. 3). Four drugs (donepezil, tacrine, metrifonate and rivastigmine) have shown a long-term effect extending from 1 to 4.5 years [28]. Differences between responders (*i.e.*, stable or improved patients) *versus* non-responders may reflect the level of cholinergic damage present in the brain, genetic factors (such as the presence of APOE-ε4 alleles), gender or too low levels of ChE inhibition in the brain [20, 29, 30]. The question whether rivastigmine has a protective effect on subjects suffering from mild cognitive impairment is presently evaluated in on-going trials. Direct evidence of such an effect would modify the present definition of ChEI as drugs with symptomatic effects only.

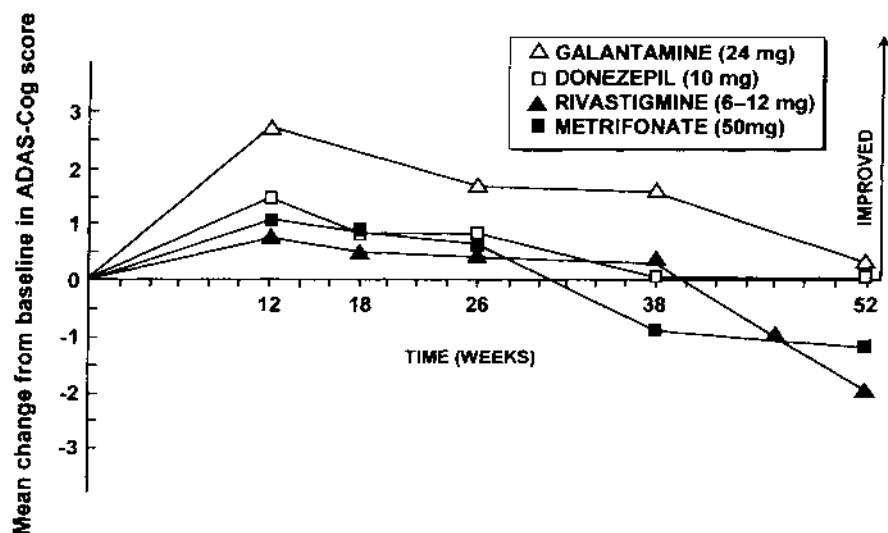


Figure 3 Stabilization effect (ADAS-Cog) of 12 month treatment with four cholinesterase inhibitors. The patients change little cognitively (ADAS-Cog) from baseline during this period.

Effects of cholinesterase inhibitors on behavior

Neuropsychiatric symptoms and functional deficits contribute greatly to the disability associated with AD. Neuropsychiatric symptoms occur in almost 90% of AD patients and are the most important factor of stress in caregivers. Biochemical and pharmacological considerations suggest the involvement of cholinergic deficiency in the mechanism of psychotic symptoms in AD. Ameliorating cholinergic function with ChEI should therefore decrease these problems.

Several studies have confirmed the beneficial effects of ChEI on neuropsychiatric symptoms in AD patients as measured by changes in the Neuropsychiatric Inventory (NPI) Scores [23, 27, 31, 32]. Significant improvement was seen for four drugs (rivastigmine, donepezil, galantamine and metrifonate) in delusions, hallucinations, apathy, motor agitation, depression and anxiety. Based on meta-analyses of 29 randomized controlled trials (16 with neuropsychiatric outcomes and 18 with functional outcomes) it is seen that ChEI exert a beneficial effect in treating neuropsychiatric symptoms and reducing functional impairment in patients with mild to moderate AD living in the community (Tab. 3) [33]. Thus, these studies support the hypothesis of a cholinergic link between cognitive and behavioral deficits in AD. They also demonstrated that the attenuation of such symptoms reduces caregiver burden, delays nursing home placement and in consequence could decrease the costs of patients care.

Table 3. The effect of cholinesterase inhibitors on functional impairment of Alzheimer's patients: A summary of 25 trials [33]

-
- OUTCOME MEASURE = ADL*, 13 trials
 - 11 trials show improvement *versus* placebo
 - OUTCOME MEASURE = IADL**, 12 trials
 - 11 trials show improvement *versus* placebo
 - No difference in efficacy among cholinesterase inhibitors
-

* ADL: activity of daily living; IADL**: instrumental activities of daily living

Combination therapy: cholinesterase inhibitors and anti-psychotics

Antipsychotic medication is more and more often prescribed in elderly and in AD patients particularly in a nursing home setting. Up to recently the most used neuroleptic has been haloperidol. Haloperidol a typical neuroleptic, has been progressively replaced by atypical neuroleptics such as risperidone, olanzapine and quetiapine.

Recent studies indicate that behavioral symptoms such as hallucinations and agitation can be treated with a combination of antipsychotics and ChEI

and in some cases with ChEI only [35] by combining rivastigmine with either antipsychotics, anxiolytics or antidepressants were able to reduce or terminate the use of these medications after 52 weeks of rivastigmine treatment in a nursing home study. In a second US study [36], over 50% of institutionalized patients, receiving antipsychotics, antidepressants or anxiolytics in combination with rivastigmine, reduced the dose or ceased the treatment. In a third study of patients with severe AD, discontinuation of atypical antipsychotics (risperidone, olanzapine and quetiapine) was possible after eight months treatment with rivastigmine [35]. In a randomized, 20 week open label trial with AD and vascular dementia (VD) patients with behavioral disturbances being treated with rivastigmine, a combination of rivastigmine with risperidone or risperidone alone, showed that the improvement in behavior was greatest when rivastigmine was co-administered with risperidone than when the drugs were given individually, suggesting a synergistic effect of the two drugs [37].

These results suggest some benefits of a concomitant use of atypical neuroleptics together with ChEI; however, a recent short communication reports of extrapyramidal side-effects seen with a combination of risperidone and donepezil [38].

Differences in cholinergic effects among cholinesterase inhibitors

As mentioned above, cholinomimetic therapy is based on the principle that brain ChE inhibition increases synaptic ACh levels which may lead to cognitive improvement. Ideally, the correlation between cognitive effects and level of AChE or BuChE inhibition would be best observed in the brain or in the cerebrospinal fluid (CSF) [12] (Fig. 2). However, as CSF monitoring is difficult to achieve, peripheral inhibition of AChE in red blood cells (RBC) or plasma BuChE has been studied as an indirect measure of the drug effect. Inhibition varies between 30% and 80% depending on dose and pharmacokinetic characteristics of the compound. For some drugs (donepezil and metrifonate) the mean level of peripheral ChE inhibition is 65–70% and could be safely brought to as high as 90%. For other drugs, such as tacrine, the practical limit of inhibition can be as low as 30% and may be increased only at the expenses of severe side-effects. There is little correlation between central AChE inhibition and side-effects; the severity of side-effects is mainly due to peripheral inhibition [20, 39].

A direct clinical implication of this relationship is that drugs producing high levels of central AChE inhibition (or BuChE inhibition) at a low dose with a short 1/2 life (see below) will produce only mild peripheral cholinergic side-effects. A high increase of brain ACh may be achieved within a full range of therapeutic potency. As an example, rivastigmine at doses of 6 mg (corresponding to 62% AChE inhibition in CSF) produces a significantly greater improvement in cognitive function than at doses between 1 to 4 mg [40].

Pharmacological properties of cholinesterase inhibitors

Pharmacological properties and differences of ChEI affect both safety and clinical efficacy of these agents. These properties are related to the way the inhibitor interact with the enzyme, to the characteristic of the enzyme and its localization in brain. Cholinesterases hydrolyze choline-esters into the respective acid (such as acetic acid for ACh) and choline. These enzymes are widely distributed in different tissues of vertebrates and invertebrates. An important feature distinguishing BuChE present in serum and glia from AChE present in neurons and erythrocytes is its kinetics toward concentrations of ACh. BuChE is less substrate-specific for ACh than AChE. Butyrylcholinesterase catalyzes the hydrolysis of both ACh and BuCh as well as of their analogues; however, its affinity for ACh is less than that for BuCh. A wide range of inhibition (measured in rat brain and plasma or in human plasma and erythrocytes) of the two types of ChEI is seen for various ChEI with a different substrate specificity (Tab. 4).

Particularly interesting from the clinical point of view is the relative rate of inhibition shown by several ChEI for BuChE and AChE. We observe (Tab. 5) that most inhibitors presently utilized for AD therapy with the exception of donepezil, are not selective for AChE. However, they all show a similar degree

Table 4. Selectivity of cholinesterase inhibitors in humans

Compound	AChE ^a IC50 ^c	BuChE ^b IC50	BuChE/AChE ^d	Clinical dose (mg/day)
BW 284 C51	18.8	48 000	2 553	
Huperzine	47	30 000	638	0.15–0.8
Donepezil	22	4 150	186	5–10
Phenserine	22	1 560	70	
Metrifonate	800	18 000	22.5	25–80
Galantamine	800	7 300	9	30
Rivastigmine	48 000	54 000	1.1	6–12
Physostigmine	28	16	0.6	36
Tacrine	190	47	0.25	80–160
Eptastigmine	20	5	0.25	45–60
Cymserine	758	50	0.7	
Iso-ompa	34 000	980	0.03	
Bisnorcymserine	110	1.0	0.009	
Hetopropazine	260 000	300	0.001	
Phenylethylcymserine	30 000	6	0.0002	
Bambuterol	30 000	3	0.0001	
MF-8622	100 000	9	0.00009	

^aHuman erythrocytes; ^bHuman plasma; ^cIC50 = concentration of drug required to inhibit enzyme activity by 50%; ^dThe higher the ratio the greater the selectivity for AChE.

Table 5. Pharmacological properties of cholinesterase inhibitors

Compound	Type of inhibition	Specificity of substrate	Chemical structure
Physostigmine	Pseudo-irreversible	ACh-BuCh	Carbamate
Tacrine	Reversible	ACh-BuCh	Aridine
Metrifonate	Irreversible	ACh-BuCh	Organophosphate
Donepezil	Reversible	ACh	Piperidine
Rivastigmine	Pseudo-irreversible	ACh-BuCh	Carbamate
Galantamine	Reversible	ACh-BuCh	Phenantrene
Huperzine A	Reversible	ACh	Pyridine

of clinical efficacy. Considering the drastic decrease in AChE activity taking place in the brain of advanced cases of AD patients (reaching 5% AChE levels at autopsy in some regions), and the large pool of BuChE available in glia neurons and neuritic plaques, it may not be an advantage for a ChEI to be selective for AChE. On the contrary, a good balance between AChE and BuChE inhibition may result in higher efficacy and allow a longer therapeutic use throughout the course of the disease. A very few selective inhibitors of BuChE are known and none has been tested clinically in placebo controlled trials. As it can be seen in Table 5, ChEI used in therapy belong to different classes with distinct differences in their chemical structure. ChEI can also be classified on the basis of the nature of the bonds formed between the agent and the enzyme in the complex (reversible, non-covalent) or the conjugate (carbamoyl or phosphoryl) (Tab. 5). Tacrine, donepezil and huperzine-A are high affinity, non-covalent inhibitors binding at the active center of the enzyme and occupying the choline binding subsite. Carbamoylating agents such as physostigmine and rivastigmine react with the active center serine to form a carbamoyl ester. On the other hand, phosphorylating agents such as metrifonate (DDVP is the active inhibitor) react covalently with the enzyme to form an inactive phosphoryl enzyme. This classification provides a clearer definition of the mechanism and the duration of action of the inhibitor.

The present knowledge of the molecular configuration of the two enzymes active sites allow to design compounds possessing well-balanced AChE-BuChE specificity, high CNS penetration and low peripheral and central cholinergic toxicity. Some of the second generation ChEI have demonstrated interesting beta-amyloid inhibitory characteristics.

Pharmacokinetic differences

A summary of pharmacokinetic properties of five ChEI is presented in Table 6 [17, 20, 25, 40–42]. Several important differences are apparent with regard to metabolism as well as other characteristics. While tacrine, galantamine and

Table 6. Comparison of pharmacokinetic properties of cholinesterase inhibitors after oral dosage in humans*

Drug	Plasma conc. (ug/L) C _{max}	Time to peak (h) T _{max}	Elimin. 1/2 life (h) T _{1/2}	Metabolism
Tacrine	—	1–2	2–4	hepatic (P450)
Donepezil	30–60	3–4	73	hepatic (P450)
Rivastigmine	114	1.7–1	5	non-hepatic
Metrifonate	500	0.5	2	non-hepatic
Galantamine	543	0.5	4.4–5–7	hepatic (P450)

*Modified from [20].

donepezil are metabolized through the hepatic route (P-450), rivastigmine and metrifonate are not. This difference is clinically important since elderly patients show decreased hepatic metabolism and therefore drugs not hepatically metabolized should be preferred. Another important characteristic is the difference in drug elimination with a half life (T_{1/2}) between 2 to 73 hours. Such differences are important as a short T_{1/2} reduces the time of exposure of the peripheral pool of ChE to the inhibitor decreasing side-effects. Galantamine and metrifonate have maximal bioavailability (100% and 90% respectively) and lowest plasma protein binding (10% and 20% respectively). With 96%, plasma protein binding is highest for donezepil. Since elderly patients are generally treated with several drugs simultaneously, this factor is of particular interest in relation to drug interactions.

Pharmacokinetic properties may also cause important differences in efficacy and severity of side-effects. To maximize therapeutic central effects and to minimize peripheral (bronchial, muscular, gastro-intestinal and cardiac) side-effects, elimination half life should be short (around 1–2 hours). The effective dose should be low but able to produce a substantial CNS enzyme inhibition (60–80%) and a steady (small diurnal/nocturnal variations) and long-lasting (several days) level of inhibition. Irreversible ChEI (such as metrifonate) satisfy such criteria more closely than reversible ones.

Long-term effects of cholinesterase inhibitors

Prolonged clinical efficacy of ChEI is deduced from the two observations. Firstly, if drug treatment is interrupted, the cognitive effect may continue for three to four weeks even in the absence of ChE inhibition. Secondly, as mentioned above, treatment benefits can be maintained in a number of patients for at least one year (Fig. 3). This suggests that ChEI effect may not be related exclusively to an elevation in brain acetylcholine levels but also to a modification of the amyloid-linked pathology in AD.

Clinical data support a stabilizing effect of cholinesterase inhibitors

The benefit of ChEI treatment has been previously considered to be exclusively symptomatic and cognitive. It has now been demonstrated that improvement involves cognitive as well as behavioral symptoms [43, 44]. In many patients, cognitive improvement is significant up to 12 months (Fig. 3). Several clinical studies have demonstrated that the drug effect in many patients can be seen for as long as two years (Fig. 3, Table 7) [45–56]. This long-term effect translates into improved activity of daily living of the patient and reduced emotional impact for the caregiver as well as in reduction in care costs. Two year open-label data from a donepezil trial reveal a decline in ADAS-Cog from the base line 50% lower than the predicted value [56]. Untreated patients progress more rapidly than treated ones and the treatment effect seems to be related to the dose [56]. Average annual rate of decline for patients with a higher dose of rivastigmine is almost 50% lower (4.5 ADAS-Cog points/year) than that of patients treated with a lower dose (8.2 ADAS-Cog points/year) [56]. Increasing the dose of rivastigmine reduces the rate of cognitive decline over a three year period suggesting a reduction in the rate of progression of cognitive deterioration [50, 55]. Clinical data also indicate that rapidly progressing patients show the strongest drug effect, therefore, both disease stage and dose of the ChEI seem to play a role in altering the course of the disease [56]. Stabilization of cognitive deterioration suggests either a protective and struc-

Table 7. Long-term efficacy of cholinesterase inhibitors in Alzheimer's disease patients

References	Drug	Number of participants	Maximum treatment duration/year	Cognitive test paradigms	Benefit difference
[45, 54, 55]	Donepezil	1600	2	ADAS-COG	positive
[46]		133	4.9	ADAS-COG, CDR	Positive
[47]		431	1	ADFA-C-CDR	Positive
[48]		286	1	GBS-MMSE	Positive
[49]	Tacrine	25	1	MMSE-EEG	Positive
[50]	Meflunitone	432	1	ADAS COG-CIBIC plus, MMSE	Positive
[51, 56]	Rivastigmine	2149	2	ADAS-COG, MMSE, CIBIC+, GDS.	Positive
[52]	Galantamine	44	3	ADAS-COG	Positive
[53]		636	1	ADAS-COG	Positive

Studies [47, 48, 50] are prospective, placebo controlled, double blind.

Total number of study participants = 4258. "Positive" indicates statistically significant clinical improvement from baseline for that specific measure.

tural effect or a long-term improvement of cholinergic synaptic function. The gradual return to the predicted deterioration-line after wash-out of the drug also suggests additional non-cholinergic effects [51].

Long-term stabilizing effects of cholinesterase inhibitors

Recent data from 12–24 month open trials and one randomized placebo-controlled trials suggest that optimization and maintenance of clinical effects for two years or more is a feasible goal in many patients (Tab. 7). Figure 3 reports the effect on the mean change in ADAS-Cog score of a 12 month treatment with four ChEI in clinical use (donepezil [44], galantamine [52], rivastigmine [50], metrifonate [49]). At twelve months, the data show no statistical difference from base line for all four ChEI (Fig. 3). The results of several clinical studies (placebo-controlled and open label) for periods longer than one year (up to three years) are reported in Table 7 [45–56]. These data indicate that benefit differences can be maintained in a number of patients for up to 12–24 months for five different inhibitors (donepezil, tacrine, metrifonate, rivastigmine and galantamine). In terms of global improvement in the ADAS-Cog score, this may sum to a total 15–20 point gain which represents a 18–24 month difference in disease history from placebo-treated patients. How to interpret this improvement? Is it the result of slowing down increase of disability or is it an expression of delaying progression of the disease?

Pre-clinical data supporting a non symptomatic effect of cholinesterase inhibitors

The amyloid precursor protein (APP) pathway which generates (A-beta)-amyloid is regulated by the sequential action of three enzymes (alpha, beta and gamma secretases). Alpha secretase cleaves APP within the A-beta sequence and releases soluble N-terminal non-aggregating fragments (sAPP). Numerous studies have shown that stimulation of sAPP release is associated with reduced formation of amyloidogenic peptides. Muscarinic-agonist induce sAPP secretion through activation by carbachol of m₁ and m₃ (but not m₂ and m₄) receptor subtypes increases sAPP release in human embryonic cell lines [59]. Activation of the pathway that cleaves APP decreases the release of beta-A fragments and may slow down amyloid formation in the brain. On the basis of results obtained from rat superfused cortical slices demonstrating an increased release of sAPP in response to muscarinic stimulation, we proposed an effect of ChEI on sAPP secretion acting through the same pathway [57].

Racchi et al. [58] using neuroblastoma cells and Pakaski et al. [61] using primary cultures of rat basal forebrain neurons have shown that short-term treatment with reversible and irreversible ChEI such as ambenonium and metrifonate or its metabolite DDVP increases sAPP release into the conditioned

media and elevates levels of protein kinase C (PKC). These studies have demonstrated that this effect on APP is consistent with AChE inhibition and with indirect muscarinic-mediated cholinergic stimulation. In addition, short-term or long-term stimulation do not result in changes in APP mRNA expression either in cortical slices or neuroblastoma cells [57–59] and in down-regulation of the response to cholinergic stimulation of muscarinic receptors [60]. These results suggest that ChEI promote the non-amyloidogenic route of APP processing through a stimulation of alpha-secretase activity mediated through PKC [61]. This demonstrated feature of ChEI and of muscarinic agonists and their ability to enhance the release of non-amyloidogenic soluble derivatives of APP *in vitro* and *in vivo* suggests a slowing down in the formation of amyloidogenic compounds in brain of AD patients [20, 57, 59, 62].

Effects of cholinesterase inhibitors on A β metabolism independent of cholinesterase inhibition

Recent data demonstrate that the effect of ChEI on A-beta metabolism does not necessarily relate to ChE inhibition [63]. Phenserine, a carbamate-type of ChEI, reduces beta-APP levels *in vivo* and also decreases secretion of soluble beta-APP and beta-A into the conditioned media of human neuroblastoma cells. However, phenserine action is neither mediated through classical receptor signalling pathway, involving extracellular signal-regulated kinase or phosphatidylinositol 3-kinase activation, nor is associated with ChE inhibition. Phenserine reduces A-beta levels by regulating beta-APP translation by a putative interleukin-1 or TGF-beta responsive element located within the 5'-UTR [63]. This effect is translated in the cortex of transgenic mice (APPswc + PSI) with a 53% reduction of A-beta 1-42 peptide which is evident following a three week treatment with phenserine [64]. These results suggest that phenserine regulates APP protein expression at the post-transcriptional level as it suppresses APP protein expression without altering message levels.

Figure 4 summarizes the relationship between AChE and beta-amyloid in the brain of AD patients [62]. Accordingly, AChE which is present in a glycosylated form associated with the amyloid core of neuritic plaques [64, 65] is stimulated in its synthesis by beta-amyloid. A-beta stimulates beta-amyloid accumulation in or near to the plaque [65–68] (Fig. 4). An indirect support for this hypothesis is the finding of Rees et al. [69] that double transgenic mice over-expressing human amyloid precursor protein and human AChE develop earlier and larger plaque burden than single transgenic animals. On the other hand, AChE is increased in the brains of transgenic mice expressing the C-terminal fragment of the A-beta precursor protein [70]. These results suggest that a direct interaction between A-beta and AChE can accelerate and promote amyloid deposition in brain. Therefore, drugs designed to target this particular protein-protein interaction could retard progression of AD.

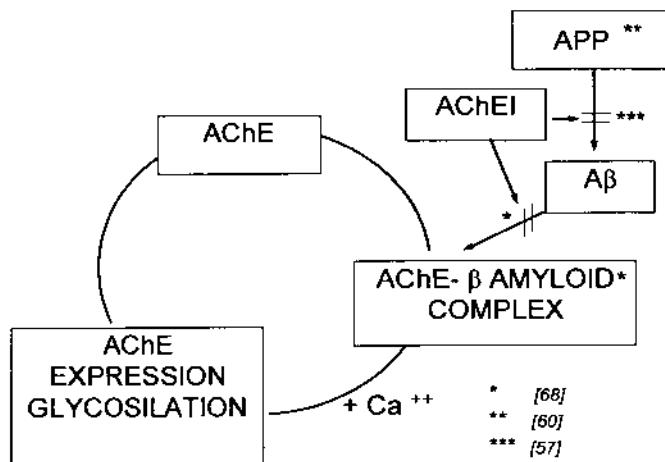


Figure 4. Proposed beta-amyloid cycle. AChE co-localizes with beta-amyloid and accelerates beta-amyloid formation and deposition in AD brain. Beta-amyloid increases AChE and in brain. Inhibition of AChE activity inhibiting amyloid precursor protein (APP) release, reduces beta-amyloid deposition. Inhibition of AChE activity by decreasing APP release, or by AChE-beta-amyloid interaction reduces beta-amyloid deposition (see text). This mechanism could contribute to the patient long-term cognitive stabilization seen during AChEI treatment.

New indications for cholinesterase inhibitor therapy

Treatment of mild cognitive impairment (MCI)

A natural challenge to the findings of a prolonged effect of ChEI is to investigate whether or not early treatment may alter the course of the disease by delaying its clinical onset. This approach could be of interest in subjects "at risk" such as asymptomatic members of familial AD pedigrees or in persons with mild cognitive impairment [71, 72]. As the definition of MCI is going to differentiate into various sub-groups corresponding to various types of diagnosis, such as VD-MCI (MCI in vascular dementia) or PDD-MCI (MCI in Parkinson Disease Dementia), the treatment will also vary accordingly. Presently, only results of small studies are available suggesting a possible delayed onset of AD and VD with ChEI such as donepezil [73]. Two major multicenter studies on MCI and ChEI are in progress in US and Europe with donepezil and rivastigmine.

Dementia with Lewy bodies

Dementia with Lewy bodies (DLB) is thought to be the second most common cause of dementia after AD [74]. The disorder is characterized by progressive

fluctuating of cognitive impairment, visual hallucinations and motor features of parkinsonism. The disease presents itself from dementia with subsequent parkinsonian syndrome to parkinsonian syndrome with dementia. Studies have shown that neocortical cholinergic activity is more severely depleted in DLB than in AD, and that this deficit also affects the caudate nucleus, the thalamus and the brain stem [74]. It is likely this pattern is related to the occurrence of hallucinations characteristic for the disease. As typical neuroleptic treatment is contra-indicated in DLB, cholinergic therapy with rivastigmine has been investigated in 120 patients in a multicenter study with statistically and clinically significant effects on behavior [75]. At least 10 other smaller studies during the period 1998–2002 are supporting this beneficial effect of ChEI on DLB patients. Clinical data show that tolerability of ChEI in DLB appears similar to AD with some gastrointestinal effects and muscle cramps. Tremor may be present at higher dose. Particularly responsive to ChEI treatment are hallucinations and delusions.

Parkinson's dementia

Parkinson disease (PD) patients may suffer from cognitive impairment and behavioral problems such as apathy, personality changes and visual hallucinations. There is currently no recommended treatment for PD dementia (PDD). The observation that PDD patients have extensive cholinergic deficits led to the hypothesis that ChEI may provide benefits for patients with this condition. The treatment of PDD with ChEI (tacrine, donepezil, rivastigmine) has shown variable results from general improvement of cognition and psychotic symptoms to no change or even worsening of motor responses. Although some authors have concluded that ChEI have a beneficial effect on the cognitive state of PDD patients and do not aggravate motor function, larger studies are needed in order to demonstrate a clear benefit [76].

Vascular dementia

Vascular dementia (VD) accounts for approximately 20–30% of dementia cases and there is a large degree of clinical and pathological overlap between VD and AD. The presence of a cholinergic deficit in VD, similar to that observed in AD, is suggested by reductions in cholinergic markers. Enhancing the availability of endogenous acetylcholine by inhibition of cholinesterase increases cerebral blood flow. Clinical trials with all three inhibitors (donepezil, rivastigmine and galantamine) have shown that this effect is possible (Tab. 8).

One first attempt to treat vascular dementia with a ChEI (rivastigmine) was published with encouraging results [77] and, more recently, positive findings have been reported in the treatment of vascular and mixed dementia with galantamine [78], donepezil [79] and rivastigmine for 12 months [80].

Table 8. Vascular dementia: Treatment with cholinesterase inhibitors

Drug	Clinical effect	Reference
Rivastigmine	Improvement in cognition and behavior	[77, 80]
Donepezil	Cognitive effect	[80]
Galantamine	Cognitive and functional effect	[79]

However, clinical evaluation of these cases remains difficult in the absence of consensus on valid criteria for the diagnosis of vascular dementia.

Down's syndrome

Cholinesterase inhibitor treatment might also be indicated in Down's syndrome [81]. Genetic (chromosome 21), neuropathological and neurochemical similarities between Down's syndrome and AD as well as the presence of cognitive impairment, have motivated the use of cholinergic therapy in this disorder. Four published trials on a small number of patients (3–30) for a period of 8–40 weeks demonstrated a decrease in confusion and improvement of cognition [82–85] (Tab. 9).

Table 9. Down's syndrome: Effect of donepezil

Reference	Number of patients; duration; type of trial	Clinical effect	Side effects
[82]	3 patients; 8–24w; Open	Decreased confusion	Agitation incontinence
[83]	9 patients; 40w; open	Cognitive improvement	
[84]	30 patients; 24w; double blind placebo	Cognitive improvement	G.I.
[85]	6 patients; 24w; double blind placebo	Cognitive improvement	G.I.

Traumatic brain injury

Traumatic brain injury is the most common cause of death in subjects under the age of 40. Loss of hippocampal cells and reduction of acetylcholine levels and of muscarinic receptors can be attenuated in experimental animals by using ChEI such as rivastigmine [86]. The drug improves blood perfusion in

ischemic areas and cholinergic transmission in cortex and hippocampus by increasing cholinergic activity in cerebral vessels [86]. This is the same mechanism invoked for treatment of VD. Several small trials with acute physostigmine treatment [87, 88] or chronic treatment (three week to two years) with donepezil have shown improvement in attention, verbal memory, general cognition and behavior [89–91] (Tab. 10).

Table 10. Traumatic brain injury: Treatment with cholinesterase inhibitors

Reference	Drug	Number of patients and duration of treatment	Clinical effect
[87]	Physostigmine	1 patient, acute	Improved verbal memory
[88]	Physostigmine	16 patients/6 weeks	Improved attention
[89]	Donepezil	2 patients/3 weeks	Improved memory
[90]	Donepezil	53 patients/2 years	Improved cognition
[91]	Donepezil	4 patients/16 weeks	Improved memory and behavior

Korsakoff's disease

Wernicke-Korsakoff disease is characterized by an amnestic state resulting from selective lesions in the limbic system with impairment of episodic memory. Basal forebrain nuclei are also affected. The aetiology is thiamine deficiency in alcoholic patients. In two of the three studies performed with donepezil, an improvement in memory has been reported [92–94]. However, the number of treated patients is too small to show evidence of clinical effect.

Delirium

Delirium is a common complication of dementia with fluctuation of attention and consciousness which may produce considerable morbidity. It is not always reversible and there is no specific treatment. The "scopolamine type" of dementia is an acute cognitive disorder which could be classified as a scopolamine-induced delirium in old age triggered by a muscarinic antagonistic effect. Most often, delirium is a consequence of a general medical condition and the direct cause of this syndrome is unknown. Cases of post-narcotic delirium, somnolence or coma being interpreted as central cholinergic syndromes have been reversed by using physostigmine [95]. More recently delirium of various origin (dementia, opioids, lithium etc.) has been reversed with ChEI such as donepezil or rivastigmine [96–99] (Tab. 11). Based on these results, it would be of great interest to test the effect of ChEI administered prior to general anaesthesia to prevent the occurrence of delirium.

Table 11. Delirium: Treatment with cholinesterase inhibitors

Reference	Drug	Etiology	Clinical effect
[95]	Physostigmine	Midazolam, fentanyl, other	5 patients, reversal
[99]	Donepezil	Dementia	1 patient, reversal
[96]	Donepezil	Opioids	6 patients, decreased sedation
[97]	Rivastigmine	Lithium	Reversal
[98]	Various ChEI	Various origins	Reversal

Migraine

The antinoceptive activity of donepezil which is prevented by muscarinic antagonists has been demonstrated in experimental animals. The analgesic effect of donepezil was investigated in patients suffering from migraine. The drug was effective in reducing the number of attacks and severity of pain [100].

Can cholinesterase inhibitors be switched or combined?

According to the report of the British National Institute for Clinical Excellence (NICE) about 50% of the patients treated with ChEI (donepezil, rivastigmine and galantamine) show evidence of improvement. This is probably an underestimation depending on the low sensitivity of the outcome measures particularly when they are applied to short-term (three months or shorter) observations. The decision to discontinue the treatment is based on early intolerable side-effects or lack of an initial therapeutic effect, loss of therapeutic effect during long-term treatment or occurrence of tolerability problems. As a result, patients are often tried on one drug only and if unsuccessful, this is a definitive trial. This decision is taken in spite of the fact that clinical experience shows that when a particular drug is ineffective or poorly tolerated, another agent belonging to the same pharmacological class may still result effective and safe. An open retrospective study of patients in the United Kingdom showed that 55% of patients who could not tolerate donepezil due to side-effects or did not show a sustained therapeutic effect, benefited from treatment with rivastigmine [101]. These results suggest that both therapeutic effect and side-effects of donepezil and rivastigmine may be independent of one another.

Several switching studies have confirmed that over 50% of patients may benefit from treatment with a second ChEI [102, 103]. A *post hoc* analysis of a five-month trial showed that a previous failure to respond to a ChEI did not predict response to a second one (galantamine) [104]. The same result was obtained switching from memantine, an NMDA antagonist, to donepezil supporting the idea that the mechanism of action of the two drugs may be different [105].

Switching from one ChEI to another is worthwhile when intolerable side-effects are present or the first drug has failed to prove effective following a period of time of several months testing.

Switching should not be considered when the patient responds and have no tolerability problems. Before switching, it is important to adjust the dose to an appropriate range.

Clinical experience has shown that switching to attain these potential benefits can take place without the need for an extensive washout period, beginning with the recommended starting dose of the second agent. If the patient is experiencing tolerability problems from the first drug, a washout period may be necessary. This procedure provides continuous cholinergic stimulation and ensures that AD patients are offered the maximum benefit of the drugs currently available. Little is known about benefits of switching between drugs of different pharmacological classes or combination. A recent study suggests that combination of a ChEI (donepezil) with memantine (an NMDA antagonist) is well tolerated and may show improved efficacy. These results need to be verified by more extensive double-blind studies.

General conclusions

This review illustrates that the number of therapeutic indications for ChEI has been growing since the first use of these drugs as cognitive enhancing treatments in AD almost two decades ago. Our knowledge of ChEI has also been growing in parallel with the increased clinical use of these drugs in AD patients. First, the possibility of using either selective BuChE inhibitors or dual-action AChE-BuChE inhibitors for cognitive improvement in AD or for newer indications represents a most recent development based on new knowledge of the role of BuChE in brain function. A second possibility of switching from one ChEI to another in order to increase the number of responders is now being investigated. A third possibility is to combine ChEI with non-cholinergic drugs such as memantine with the hope of achieving potentiation of mnemonic effects (also see Chapter by Jerry L. Buccafusco and Moussa B.H. Youdim). Why is therapy with ChEI efficacious in various types of CNS disorders having different etiologies?

Cholinergic systems are more numerous and more widely distributed in the brain than any other neurotransmitter system. As an example, the nucleus basalis of Meynert represents the most substantial regulatory system of the cerebral cortex, and the ascending cholinergic system alone is capable of maintaining the neocortex operative. In particular, cholinergic projections control selective attention and conscious awareness. The multifaceted effect of ChEI in various syndromes and diseases is therefore not surprising considering the major involvement of cholinergic transmission in CNS function at various brain localizations and neurotransmitters levels. Due to the central role of the cholinergic system and the variety of cholinergic functions that are

impaired in a number of brain disorders, it is plausible to consider that in the future, ChEI might be revealed to be more efficacious in CNS disorders other than in AD.

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Drugs that target muscarinic cholinergic receptors

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Cholinergic pathways

Six groups of cholinergic neurons have been identified in the central nervous system (Ch1–Ch6) [1]. Cholinergic neurons innervating the hippocampus and cerebral cortex arise from basal forebrain nuclei, including the medial septum (MS, Ch1 group), the vertical and horizontal limbs of the diagonal band (Ch2 and Ch3) and the nucleus basalis of Meynert (NBM, Ch4) [2]. Cholinergic neurons arising from the MS project mainly to the hippocampus, a brain region that plays an important role in learning and memory function. The diagonal band projections innervate the anterior cingulate cortex and the olfactory bulb, while the NBM projects to the amygdala and the cerebral cortex. The cholinergic projection from the NBM is widespread, covering the entire cortical mantle [3]. Basal forebrain cholinergic pathways have been implicated in attention, learning, memory and cognitive function. Moreover, the degeneration of basal forebrain cholinergic neurons is a consistent neurochemical hallmark of Alzheimer's disease.

Other important cholinergic projections originate in the laterodorsal tegmental nucleus (LDTN, Ch5) and the pedunculopontine tegmental nucleus (PPTN, Ch6) and project to various brainstem and midbrain regions, including the ventral tegmental area, a brain region that regulates forebrain dopamine pathways. Although the basal forebrain system has received much attention due to its involvement in Alzheimer's disease, the brainstem cholinergic system has been found to play important roles in sensory-motor gating and cognitive function [2].

Muscarinic receptors

Muscarinic receptors play important roles in the regulation of physiological responses to acetylcholine. Five subtypes of muscarinic receptor have been identified and each subtype has a unique tissue distribution and physiological function [4]. Although there are relatively few selective ligands for individual muscarinic receptor subtypes, the development of mice lacking specific mus-

carinic receptors has helped clarify the roles of each subtype [5]. M₁ receptors are found in the cerebral cortex and hippocampus and have been proposed to modulate cognitive function and memory processes. M₂ receptors are found in the heart, where they mediate the negative inotropic effects of acetylcholine. In the central nervous system, M₂ receptors serve as autoreceptors regulating acetylcholine release and mediate the analgesic effects of muscarinic agonists [6]. M₃ receptors stimulate contractions of smooth muscle and promote secretion from exocrine glands. M₄ receptors are found in the central nervous system and regulate the release of neurotransmitters, including dopamine [6]. M₅ receptors are present in very low abundance in the brain. Although the M₅ receptor protein has been difficult to identify in brain, M₅ receptor mRNA is found in the ventral tegmental area and substantia nigra [7, 8].

Muscarinic receptors belong to the large family of G protein-coupled receptors. M₁, M₃ and M₅ receptors couple to the stimulation of phospholipase C through the G_{q/11} family of G proteins. Activation of M₁, M₃ and M₅ receptors results in the activation of protein kinase C through formation of 1,2-diacylglycerol and the release of Ca²⁺ from the endoplasmic reticulum through generation of inositol-1,4,5-trisphosphate. In contrast M₂ and M₄ receptors inhibit adenylyl cyclase activity through activation of the G_{i/o} family of G proteins. Thus, M₁, M₃ and M₅ receptors are generally excitatory while M₂ and M₄ receptors are generally inhibitory, although the net effect of muscarinic receptor activation depends largely upon the integration of acetylcholine signaling within distinct brain pathways. For example, activation of M₄ receptors in the striatum results in increased dopamine release through the inhibition of striatal GABA release [9]. Because muscarinic receptors mediate their effects through activation of second messenger systems, muscarinic receptor stimulation results in a slower rate of onset and longer duration of action as compared with nicotinic receptor activation. As a result, muscarinic receptors are considered to play a modulatory rather than a direct stimulatory role in the central nervous system.

Neural networks and physiology

The central cholinergic system is a critical component of the cortical neural network. The basal forebrain cholinergic innervation of the cerebral cortex is diffuse and extends in a roughly topographical manner throughout the cerebral cortex [10]. Muscarinic receptors, particularly the M₁ receptor subtype, are found throughout the forebrain, including the neocortex [11]. Activation of cholinergic pathways results in the modulation of excitatory and inhibitory synapses, through regulation of on-going activity in glutamate and GABA systems. For example, activation of muscarinic receptors in the rat prefrontal cortex leads to enhancement of GABA_A receptor currents through activation of a protein kinase C-dependent tyrosine kinase pathway [12]. The increased

amplitude of GABA_A receptor currents is likely mediated by either M₁ or M₄ receptors. The activation of muscarinic receptors has been proposed to shift the dynamics of cortical networks into a mode whereby afferent inputs exert a stronger influence than intracortical pathways on neural activity [13, 14].

Muscarinic receptor subtypes are found throughout the hippocampus, with M₁ receptors expressed predominantly in pyramidal neurons of the hippocampus and granule cells of the dentate gyrus [15]. The cholinergic projection from the MS helps drive the expression of the theta rhythm revealed through electrophysiological recordings of the rodent hippocampus. The theta rhythm is most pronounced during active exploratory movement and REM sleep. Lesions of the MS and muscarinic antagonists impair the expression of the theta rhythm. Induction of the theta rhythm with cholinergic agonists such as carbachol enhances synaptic plasticity [16], suggesting a potential role for muscarinic agonists in the storage of new information.

Memory function

The role of acetylcholine in memory function has been well documented. Classical muscarinic antagonists such as scopolamine and atropine impair memory function in experimental animals and in humans. Lesions of basal forebrain cholinergic pathways with specific neurotoxins such as AF64A and 192 IgG-saporin also impair memory function [17–22]. The role of individual muscarinic receptor subtypes in memory and cognitive function has been limited by the availability of agonists and antagonists selective for muscarinic receptor subtypes.

With the development of mice that lack specific muscarinic receptor subtypes, the role of individual muscarinic receptor subtypes in cognitive and memory function is currently being explored, and some recent studies provide some insight. Nathanson's group studied cognitive and memory function in mice with a null mutation of the gene coding the M₁ receptor [23]. Interestingly, the mice exhibited a phenotype characterized by both enhancements and deficits in memory function. Mice lacking the M₁ receptor exhibited impairments in non-matching-to-sample working memory tasks, yet displayed normal or enhanced performances in matching-to-sample tasks. For example, memory was impaired in mice lacking M₁ receptors on the eight-arm radial maze task as evidenced by a significant increase in errors following a 60-minute delay as compared to wild-type controls. In contrast, mice lacking M₁ receptors performed as well as wild-type mice in the Morris water maze, a paradigm that measures spatial memory function. In addition, hippocampal long-term potentiation (LTP) associated with theta burst stimulation was significantly lower in mice lacking M₁ receptors as compared with wild-type controls. Other forms of LTP were relatively unaffected in mice lacking M₁ receptors.

Neurological disorders

Muscarinic receptors have been implicated in the pathophysiology of Alzheimer's disease and other neurological disorders, including schizophrenia and drug abuse. The cholinergic hypothesis of geriatric memory dysfunction focused attention on the role of acetylcholine in memory and cognition [24]. Numerous studies indicated a consistent loss of basal forebrain cholinergic neurons in Alzheimer's patients that appeared to correlate with cognitive and memory deficits [24, 25]. Recent studies have raised questions regarding the role that cholinergic degeneration plays in the disease process, since the loss of cholinergic enzymes such as choline acetyltransferase does not precede changes in cognition and memory function in early stage Alzheimer's patients [26]. Despite such concerns, compounds that activate cholinergic systems represent a viable approach for the treatment of Alzheimer's disease [25]. As of this writing, acetylcholinesterase inhibitors are the only compounds approved for the treatment of the symptoms of Alzheimer's disease. The development of compounds that activate post-synaptic receptors directly could provide a distinct advantage over acetylcholinesterase inhibitors since activity is dependent on the integrity of cholinergic nerve terminals for the production of acetylcholine.

M_1 receptors have been a major target for drug development over the past decade based on their preferential localization in the forebrain, the potential utility of muscarinic agonists in the treatment of Alzheimer's disease and the adverse side-effects associated with activation of other receptor subtypes [24, 27-29]. Although acetylcholinesterase inhibitors have been utilized in treating the symptoms of Alzheimer's disease, efforts to develop selective muscarinic agonists have met with limited success.

Two main problems have been encountered with the muscarinic agonists tested in the clinic. First, many of the compounds lacked efficacy at M_1 receptors in the central nervous system [30]. A number of compounds were identified using muscle preparations from peripheral tissue such as rabbit vas deferens, where a population of M_1 receptors is located [31]. Activity in the rabbit vas deferens assay did not always translate into activity at M_1 receptors in brain [30]. As a result, some compounds did not exhibit efficacy in clinical studies. Second, many compounds exhibiting strong M_1 agonist activity lacked functional selectivity for M_1 receptors. These compounds produced significant side-effects in clinical studies, most notably nausea, salivation and diaphoresis due to activation of M_3 receptors. One compound, xanomeline, displayed relatively high efficacy and selectivity for M_1 receptors. Although xanomeline exhibited some utility in treating the symptoms of Alzheimer's disease in clinical trials, side-effects associated with metabolism of the parent compound limited its utility.

Despite the difficulties, selective muscarinic agonists could be useful in treating not only the symptoms, but also the underlying cause(s) of Alzheimer's disease. Muscarinic agonists promote the release of soluble forms

of amyloid precursor protein through the activation of α -secretase [32–34]. Furthermore, muscarinic agonists activate the Akt/protein kinase B cell signaling pathway [35, 36], which leads to protection from apoptosis, limits glycogen synthase kinase-3 β activity and prevents hyperphosphorylation of tau proteins [37]. Clinical studies with talsaclidine and cevimeline have demonstrated that muscarinic agonists can alter cerebrospinal fluid (CSF) levels of A β in Alzheimer's patients [38, 39]. Unfortunately, all of the muscarinic agonists tested thus far suffer from a lack of efficacy and/or selectivity, which greatly limits their therapeutic potential. The high degree of amino acid homology among the five receptor subtypes, particularly within the trans-membrane domains, has likely contributed to difficulties in developing selective muscarinic agonists.

Beyond potential usefulness in the treatment of Alzheimer's disease, muscarinic agonists could be useful in treating other neurological disorders, including schizophrenia and chronic pain [40, 41]. The M₁/M₄ agonist xanomeline produced modest effects on cognitive function, and dramatically decreased the incidence of hallucinations in Alzheimer's patients. Unfortunately, xanomeline is rapidly metabolized *in vivo*, resulting in non-selective effects associated with a high level of side-effects mediated by M₃ receptors.

The potential utility of muscarinic agonists in treating cognitive deficits associated with schizophrenia has received increased interest. Cognitive deficits such as behavioral praxis, verbal memory and verbal fluency impairments are relatively common in schizophrenia patients. Muscarinic agonists might be particularly helpful in treating cognitive deficits associated with schizophrenia. As noted by Hyman and Fenton [42], the cognitive aspects of schizophrenia have been largely ignored by the pharmaceutical industry as evidenced by the low number of clinical trials addressing cognitive function in patients with schizophrenia. The development of muscarinic agonists as cognitive enhancers may provide important symptomatic relief for patients with schizophrenia.

Muscarinic agonists

Classical muscarinic agonists such as arecoline and pilocarpine improve memory and cognitive function in experimental animals. Unfortunately, most of the classical muscarinic agonists are non-selective and suffer from a short duration of action. Over the past twenty years, several selective muscarinic agonists have been identified and some have been tested in clinical trials for their ability to enhance memory and cognitive function in Alzheimer's patients.

Cevimeline, among the first selective M₁ agonists to be identified, is a quinuclidine derivative that enhances memory function in both rodents [43] and primates [44]. Cevimeline has been approved for the treatment of xerostomia associated with Sjögren's disease. Derivatives of cevimeline also display cog-

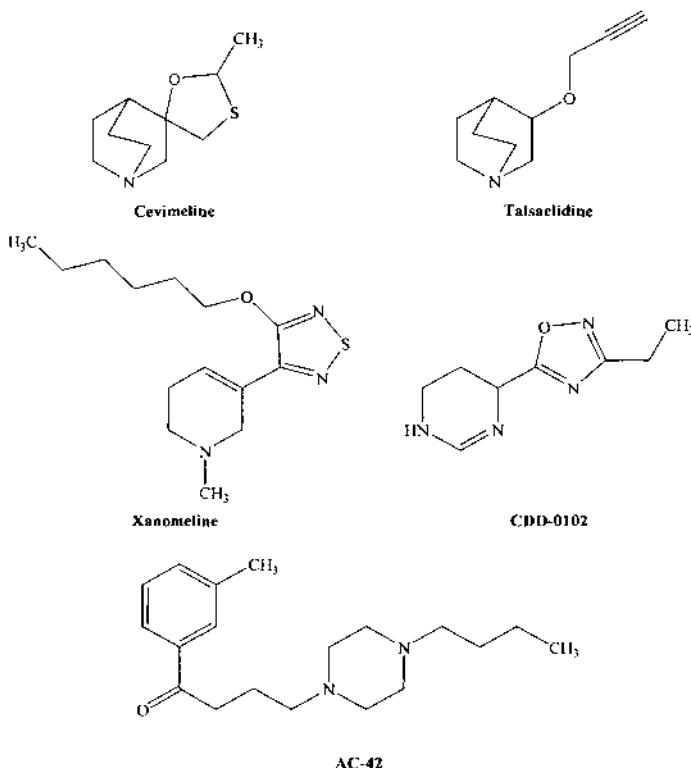


Figure 1.

nitive enhancing effects in rodents exhibiting cognitive impairments [45]. Another quinuclidine derivative, talsaclidine, also enhances memory function in the non-match-to-sample eight arm radial maze task in rodents [46] and in a delayed match-to-sample task in primates [46, 47].

Xanomeline shows functional selectivity for M_1 receptors [48], and enhances memory function on a paired-run alternation task in rats [22]. Xanomeline produced some cognitive benefits in Alzheimer's disease patients, particularly on the cognitive subscale of the Alzheimer's disease Assessment Scale (ADAS-Cog) [49, 50], although unwanted side-effects greatly limited its therapeutic utility. The novel amidine derivative, 5-(3-ethyl-1,2,4-oxadiazol-5-yl)-1,4,5,6-tetrahydropyrimidine (CDD-0102), also exhibits functional selectivity for M_1 receptors [21, 51] and enhances memory function on a paired-run alternation task in rats [48]. CDD-0102 displays a low side-effect profile in rodents, but its functional selectivity in humans remains untested.

The clinical utility of muscarinic agonists will depend on the development of compounds with improved selectivity for individual muscarinic receptor subtypes. Recent studies with bivalent derivatives of xanomeline have suggested a new approach for the design and development of truly selective mus-

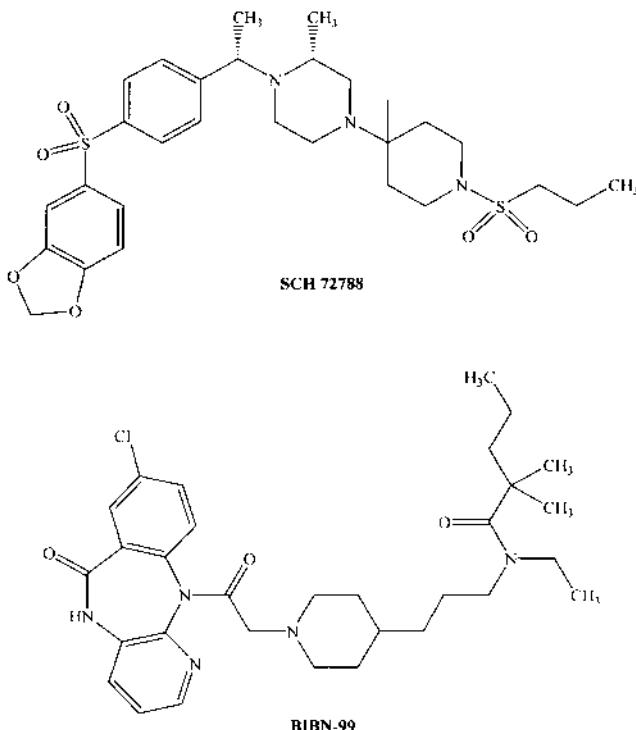


Figure 2.

carinic agonists [52, 53] by taking advantage of the relatively low homology within the extracellular loops of muscarinic receptor subtypes. While small molecules interact mainly with highly conserved residues buried within the transmembrane domains, larger molecules can interact with potentially unique accessory binding sites on different receptor subtypes. Small structural modifications of the bivalent ligands yield compounds with agonist activity and selectivity for M_1 versus M_3 or M_5 receptors [52, 53]. These compounds serve as useful lead compounds for the next generation of selective muscarinic agonists.

Using a high-throughput functional screening approach to identify M_1 agonists, Spalding and colleagues [54] have characterized a unique compound (4-n-butyl-1-[4-(2-methylphenyl)-4-oxo-1-butyl]-piperidine, AC-42) that appears to bind to an ectopic site on M_1 receptors. The screening approach utilized assesses agonist activity by measuring cell growth, rather than screening for interaction with receptors by displacement of radiolabeled antagonists in traditional receptor binding assays. In this manner, compounds can be identified that do not compete for the acetylcholine binding site, and thus may exhibit receptor subtype selectivity. The compound identified through the novel screening approach activates M_1 receptors yet does not display significant

activity at other muscarinic receptor subtypes. Such compounds may have distinct advantages over compounds identified through more classical drug development approaches.

Muscarinic antagonists and allosteric modulators

M_2 receptors are located on presynaptic fibers of septohippocampal neurons [15], and regulate the release of acetylcholine from cholinergic terminals [55]. Selective M_2 antagonists might be helpful in enhancing cognitive function by elevating acetylcholine release. Over the past several years, a few selective M_2 antagonists have been identified, including 4-[4-[1(S)-4-[(1,3-benzodioxol-5-yl)sulfonyl]phenyl]ethyl]-3(R)-methyl-1-piperazinyl]-4-methyl-1-(propylsulfonyl)piperidine (SCH 72788) [56, 57], which enhances acetylcholine release from the striatum and improves cognitive function in a passive avoidance paradigm in rats. Similarly, BIBN-99 reverses impairments of acetylcholine release and cognitive function in age-impaired rats [58], and enhances learning of a spatial memory task in the Morris water maze [59]. Selective M_2 antagonists may have an advantage over directly acting muscarinic agonists since they indirectly modulate on-going cholinergic activity, thereby permitting activation of cholinergic receptors in a context-dependent manner [60, 61].

A wide variety of compounds modulate muscarinic receptors through interaction with allosteric binding sites, which are separate from the binding site for acetylcholine and other muscarinic agonists and antagonists. Binding to an allosteric site can enhance (positive co-operativity) or reduce (negative co-operativity) the affinity of other molecules for the receptor. For example, gallamine exhibits strong negative co-operativity with muscarinic agonists and antagonists at M_2 receptors [62]. Over the past few years, a few strychnine and brucine derivatives have been identified with positive co-operativity at muscarinic receptor subtypes [63]. Although additional work is needed to optimize affinity, selectivity and CNS penetration, such compounds could be useful in enhancing on-going cholinergic activity in a selective manner, and serve as cognitive enhancers [64].

Summary

In summary, muscarinic receptors play important roles in the expression of memory and cognitive function. Selective muscarinic agonists may be useful in enhancing cognitive function in normal individuals and in patients with neurological disorders such as Alzheimer's disease and schizophrenia. In addition, M_2 -selective antagonists could be beneficial as cognitive enhancers through the stimulation of acetylcholine release, while allosteric modulators might enhance on-going activity at muscarinic receptor subtypes. The development

of selective muscarinic agonists with improved selectivity for M₁ and M₄ receptors remains an important goal for drug discovery efforts.

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Rationale and prospects for drugs that target nicotinic acetylcholine receptors

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Introduction

Nicotinic acetylcholine receptors (nAChRs) are implicated in a variety of disorders of the human central nervous system including addiction to nicotine, Alzheimer's disease, anxiety, autism, depression, epilepsy, Parkinson's disease, schizophrenia, and Tourette's syndrome [1, 2]. Mechanisms of nAChR impairment in this disparate group of syndromes are poorly understood. Additionally, in healthy organisms nAChRs play a significant role in a number of cognitive processes including learning and memory [3, 4]. Because nAChRs are involved in normal cognitive processes as well as a complex range of central nervous system disorders, it is important to define the means by which these receptors exert their action in the brain and interact with disease-related neuropathology. It is also imperative to explore the prospects of therapeutic manipulations of nAChRs in human central nervous systems disorders.

The characteristic structure of nAChRs is a ring of five subunits arranged around a ligand-gated excitatory ion channel. Neuronal nAChR subunits are classified as α ($\alpha 2$ to $\alpha 10$) and β ($\beta 2$ to $\beta 4$) subunits. The two main neuronal categories that have been identified on the basis of function and pharmacology are: (1) heterologous pentamers, constructed from combinations of α - and β -subunits [5], and (2) homologous pentamers, constructed from one subunit type, $\alpha 7$, $\alpha 8$, and $\alpha 9$ [6]. Besides the $\alpha 4\beta 2$ nAChRs that constitute the high affinity binding sites for nicotine [7], the $\alpha 7$ nAChRs being highly permeable for Ca^{2+} are expressed widely and abundantly in the mammalian brain [8, 9]. The $\alpha 7$ nAChRs produce multiple effects at the cellular level including presynaptic modulation of neurotransmitter release [10] and post-synaptic generation of depolarizing currents [11].

The various types of nAChRs have characteristic patterns of distribution in the brain, and they have several loci on neurons, including on terminals, soma, and dendrites [12]. Neuromodulation of communicative processes in the brain is one role for nAChRs [13]. The demonstrated involvement of nAChRs in

cognitive processes, the dramatic cognitive impairment apparent in Alzheimer's disease (AD), and the mounting number of AD patients in the world are among the compelling rationales for investigating nAChRs in AD. The role of nAChRs in AD and the potential for drug therapies in this neurodegenerative disease is the focus of this chapter. Studies of nAChR subtypes in the brains of patients with AD indicate that nAChRs that participate in high-affinity nicotine binding (e.g., $\alpha 4\beta 2$) are substantially reduced but those binding to α -bungarotoxin (e.g., $\alpha 7$) are better maintained (for a review, see [14]). However, neuropathology may interact with the nAChR subtypes that survive in the AD brain and render them functionally ineffective.

Relationship of β -amyloid (A β) and nicotinic acetylcholine receptors (nAChRs)

AD is neurochemically characterized by cholinergic dysfunction, in particular affecting the nAChRs, and neuropathologically by intracellular neurofibrillary tangles and the extracellular β -amyloid (A β) plaques (for a review, see [14]). However, finding the possible link between these markers has remained elusive. Although increasing numbers of reports suggest that A β can potently inhibit various cholinergic transmitter functions independently of apparent A β neurotoxicity (for review cf. [15]), little attention has been paid to the cholinoreactive site. Nowadays there are numerous indications that chronic exposure to nicotine may protect against A β pathology (cf. [16]). In addition, it was shown recently that nicotine not only dose-dependently inhibited fibril formation from A β_{1-40} and A β_{1-42} but also disrupted preformed fibrillary A β_{1-42} *in vitro* [17]. Although epidemiological studies of tobacco use and risk of developing AD are somewhat contradictory [18, 19], a significant lower plaque density has been observed in autopsy cortical tissue of smokers as compared to non-smokers [20, 21].

In vitro studies have also indicated protective effects of nicotine against A β toxicity ([22, 23], for review cf. [24, 25]). Initial evidence for an interaction of A β with nAChRs was provided by the finding that stimulation of the $\alpha 7$ and $\alpha 4\beta 2$ nAChR subtype in cultured rat cortical neurons was able to inhibit A β cytotoxicity [22, 23]. Furthermore, Alzheimer patients carrying the Swedish amyloid precursor protein 670/671 mutation and early and excessive accumulation of A β plaques were shown to display nAChR deficits [26]. In the meantime, numerous studies have investigated the relationship of A β and nAChR expression and the mechanisms that may be involved in this interaction.

So far only few reports on human brain have addressed potential links between AD neuropathology and cholinergic dysfunction. Our own previous studies on autopsic human cortex combining *in situ* hybridization and immunohistochemistry revealed that in the frontal cortex neurons expressed $\alpha 4$ as well as $\alpha 7$ mRNA even in the vicinity of A β -immunoreactive plaques [27]. The density of $\alpha 4$ and $\alpha 7$ transcript expressing neurons was not altered

in AD. This finding is confirmed by other studies [28, 29] and may point to a lack of the impact of A β plaques on nAChR expression. By contrast to the findings on the mRNA level there is a decrease of nAChR subunit proteins in AD. Several groups by using immunohistochemistry, Western blot, and binding studies reported on a massive reduction of the $\alpha 4$ subunit, whereas less consensus exists regarding the decrease of the $\alpha 7$ subunit protein (for review see [27, 30]).

To investigate the possible impact of A β on nAChR protein expression we approached co-localization studies by two different ways. First, we combined silver staining techniques to visualize plaques with immunohistochemistry to show nAChR expressing cells. Second, we applied a double-immunohistochemical protocol. With both methods we were able to demonstrate the presence of nAChR-immunoreactive neurons in close proximity to and sometimes even within plaques. Therefore it seems to be rather unlikely that plaques interfere with nAChR expression. This result is confirmed by an investigation demonstrating no correlation of the plaque load in AD patients and nicotine binding, whereas an inverse correlation between [3 H]epibatidine binding and A β_{1-42} levels was found [21]. In addition, studies in transgenic mice [31–34] support the emerging view for a plaque-independent A β toxicity in the development of synaptic deficits in AD. The positive correlation between the decrease of synaptophysin immunoreactivity and [3 H]epibatidine binding [35] complements the picture.

The increasing evidence that soluble A β plays a more important role in the development of AD and the general acceptance that A β_{1-42} is the pathogenic A β peptide [36] led to the development of various *in vitro* model systems to study the impact of A β on nAChR expression under standardized conditions. Using primary hippocampal cultures grown under defined serum-free conditions it was shown that incubation for three days with 1 μ M A β_{1-42} caused severe morphological alterations: Neuronal cell bodies appeared to be shrunken and a remarkable retraction of dendrites as well as a loss of dendritic density was observed. The reduction of labelled processes was most prominent in the $\alpha 4$ -immunoreactive neurons, whereas $\alpha 7$ -expressing neurons seemed to be less affected (cf. [30]). Comparable morphological changes occurred after incubation with 0.5 μ M A β_{1-42} . This different behaviour may indicate a sub-unit-dependent impact of A β_{1-42} on nAChR expression.

Interaction of A β with nAChRs

The underlying mechanism of nicotinic transmission and A β interaction remained unclear until recently when investigations of Wang and co-workers shed light on this issue [37, 38]. They found that A β_{1-42} binds specifically and with picomolar affinity to the neuronal $\alpha 7$ nAChRs. So far numerous studies provide evidence for a functional block of acetylcholine (ACh)-evoked current responses by A β_{1-42} via the $\alpha 7$ nAChR-subtype in various types of cells and

in $\alpha 7$ -transfected oocytes [39–41]. At complete variance with these data, 10 pM of non-fibrillar $A\beta_{1-42}$ have been reported to activate rat $\alpha 7$ nAChRs expressed in *Xenopus* oocytes, although only upon the very first exposure, whereas a blockade of the $\alpha 7$ nAChR only occurred after application of 100 nM $A\beta_{1-42}$ [42]. An activation of $\alpha 7$ nAChRs by $A\beta_{1-42}$ was described for *Xenopus* oocytes transfected with mutant rat and human $\alpha 7$ cDNA carrying a point mutation within the pore-forming region (rat: L250T [42], human: L248T [41]). The latter finding is in good agreement with data obtained from studies with mutant $\alpha 7$ nAChR receptors carrying the particular threonine-for-leucine substitution showing that several antagonists of chick and human wild-type $\alpha 7$ nAChRs behave like agonists (for review see [41]).

Consensus exists on the ability of $A\beta 1-42$ to block α -bungarotoxin (α -Bgt) sensitive nAChR subtypes like the $\alpha 7$ nAChR [37, 39–43]. Its interaction with non- α -Bgt sensitive nAChR subtypes, however, is not clear yet. In the initial study it was demonstrated on hippocampal membranes from sporadic AD brains by immunoprecipitation and Western blot analysis that the nAChR $\alpha 7$ -subunit co-immunoprecipitated with $A\beta 1-42$, whereas the nAChR $\alpha 1$ -, $\alpha 3$ -, $\alpha 4$ -, $\alpha 5$ -, $\alpha 8$ -, or $\beta 2$ -subunit failed to yield any detectable co-immunoprecipitate with anti- $A\beta 1-42$ [37]. In functional studies some groups did not find any blockade of non- α -Bgt sensitive nAChR subtypes by $A\beta 1-42$ [40, 41], whereas other researchers found a binding affinity for the $\alpha 4\beta 2$ nAChR subtype in the range of 20–30 nM [38] or a partial blockade of non- $\alpha 7$ nAChRs at higher concentrations [39]. In one paper even a higher depression of $\alpha 4\beta 2$ than of $\alpha 7$ nAChR currents upon $A\beta 1-42$ incubation was reported [43]. In PC12 cells nanomolar concentrations of $A\beta 1-40$ and $A\beta 25-35$ significantly decreased [3 H]epibatidine and [125 I] α -Bgt binding sites as well as the mRNA and protein amounts of nAChR $\alpha 3$ -, $\alpha 7$ -, and $\beta 2$ -subunits [44]. A possible explanation of the inconsistencies in these findings may be the different $A\beta 1-42$ concentrations used or the different cell types studied [40].

Another controversial issue is the nature of the interaction between $A\beta 1-42$ and $\alpha 7$ nAChRs, as $A\beta 1-42$ has been reported to compete with α -Bgt for binding to $\alpha 7$ nAChRs [37], whereas no competitive displacement of α -Bgt by $A\beta 1-42$ was observed neither in intact mouse hippocampal neurons nor in neurons derived from chick ciliary ganglia [40]. Also Guan et al. [44] failed to displace [3 H]epibatidine and [125 I] α -Bgt by $A\beta 1-42$, $A\beta 1-40$, and $A\beta 25-35$ in PC12 cells. Further functional characterization of the interaction of $A\beta 1-42$ with $\alpha 7$ nAChRs revealed that the blockade of $\alpha 7$ nAChR function does not require the presence of an agonist. On the contrary, co-application of $A\beta 1-42$ and ACh resulted in a failure to induce blockade [40].

The formation of an $\alpha 7$ nAChR- $A\beta 1-42$ complex can be efficiently suppressed by $A\beta 12-28$, implying that the binding epitope for $\alpha 7$ nAChRs resides in the amino acid 12–28 sequence region of $A\beta 1-42$ [37]. Further studies using chimeric $\alpha 7/5HT_3$ receptors suggested that $A\beta 1-42$ interacts with the extracellular N-terminal domain of the $\alpha 7$ nAChR [40]. However, little information exists on the physical state of active $A\beta 1-42$ that binds to nAChRs. In brain

slices incubation with freshly prepared $\text{A}\beta_{1-42}$ led to a rapid onset of inhibition, which speaks in favour of a soluble, oligomeric rather than a fibrillar form of $\text{A}\beta_{1-42}$ [39].

nAChR mediated signal transduction in respect to A β

The question remains whether the observed $\text{A}\beta_{1-42}$ -induced nAChR functional changes affect synaptic transmission. It has been shown that $\text{A}\beta_{1-42}$ affects I_{ACh} with an IC_{50} around 100 nM ($\sim 450 \text{ ng ml}^{-1}$) [39, 41, 42], although an IC_{50} of about 7.5 nM has been described for rat hippocampal neurons [40]. The concentration of $\text{A}\beta$ peptides in brain tissue from AD patients (2–20 μM) is higher than that required for maximal $\alpha 7$ nAChR blockade [45], but most $\text{A}\beta$ peptides are concentrated in the $\text{A}\beta$ deposits, and their exchange with interstitial fluid and their proximity to receptors are difficult to estimate. It is known, however, that mice genetically engineered to express $\text{A}\beta$ peptides can display synaptic toxicity that correlates with $\text{A}\beta_{1-42}$ level in the 10–100 nM range [31, 32], concentrations that are effective at blocking $\alpha 7$ nAChRs.

In a recent review it was summarized that $\text{A}\beta$ peptide can be directly neurotoxic, induce oxidative stress, incite an inflammatory response, and alter calcium homeostasis. These events might be mediated by direct interaction of $\text{A}\beta$ aggregates with cellular membranes, or by binding of $\text{A}\beta$ to microglial and neuronal cellular receptors [46]. High levels of $\text{A}\beta_{1-42}$ in AD may promote interactions with cholinergic neurons that express $\alpha 7$ nAChRs. The high affinity binding of $\text{A}\beta_{1-42}$ to $\alpha 7$ nAChRs may result in the inhibition of ACh release and may alter Ca^{2+} -homeostasis for the affected cholinergic neuron. These significant and chronic physiological perturbations may lead to stress and even neurodegeneration.

An important role for $\alpha 7$ nAChRs in facilitating the entry and intraneuronal accumulation of $\text{A}\beta_{1-42}$ via endocytosis has been described [47]. The rate and extent of $\text{A}\beta_{1-42}$ internalization was directly related to the amount of $\alpha 7$ nAChR protein and effectively blocked by α -Bgt and by the endocytosis inhibitor phenylarsine oxid. Furthermore, the $\alpha 7$ nAChR was co-localized with $\text{A}\beta_{1-42}$ in prominent intracellular aggregates [47]. Based on these data the authors [47] suggest that internalization of $\text{A}\beta_{1-42}$ occurs predominantly in neurons expressing the $\alpha 7$ nAChR and may be facilitated by the high-affinity binding of $\text{A}\beta_{1-42}$ to $\alpha 7$ nAChRs on neuronal cell surfaces followed by endocytosis of the resulting complex. According to the authors their data provide a plausible explanation for the selective vulnerability of neurons expressing the nAChR $\alpha 7$ subtype in AD brain. The data are also interpreted to suggest that $\text{A}\beta_{1-42}$ is the dominant $\text{A}\beta$ peptide in intracellular accumulation and amyloid plaques [47]. However, the nAChR subtype affected in AD most severely is the $\alpha 4\beta 2$ -subtype providing the high-affinity nicotine binding sites.

Further evidence for the view that high affinity $\alpha 7$ nAChR- $\text{A}\beta_{1-42}$ interaction may be a critical step leading to AD pathology arises from the findings of

the nAChR-mediated protection against glutamate- and A β -induced neurotoxicity (for review see [24]). Stimulation of nAChRs with nicotine or epibatidine resulted in a prevention of A β -induced cytotoxicity [22, 23, 37]. Evidence for a nAChR-mediated protection against the A β -enhanced glutamate neurotoxicity was provided by the finding that α -Bgt suppressed this protection which therefore seems to be mediated via the α 7 nAChR subtype. It is supposed that nicotine activates α 7 nAChRs to stimulate the Src gene family, which in turn activates phosphatidylinositol 3-kinase (PI-3-K). PI-3-K phosphorylates Akt, which causes upregulation of Bcl-2 and Bcl-x preventing cells from neuronal death induced by A β and glutamate [24, 48]. Further insights into the underlying mechanisms of the nAChR-mediated neuroprotection were given recently by the findings of Shaw et al. [49] in PC12 cells. The authors provide evidence that nicotine stimulation of the α 7 nAChRs transduce signals to PI-3-K and Akt via Janus kinase 2 (JAK2) in a cascade that results in neuroprotection. Exposure to A β leads to an activation of the apoptotic enzyme caspase-3 and cleavage of the DNA-repairing enzyme poly-(ADP-ribose) polymerase. This apoptotic cascade is inhibited by nicotine through JAK2 activation. Pre-treatment of cells with angiotensin II was able to block the nicotine-induced activation of JAK2 via the AT₂ receptor and completely prevented α 7 nAChR-mediated neuroprotective effects [49]. These findings identify novel mechanisms of receptor interactions relevant to neuronal viability and suggest novel therapeutic strategies to optimise neuroprotection [49].

A possible link between A β_{1-42} binding to α 7 nAChRs and cognitive impairment in AD was suggested by a paper showing that A β_{1-42} is able to promote MAP kinase activation by inducing Ca²⁺ influx through mutant L250T α 7 nAChRs, thereby interfering with long-term potentiation processes [50]. Contrary to the wild-type receptor that is blocked by A β_{1-42} , the mutant receptor is activated. Therefore, the significance of the interaction between A β_{1-42} and α 7 nAChRs for the etiology or the pathogenesis of AD is unclear [41] and needs further elucidation.

Current therapy strategies targeting A β in the brain

Besides AChE-inhibitor approaches the most intensive therapeutic efforts thus far have been directed toward a decrease of A β in the brain. Procedures inhibiting A β formation or modulating A β production, assembly and/or removal might be useful as treatments for preventing AD or having a meaningful impact on disease progression. Immunization with A β of aged transgenic PDAPP-over-expressing mice led to a reduction in A β deposits, whereas immunization of young mice prevented formation of A β deposits [51]. In addition, in TgCRND8 mice (K670N/M671/L and V717F human β APP₆₉₅) a reduction of the cerebral fibrillar A β and of cognitive dysfunction was documented, without, however, altering the total level of A β in the brain after A β immunization [52]. Passive immunization of PDAPP transgenic mice with

m266, an antibody directed to the central part of the A β , can rapidly reverse memory deficits but without altering brain A β burden [53]. These promising results have led to the development of an A β_{1-42} -compound (AN-1792, Elan Pharmaceuticals), as a potential vaccine to treat AD. AN-1792 was undergoing Phase IIa trials in 360 AD patients, as it had to be stopped in January 2002 due to meningio-encephalitic presentation in about 5% of the study group participants [46, 54]. Very recently after the death of the first patient participating in this study an analysis of human neuropathology became available [55]. Some of the findings strongly resemble the changes seen after A β -immunotherapy in mouse models of AD. First, there were extensive areas with a low density of A β plaques without plaque-associated dystrophic neurites and GFAP-immunoreactive astrocytes. Second, remaining A β -immunoreactivity was associated with microglia in areas devoid of plaques. Third, there was persistence of cerebrovascular amyloid. Additional features that were not predicted by the mouse models of A β -immunotherapy comprise (1) a CD4+ lymphocytic meningoencephalitis, (2) persistence of neurofibrillary tangles and neuropil threads in areas devoid of plaques, and (3) extensive macrophage infiltration of cerebral white matter [55].

A possible alternative for plaque clearance by immunization with A β may be the stimulation of nAChRs. Chronic treatment using high doses of nicotine in the diet of APPsw transgenic mice carrying the Swedish mutation of the human amyloid precursor protein [Tg(Hu.App695.K670N-M671L)2576] led to a selective reduction in A β_{1-42} positive plaques by more than 80%, but there were no changes in soluble A β_{1-40} or A β_{1-42} levels [56]. However, there was also significant weight loss in these animals. Their findings led the authors to conclude that chronic nicotine administration can be added to an increasing number of treatments that are able to reduce A β deposition in animal models and that nicotinic drugs directed towards select nAChR subtypes represent a feasible neuroprotective therapeutic approach in AD [56]. Whether the chronic nicotine treatment of APPsw mice has any effect on cognitive improvement, however, needs to be further elucidated.

Designing compounds that distinguish individual receptor subtypes is a highly desirable therapeutic strategy for redressing some of the degenerative effects associated with AD. To the extent that $\alpha 7$ nAChRs represent an early molecular casualty of the disease, they should be considered a high-priority target for drug design [40].

An early attempt to target $\alpha 7$ nAChRs used a compound designed from a naturally-occurring substance: A synthesized analog of the marine natural product anabasine [57] called GTS-21 (3-(2,4-dimethoxybenzylidene) anabasine) that preferentially interacts with $\alpha 7$ nAChRs. When GTS-21 was introduced to cultured rat cortical neurons, it protected neurons against A β -induced death [22]. These results suggest that $\alpha 7$ nAChR activation can play an important role in neuroprotection against A β neurotoxicity. Activation of $\alpha 7$ nAChRs may be able to protect neurons from degeneration induced by A β and may have effects that counter the progression of AD. In a subsequent study,

Kihara et al. [23] reported that nicotine neuroprotection could be blocked by an $\alpha 4\beta 2$ nAChR antagonist, suggesting a neuroprotective effect for $\alpha 4\beta 2$ nAChRs as well as $\alpha 7$ nAChRs.

Current therapy strategies with nAChRs targeting learning and memory

A role for nAChRs has been demonstrated in a number of forms of cognition including attention [58, 59], sensorimotor gating [60–62], and learning and memory [63–66]. These studies have been carried out in several mammalian species, including humans. Because of the central role that memory loss plays in AD, the focus here is on nAChR involvement in learning and memory. Among the novel approaches to cognition enhancement is the application of agonists to nAChRs, in particular the $\alpha 7$ nAChR, and allosteric modulation of nAChRs.

Role for nAChRs in working memory

Repeatedly it has been shown that working memory is facilitated by both acute injections of nicotine [67, 68] and chronic infusion of nicotine [69–72]. Nicotine's facilitatory effect on working memory is blocked by the nAChR antagonist, mecamylamine [67, 68, 72, 73]. Mecamylamine is not selective to one specific nAChR subtype. It appears to inhibit preferentially first $\alpha 3$, then $\alpha 4$, and finally, $\alpha 7$ subunits and may likewise block $\beta 2$ and $\beta 4$ nAChR sub-units [74].

Initial behavioral pharmacology research on the nicotinic cholinergic system used this broad-spectrum approach with agonists (nicotine) and antagonists (mecamylamine). More recent research has used a receptor-targeted approach. In the case of working memory as assessed by the 8- and 16-arm radial maze in rats, the approach using focal brain infusions and antagonizing the broad-spectrum agonist, nicotine with receptor-targeted antagonists has demonstrated that both $\alpha 7$ and $\alpha 4\beta 2$ nAChRs in the ventral hippocampus are critical [71]. The work of Levin and associates demonstrates the utility of infusing drugs focally in the brain to identify sites of drug action. Experiments infusing antagonists specific to $\alpha 7$ nAChRs (methyllycaconitine) or to $\alpha 4\beta 2$ nAChRs (dihydro-beta-erythroidine) into the ventral hippocampus demonstrated that both types of nAChRs are critical for working memory function measured by the radial arm maze in rats [69–71].

Role of nAChRs in associative learning and memory

Brain structures and systems of demonstrated involvement in eyeblink classical conditioning in rabbits and humans are compromised during the progres-

sion of AD. Patients diagnosed with probable AD are severely impaired in eyeblink conditioning beyond the impairment observed in normal aging [75-77]. For a decade, it has been our working hypothesis that disruption of the septohippocampal cholinergic system and selective loss of hippocampal pyramidal cells impair acquisition of eyeblink classical conditioning in AD beyond the impairment observed in normal aging. Additional evidence that septohippocampal lesions disrupt conditioning in humans comes from patients with a common cerebral aneurysm: aneurysm of the anterior communicating artery. The anterior communicating artery vascularizes the basal forebrain, and survivors of an aneurysm of this artery often display some degree of anterograde amnesia. Anterior communicating artery aneurysm survivors often sustain basal forebrain lesions that include lesions of the medial septum. Six patients with such lesions were tested in the delay eyeblink conditioning procedure and showed significant impairment [78].

In addition to septohippocampal disruption, AD-related injury to the cerebellum may play a role in impairing delay eyeblink classical conditioning in AD. Purkinje cell loss in the AD cerebellum is significantly greater than in age-matched control brains [79, 80], and there is exceptional vulnerability of the ACh system and ACh-receptive cells in AD that may be associated with nAChRs. With the mounting evidence for a role for ACh in the cerebellum as well as the hippocampus, the possibility exists that impaired eyeblink conditioning in AD occurs at least in part because of impairment in ACh modulation and nAChR function in the essential cerebellar circuitry.

Experiments with muscarinic ACh receptor agonists [81] and antagonists [82] and nicotinic ACh receptor agonists [83] and antagonists [84] have demonstrated a role for ACh in the model system of eyeblink classical conditioning in the rabbit. Eyeblink conditioning reveals natural age-related deficits in several non-human mammals, and the similarities between age differences in eyeblink conditioning in these animal species and humans are striking. Moreover, delay eyeblink conditioning is impaired profoundly in patients with AD, making the procedure relevant for preclinical studies of cognition-enhancing drugs. In addition to parallels with human behavior and neurobiology, the model system of eyeblink classical conditioning possesses a considerable advantage over the behavioral models commonly used preclinically: The essential neural circuitry in the cerebellum has been identified along with modulatory circuits in hippocampus and cortex.

The standard format for the presentation of stimuli in eyeblink classical conditioning is called the delay procedure. The subject is presented with a neutral stimulus such as a tone or light, called the conditioned stimulus (CS), for a short duration usually less than one second. Before the CS expires, the unconditioned stimulus (US) is presented concurrently, and the briefly coinciding CS and US co-terminate 50 to 100 ms later. The US, either a shock to the infra-orbital region of the eye or a corneal airpuff, always elicits from the organism an eyeblink or nictitating membrane (NM) unconditioned response (UR). With the repeated pairing of the CS and the US, the subject learns to

blink to the tone before the onset of the US. This learned response is called a conditioned response (CR).

The site essential for acquisition and retention of the classically conditioned eyeblink response in rabbits is the cerebellar interpositus nucleus ipsilateral to the eye receiving the US. In humans, this nucleus becomes two deep cerebellar nuclei (emboliform and globose). Cerebellar cortex ipsilateral to the US also contributes to the process of acquisition, such that an intact cerebellar cortex enables acquisition to occur at a faster rate. The hippocampus itself is normally involved during acquisition in the delay procedure, however, in a complex modulatory role. The role of the hippocampus during acquisition in delay eyeblink conditioning seems paradoxical in that conditioning proceeds normally in animals with bilateral removal of the hippocampus, but manipulation of hippocampal function (in an intact hippocampus) with drugs can facilitate or impair acquisition considerably. For example, the muscarinic cholinergic antagonist scopolamine impairs acquisition in the delay procedure only when the hippocampus is intact [85]. Likewise, the cognition-enhancing drug, nefiracetam ameliorates learning impairment in older rabbits in the delay procedure only when the hippocampus is intact [86]. This modulatory role for the hippocampus may be particularly significant in AD, since, in humans, AD appears to alter hippocampal neuronal function and cause a major disruption of the brain cholinergic system. Eyeblink conditioning impairment in AD may reflect medial-temporal lobe atrophy and central nervous system cholinergic dysfunction that occurs early in disease progression.

Identification of nAChRs as the cholinergic receptors impaired in AD led to a test of an antagonist to nAChRs in the animal model of eyeblink classical conditioning. Mecamylamine is a central nervous system nicotinic antagonist that binds to a site on the receptor other than the ACh recognition site. Papke et al. [74] demonstrated with electrophysiological recordings of nAChRs expressed in *Xenopus* oocytes that the residual inhibition produced by 10 μ M mecamylamine was greatest for human β 2-containing receptors and least for α 7 nAChRs.

Using a 0.5 mg/kg dose of mecamylamine, a role for nAChRs in eyeblink conditioning in young rabbits was demonstrated [84]. The acquisition of conditioned eyeblink responses was severely disrupted so that young rabbits learned at a rate comparable to older rabbits. The deleterious effect of mecamylamine on eyeblink conditioning was not accompanied by a measurable change in brain nAChR concentration. These results in combination with studies using the muscarinic acetylcholine receptor (mAChR) antagonist, scopolamine, suggest that nAChRs as well as mAChRs are involved in the modulation of eyeblink classical conditioning.

Partial agonism of α 7 nAChRs

Results with the nAChR antagonist, mecamylamine led to the prediction that nAChR agonists would facilitate eyeblink conditioning in older rabbits. The

effect of GTS-21, a selective nicotinic agonist acting primarily at the $\alpha 7$ nAChR subtype [87, 88], on learning was tested using the 750-ms delay eyeblink conditioning procedure [89]. There were 15 daily subcutaneous injections administered 15 minutes before behavioral training. At dosage levels of 0.5 and 1.0 mg/kg, GTS-21 acted as a cognition-enhancing agent in older rabbits, resulting in eyeblink conditioning performance comparable to young rabbits. The cognition-enhancing effect of GTS-21 upon eyeblink conditioning was not accompanied by a measurable change in brain nAChRs.

The effect of GTS-21 on acquisition, retention, and relearning was also examined with a focus on the duration of the effect of GTS-21 as assessed by retention and relearning [90]. First there were 15 sessions of acquisition training with injections of 0.5 mg/kg GTS-21 or vehicle. Then drug administration ended and older rabbits were tested for retention and relearning six weeks and 13 weeks after the beginning of the experiment. Acquisition of CRs was significantly better in GTS-21-treated rabbits. During the first tone-alone retention session in week six of the experiment, rabbits initially treated with GTS-21 produced significantly more CRs than vehicle-treated rabbits. There were no group differences in retention at the 13-week retest. Differences in relearning were numerically greater for GTS-21 treated older rabbits, but these effects did not attain statistical significance. Results indicated that treatment with GTS-21 ameliorated learning and memory beyond the period when the drug was actually administered.

Given the results outlined above confirming that nAChRs are involved in the modulation of acquisition and retention in eyeblink classical conditioning, reversal of the nAChR antagonist mecamylamine with nicotine or GTS-21 was explored [91]. Young rabbits were injected with 0.5 mg/kg mecamylamine in combination with nicotine or GTS-21 and compared to vehicle-treated rabbits. Control groups were tested in the explicitly unpaired condition. Both GTS-21 and nicotine reversed the deleterious effect of mecamylamine on acquisition of CRs enabling the rabbits treated with mecamylamine and the agonists to perform at the level of vehicle-treated control rabbits (Fig. 1). Combinations of GTS-21 or nicotine and mecamylamine did not cause sensitization or habituation in the explicitly unpaired condition. That fact that mecamylamine inhibits preferentially first $\alpha 3$, then $\alpha 4$, and finally $\alpha 7$ subunits and may likewise block $\beta 2$ and $\beta 4$ nAChR subunits [74] suggests that the reversal by GTS-21 and nicotine of mecamylamine's antagonistic effect was via $\alpha 7$ nAChRs. There is some evidence that GTS-21 actually antagonizes $\alpha 4\beta 2$ nAChRs in addition to serving as an agonist for $\alpha 7$ nAChRs [87]. If $\alpha 7$ nAChRs are of primary importance in eyeblink conditioning, then selective $\alpha 7$ antagonists such as methyllycaconitine should impair acquisition to a greater extent than selective $\alpha 4\beta 2$ antagonists such as dihydro-beta-erythroidine. Demonstration that eyeblink conditioning relies more on $\alpha 7$ nAChRs than on $\alpha 4\beta 2$ nAChRs would make this behavior a sensitive index of the drugs that are $\alpha 7$ agonists under development to treat AD.

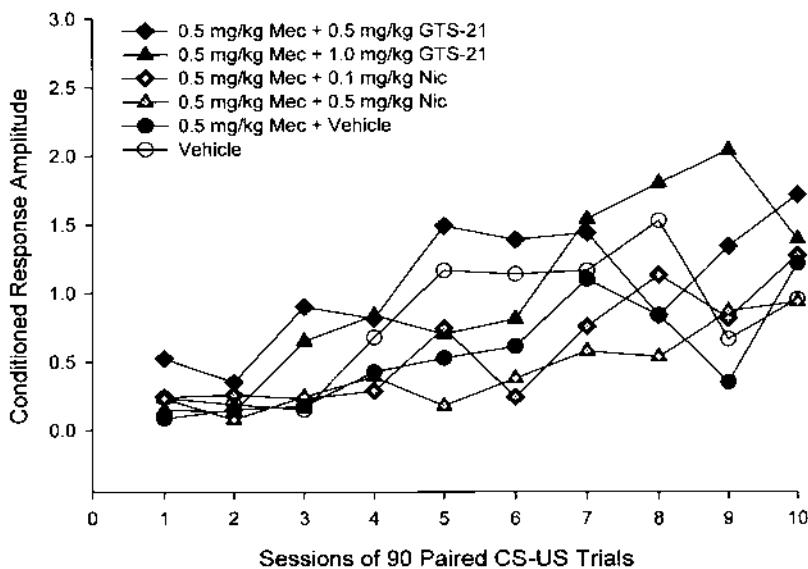


Figure 1. Mean amplitude of conditioned responses (CRs) in 58 three-month-old rabbits for the various drug treatment groups for the first 10 daily training sessions of 90 trials/session. Each group consisted of a minimum of eight rabbits. Rabbits received daily subcutaneous injections of sterile saline vehicle, 0.5 mg/kg mecamylamine (Mec) alone, or 0.5 mg/kg mecamylamine in combination with 0.5 or 1.0 mg/kg GTS-21 or 0.25 or 0.5 mg/kg nicotine (Nic) 15 min before training. The group treated with 0.5 mg/kg Mec had significantly fewer CRs than the vehicle-treated group ($p = 0.005$). Early in training (Sessions 2–3) rabbits treated with mecamylamine and GTS-21 actually had numerically higher CR amplitudes than all other groups, but by Session 4 vehicle-treated animals began to show superior performance to the mecamylamine-alone group. Rabbits treated with agonist (GTS-21 or nicotine) antagonist (mecamylamine) combinations performed similarly to vehicle-treated rabbits and better than rabbits treated with the antagonist alone [91].

Allosteric modulation of nAChRs

Allosteric modulators, such as galantamine (Reminyl®; Inteligen, Janssen) and physostigmine employ a dual action at the cholinergic synapse. First, they act as acetylcholinesterase (AChE) inhibitors. Second, they also act as agonists at presynaptic nAChRs, enhancing the release of ACh. Functionally unique features of allosterically potentiating ligands include the ability as assessed with patch-clamp recordings to induce single-channel activity indistinguishable from the single-channel activity induced by ACh. With allosteric potentiation, galantamine and physostigmine induced single-channel activity in excised patches from various cells [92–94] that could not be blocked by established nAChR antagonists like mecamylamine.

Given that an $\alpha 7$ nAChR partial agonist, GTS-21 ameliorated learning deficits in older rabbits, the aim was to determine if nicotinic agonism using a different mechanism of action would be effective in the eyeblink classical con-

ditioning paradigm. With physostigmine, a wide range of doses (0.0005 to 2.0 mg/kg) were effective in ameliorating eyeblink conditioning deficits in older rabbits [95]. Control tests of rabbits in explicitly unpaired conditions demonstrated that non-associative factors could not account for the results. This allosteric modulator had dramatic effects on associative learning in older rabbits (see Fig. 2).

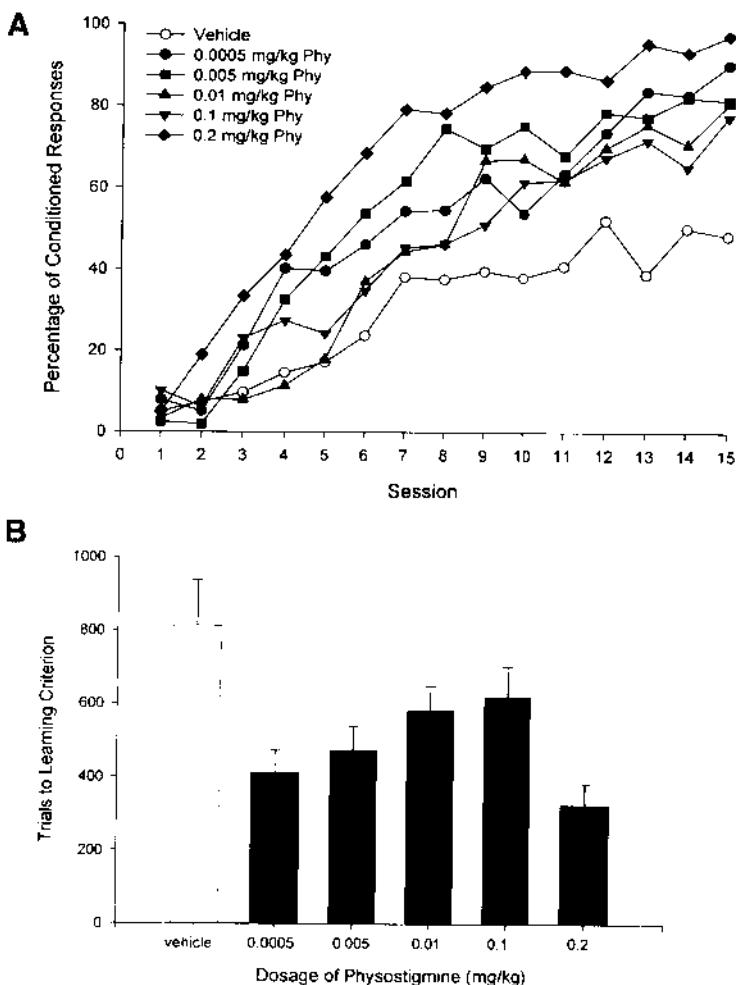


Figure 2. (A) Percentage of conditioned responses (CRs) of a total of 59 older rabbits (mean age = 28 mo., s.d. = 5.0 mo) treated with five doses of physostigmine (Phy) or vehicle over 15 daily training sessions in the 750 ms delay eyeblink classical conditioning procedure. There were a minimum of seven older rabbits in each of the Phy-treated groups and 12 rabbits in the vehicle-treated group. (B) The number of training trials to a learning criterion of eight CRs in nine consecutive trials in the 59 rabbits shown in the left panel. Physostigmine at a range of doses significantly improved learning in older rabbits [95].

Galantamine doses of 0.0, 1.0, 2.0, 3.0, and 4.0 mg/kg were tested in 10 daily sessions in old rabbits in the 750 ms delay eyeblink classical conditioning procedure [96]. A dose of 3.0 mg/kg galantamine was effective in improving conditioning in older rabbits, enabling them to achieve learning criterion rapidly and to produce a very high level of learning performance. Like physostigmine, galantamine had no effects on sensitization, habituation, or motor aspects of the task.

Additional experiments with galantamine were carried out to compare the efficacy of the drug in young and older rabbits and to evaluate retention and relearning [83]. In one experiment, young and older rabbits were administered 3.0 mg/kg galantamine for 15 days during conditioning in the 750 ms delay procedure. Galantamine significantly improved acquisition in both young and older rabbits. AChE levels in the brain were reduced, and nAChR binding was increased. There was a statistically significant correlation between brain AChE levels and trials to learning criterion, $r = 0.621$, $p = 0.007$. Neither the correlation between trials to learning criterion and plasma AChE, nor the correlations between trials to learning criterion and B_{max} or K_D attained statistical significance.

In another experiment, older rabbits were tested over a 15-week period in four conditions. Groups of rabbits received 0.0 (vehicle), 1.0, or 3.0 mg/kg galantamine for the entire 15-week period or 3.0 mg/kg galantamine for 15 days and vehicle for the remainder of the experiment. There were 15 daily conditioning sessions and subsequent retention and relearning assessments spaced at one-month intervals. The dose of 3.0 mg/kg galantamine ameliorated learning deficits significantly during acquisition and retention in the group receiving 3.0 mg/kg galantamine continuously. Nicotinic receptor binding was significantly increased in rabbits treated for 15 days with 3.0 mg/kg galantamine. All galantamine-treated rabbits had lower levels of brain AChE. The efficacy of galantamine in a learning paradigm severely impaired in AD was consistent with outcomes evaluating galantamine in clinical studies.

The single-channel activity of galantamine could not be blocked by antagonists to nAChRs suggesting by inference that the activity was induced through a separate allosteric site from the site for ACh and competitive ligands. Mecamylamine blocks many well-established AChE inhibitors tested with these electrophysiological techniques [92, 94]. Patch-clamp recordings of single ion channel activity demonstrated that donepezil, but not galantamine, could be blocked by mecamylamine. At the whole organism, behavioral level an experiment was carried out to determine whether galantamine, but not donepezil, could reverse mecamylamine-induced learning impairment. Young rabbits received delay eyeblink conditioning after one of six drug treatments: 0.5 mg/kg mecamylamine, 3.0 mg/kg galantamine, 3.0 mg/kg donepezil, 0.5 mg/kg mecamylamine plus 3.0 mg/kg galantamine, 0.5 mg/kg mecamylamine plus 3.0 mg/kg donepezil, or sterile saline vehicle [97]. Galantamine, but not donepezil, facilitated learning in young rabbits. However, both galantamine and donepezil reversed the deleterious effects of mecamylamine on

learning. Significant differences in plasma (but not brain) AChE levels were detected among the drug treatment groups. Fifteen daily injections of mecamylamine, galantamine, or donepezil, alone or in combination did not produce statistically significant changes in nAChR binding. One possible interpretation of these results is that donepezil affected nAChRs by raising the synaptic level of ACh and hence, the probability of receptor activation, whereas galantamine bound to distinct allosteric sites not blocked by mecamylamine. It may be possible to facilitate learning in young rabbits with allosteric modulation (galantamine), but not with AChE inhibition alone (donepezil).

Summary and conclusions

The widespread involvement of nAChRs in disorders of the human nervous system makes these receptors likely targets for drug therapy. In this chapter we focused on the involvement of nAChRs in AD and the relationships between A β and nAChRs. Our demonstration in brain autopsy tissue from humans with AD of the presence of nAChR-immunoreactive neurons in close proximity to and sometimes even within plaques makes it appear rather unlikely that plaques interfere with nAChR expression. This perspective receives confirmation from a number of additional studies investigating relationships between plaque load and nicotine binding in humans and transgenic mice.

Whereas A β plaques may not impair nAChRs, there is increasing evidence that soluble A β interacts with nAChRs to cause morphological alterations and impairment of function. Consensus exists on the ability of A β_{1-42} to block α -Bgt sensitive nAChR subtypes like $\alpha 7$ nAChR. The formation of an $\alpha 7$ nAChR-A β_{1-42} complex can be efficiently suppressed by A β_{12-28} , implying that the binding epitope for $\alpha 7$ nAChR resides in the amino acid 12–28 sequence region of A β_{1-42} . The interaction of soluble A β with non- α -Bgt sensitive nAChR subtypes, however, is not yet clear.

It is likely that the observed A β_{1-42} -induced functional changes in nAChRs affect synaptic transmission. For example, the high affinity binding of A β_{1-42} to $\alpha 7$ nAChRs may result in the inhibition of ACh release and may alter Ca $^{2+}$ -homeostasis for the affected cholinergic neuron. Chronic physiological perturbations such as inhibition of ACh release and altered Ca $^{2+}$ -homeostatic stress neuronal function and even lead to neurodegeneration. High affinity $\alpha 7$ nAChR-A β_{1-42} interaction may be a critical step leading to AD pathology. In this regard, using a broad-spectrum agonist (nicotine) and an $\alpha 7$ receptor-targeted agonist (GTS-21) is neuroprotective against A β -enhanced glutamate neurotoxicity.

A possible alternative for plaque clearance by immunization with A β may be the stimulation of nAChRs. In particular, chronic stimulation of $\alpha 7$ nAChRs has significant therapeutic potential. In addition to its potential to affect AD neuropathology, chronic stimulation of $\alpha 7$ nAChRs has potential to reduce cogni-

tive impairment in AD. Learning and memory are among the cognitive processes affected early in AD. Animal studies demonstrated that both $\alpha 7$ and $\alpha 4\beta 2$ nAChRs in the ventral hippocampus are critical for normal working memory. Functional blockade of $\alpha 7$ nAChRs and loss of $\alpha 4\beta 2$ nAChRs likely contribute significantly to the documented impairment of attention and working memory in AD patients. Associative learning as assessed by eyeblink classical conditioning is severely impaired in AD, and preclinical experiments with a partial $\alpha 7$ agonist and with allosteric modulators of nAChRs demonstrate dramatic therapeutic effects. Targeting $\alpha 7$ nAChRs to treat AD has a range of potential benefits, as does the targeting of selected individual receptor subtypes affected in diseases such as addiction to nicotine, anxiety, autism, depression, epilepsy, Parkinson's disease, schizophrenia, and Tourette's syndrome.

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Drugs that target catecholaminergic receptors

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Introduction

Several small catecholaminergic (CA) nuclei in the midbrain contain neurons project to broad areas of the diencephalon and telencephalon. These neurons using the neurotransmitters, dopamine (DA) and norepinephrine (NE) play important roles in a wide variety of neurobehavioral functions including cognition. DA neurons from the substantia nigra (SN) project primarily to the striatum, while DA neurons from the nearby ventral tegmental area (VTA) project more broadly to the nucleus accumbens, thalamus, hippocampus and frontal cortex. NE neurons from the locus coeruleus (LC) project to broad areas of the cortex, limbic system and thalamus. These projections serve as the basis of CA involvement in cognitive function particularly in aspects of the relationship of arousal and attention to salient sensory cues [1]. CA neurons and their post-synaptic receptors are targets for therapeutic treatments for a variety of cognitive disorders [2].

Disruptions of CA systems are known to cause cognitive impairment. Parkinson's disease, in which there is a substantial dying off of midbrain DA neurons, is most well-known as a disorder of motor function, however, there are often pronounced cognitive impairments in Parkinson's disease. Schizophrenia is also known to involve disruptions of DA innervation. This classic psychotic disorder has substantial components of cognitive dysfunction as well. Unfortunately, the DA blockers often used for schizophrenia can cause further cognitive impairment. Attention deficit hyperactivity disorder (ADHD) has been found to involve alterations of DA and NE, particularly in the frontal cortex. Cognitive impairment is a hallmark of this syndrome.

CA agonists have been shown to improve cognitive function. Parkinson's disease medications and DA agonists can improve cognitive function as well as motor function. Methylphenidate and amphetamine indirect CA agonists significantly improve attentional function in people with ADHD; NE agonists have been found to improve cognitive function in a variety of disorders including Alzheimer's disease, schizophrenia and Parkinson's disease. Better basic understanding of the receptor subtypes and their locations critical for cognitive improvement will be valuable for further development of therapeutics.

Preclinical research

Animal models have contributed greatly to our knowledge of the role of CA systems in cognition. Both rodent and primate studies have used lesion and genetic methods as well as neurobehavioral pharmacology to determine the role of DA and NE neural systems in memory and attentional function. Many tasks, including active and passive avoidance procedures, spatial memory paradigms such as the radial-arm maze in rodents and delayed matching to sample in monkeys and tests of selective and sustained attention, have been used to illustrate the involvement of DA and NE systems in cognition [3–6]. Local infusion studies using receptor subtype selective agonists and antagonists have given insight into the roles CA systems play in the neural basis of cognition. These experimental studies help form the knowledge base for development of new drug therapies for cognitive impairment.

Transmitter selective lesions and more recently genetic knockout methods have been key in demonstrating the importance of CA systems in cognitive function. Lesion-induced disruptions of CA systems have been shown to cause cognitive impairment. DA lesions in the frontal cortex using 6-hydroxy-dopamine (6-OHDA) have been shown to cause memory and attentional impairment [7, 8]. 1-Methyl-4-phenyltetrahydropyridine (MPTP) lesions in rats, which caused reduced DA in both the striatum or frontal cortex, causes specific working, but not reference memory impairments [9].

Specific involvement of CA receptor subtypes can be determined with the use of genetic knockout studies. Involvement of D₂ and D₃ DA receptors in working memory was shown in a study of mice with genetic knockout of D₂ or D₃ receptors. D₂ or D₃ KO mice had significant working memory impairments in spatial alternation [10]. Methamphetamine treatment reversed the working memory impairment in the D₂ knockout mice but not the D₃ knockout mice. Future studies with conditional knockout and knockdown mice can lend further insight into the CA mechanisms in cognitive function.

Neurobehavioral pharmacology experimental animal studies have contributed greatly to our understanding of the roles DA and NE play in cognitive function and can aid the development of CA-based therapeutics for cognitive dysfunction. A wide variety of rodent studies have shown that DA and NE antagonists generally impair learning and memory while some DA and NE agonists can improve learning and memory [11]. DA systems are particularly important for reward associations with learning [12]. NE systems play a prominent role in emotionally relevant memory [13]. Both CA transmitters have been shown to be involved in memory function. Local infusions of D₁ but not D₂ antagonists in the frontal cortex of monkeys significantly impair memory [14]. Lesions of DA projections to the hippocampus impaired spatial memory function [15]. In rats we have found that local infusion of D₂ but not D₁ antagonists impaired working memory [16].

DA drug effects may be altered by frontal cortical brain damage. Lesions of frontal DA projections significantly increase DA in the nucleus accumbens

[17]. Lesions of the medial frontal cortex significantly impaired choice accuracy on the 5-choice attentional task [18]. This impairment was significantly reversed by systemic administration of the D₂ antagonist sulpiride at a dose that significantly impaired choice accuracy in sham operated rats.

DA systems in the ventral hippocampus are involved in working memory function. D₂ drugs had very clear and consistent effects on choice accuracy [16]. The D₂ agonist quinpirole caused a significant dose-related improvement in radial-arm maze choice accuracy over the 1.1–10 µg/side dose range (Fig. 1). In a complementary fashion, the D₂ antagonist raclopride caused a significant dose-related impairment in radial-arm maze choice accuracy over the 0.19–1.67 µg/side dose range (Fig. 1). In contrast, infusions of D₁ drugs (the D₁ agonist dihydrexidine or the D₁ antagonist SCH 23390) into the ventral hippocampus at the dose ranges examined did not have any discernible effects on choice accuracy in the radial-arm maze. The pharmacological study showing the positive relationship between D₂ hippocampal activation and better memory performance is supported by receptor binding experiments. Higher levels of D₂ binding in the hippocampus are correlated with better memory performance on the radial-arm maze [19]. Interestingly, a similar

Effects of ventral hippocampal D2 agonist and antagonist local drug infusions on working memory in the radial-arm maze

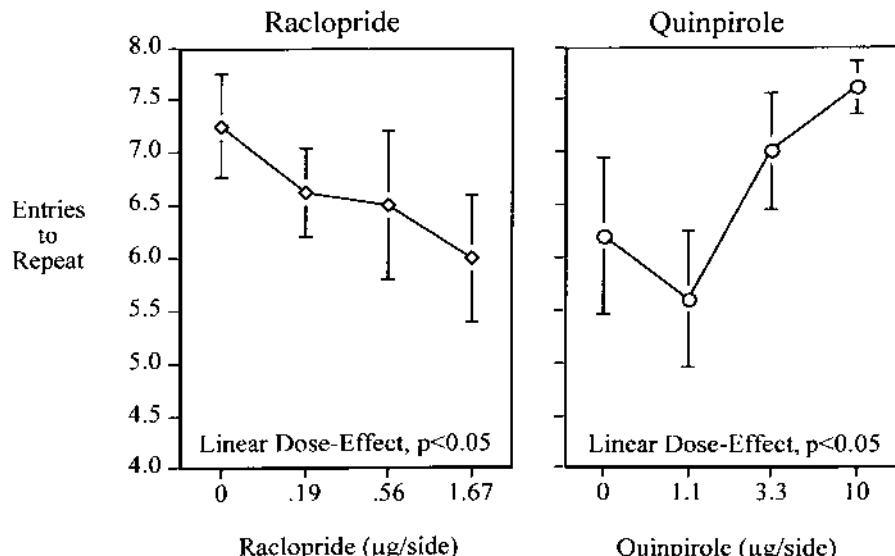


Figure 1. Working memory choice accuracy in the 8-arm radial maze (entries to repeat, mean \pm SEM) after local infusions of the D₂ antagonist raclopride or the D₂ agonist quinpirole [16].

correlation was also seen for D₂ receptors in the striatum another DA projection area which has classically been thought to be only involved in motor function.

As more receptor subtype selective ligands are developed, further animal studies will be important in determining the neural bases of CA involvement in cognitive function. In particular, local infusion methods can be used to determine the circuit basis for CA interactions with other transmitter systems in the programming of cognitive function. These same animal models represent key elements in determining the safety and efficacy of novel CA ligands before they can be used in human clinical populations. Haloperidol-induced memory impairment is clearly seen in experimental rat models. Haloperidol significantly impaired working memory performance on the radial-arm maze. This effect is seen with either acute injection or chronic infusion [20–22]. The atypical antipsychotic clozapine has also been shown in rat models to cause memory impairment [20]. Very exciting is the finding that direct DA agonists could be useful in attenuating the degeneration in Parkinson's disease. The DA agonist pramipexole has shown efficacy in reducing neurotoxicity after exposure of experimental animals to MPTP or 6-OHDA [23].

Working memory was impaired by DA depletion of the hippocampal formation via injections of 6-OHDA [15]. Local infusions of D₁ antagonists into the frontal cortex of monkeys caused a significant impairment in working memory [14]. D₁ agonist treatment improved memory performance in young adults as well as aged monkeys [24]. Local infusions of D₂ agonists into the caudate nucleus improved memory in rats [25].

NE depletion with DSP-4 caused significant learning impairments in aged rats [26]. NE α₂ agonists such as idazoxan, clonidine and guanfacine have been shown to significantly improve attention and working memory in rodent and primate models [27–30]. NE α₂ agonist treatment has also been found to reverse cognitive impairments in animal models [31]. The NE α_{2a} receptor seems to be particularly important for the cognitive improvement shown by this class of drugs [32]. The frontal cortex appears to be the critical target area [33]. NE α₂ agonist therapy has been found to be effective in humans with dementia [34], Alzheimer's disease [35], Parkinson's disease [36] and schizophrenia [37].

Clinical research

Research in humans has also demonstrated the involvement of CA systems in cognitive function. Studies in normal human adults using imaging and pharmacological techniques have shown critical CA involvement in cognitive function. Clinical studies of patients with Parkinson's disease, Alzheimer's disease, schizophrenia, attention deficit hyperactivity disorder and age-associated memory impairment have shown CA involvement in cognitive dysfunction and the usefulness of CA-based therapeutics.

Parkinson's disease

Parkinson's disease is classically thought of as a motor disease. However, there are also often prominent cognitive impairments [38–41]. Parkinson's disease patients show deficits in visuospatial processing, motor planning, executive function, selective attention, mental flexibility and at later stages memory function [42]. Often these symptoms do not respond well to traditional DA therapy [43]. Direct DA agonist therapy such as pergolide, a non-specific DA receptor agonist, has been found to have mixed effects on cognitive performance in normal adults [44]. It improved working memory performance in already high performing subjects, but lowered scores in subjects with low baseline performance. In addition, the same dose of pergolide significantly impaired verbal fluency. Possibly DA receptor subtype-selective drugs would have a more pervasive cognitive enhancing effect.

Alzheimer's disease

Cholinergic systems are not the only systems which are impaired in Alzheimer's disease. DA systems also show decline. DA treatments, which have long been used in Parkinson's disease, are being found also to have cognitive-improving effects [45]. Both ACh and DA systems are disrupted by β -amyloid [46]. NE systems have also been shown to be impaired in patients with Alzheimer's disease. The decline in NE in the mid-temporal cortex has been shown to significantly associate with cognitive impairment [47]. Decreased NA cell number in the locus coeruleus was also shown to be related to behavioral aggressiveness in demented patients.

Attention deficit hyperactivity disorder

ADHD has been shown to involve impairments in frontal CA stimulation. The use of indirect CA agonists such as methylphenidate and amphetamine are the standard treatment for ADHD and significantly improve symptoms of inattention [42]. Long known in adolescents and more recently adults with ADHD [48, 49]

Schizophrenia

Cognitive impairments are seen in schizophrenics both as a result of the disease process and its treatment. Attentional deficits have long been known to accompany schizophrenia [50]. Problems in disregarding irrelevant stimuli seems to be of particular significance [42]. This cognitive impairment could even contribute to the hallmark psychotic symptoms. Unfortunately classic

antipsychotic drugs, and to a lesser degree atypical antipsychotics which are effective in reducing hallucinations, can also impair cognitive function. Classic treatments for schizophrenia have pronounced DA antagonist effects. Drugs like haloperidol have been shown to cause significant attentional and memory impairment in schizophrenia patients [51].

The use of CA agonists, either direct receptor agonists or indirect agonists such as methylphenidate or amphetamine, which have been shown to improve cognitive function in other syndromes of cognitive impairment, may have adverse effects in patients with schizophrenia because they can aggravate psychotic symptoms. This may be due to increased DA activity in the limbic system. Avoiding this action, NE α_2 agonists may be useful for improving cognitive function in people with schizophrenia [52]. In addition, further development of DA agonists, which have selective actions in the frontal cortex without increasing limbic DA activity, is warranted.

Age-associated memory impairment

In the non-demented elderly there can be a modest decline in memory function, which has been termed age associated memory impairment or mild cognitive impairment [53, 54]. DA function has been shown in imaging studies to be associated with declines in cognitive function including learning and memory [55]. It may be possible to provide symptomatic relief of this modest cognitive impairment with direct or indirect CA agonists, although safety and efficacy trials need to be conducted first.

Conclusions

Direct and indirect agonists of the CA systems DA and NE have been shown to improve cognitive function including attention and memory. They are useful therapeutic treatments for syndromes of cognitive impairment such as ADHD, Alzheimer's disease, Parkinson's disease and schizophrenia. Further development of medications acting on CA systems will depend on receptor subtype selectivity, so that the cognitive enhancement can be achieved with minimal side-effects of motor disruption, appetite dysfunction and abuse liability. CA systems play vital roles in the neural substrate of cognitive function. Disruptions of these systems are known to cause cognitive impairment. CA agonists can effectively reverse this dysfunction.

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Drugs that target serotonergic receptors

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Introduction

Of the neurotransmitter substances characterized to date, serotonin (5-hydroxytryptamine-5-HT) is perhaps one of the most extensively studied. Initial reports of its actions on smooth muscle appeared more than 50 years ago and it is now known to have widespread peripheral and central physiologic actions. In the periphery, the amine is released from platelets to activate clotting cascades and blood vessel constriction; in the gastrointestinal tract it inhibits gastric acid production and stimulates the contractions of smooth muscle [1]. In the central nervous system, 5HT is known to regulate or influence such diverse processes as activity patterns and circadian rhythm, food intake, sleep-wake cycles, aggressive behaviors, locomotion, thermoregulation, nociception, and sexual activity [2].

In humans, 5HT is well-known to play a key role in mood and emotion, and a number of psychiatric conditions including anxiety, depression, schizophrenia, and anorexia nervosa. While undoubtedly involved in attention, learning, and memory [3] these roles of 5HT are probably the least understood when compared to those of other major neurotransmitters, particularly other monoamines [4]. The complex interactions between 5HT neurons and other neuronal phenotypes, 5HT receptor heterogeneity, and the conflicting results of behavioral experiments have made functional assessments of 5HT in cognitive processes particularly difficult. There have been numerous conflicting or confusing findings regarding the effects of serotonin. For example, stimulation of the dorsal raphe and central injection of 5HT was found to impair learning and memory [5, 6], whereas elevated synaptic 5HT with re-uptake inhibitors was found to enhance memory [5, 7]. In contrast, Jaffard et al. [8] reported that 5HT re-uptake inhibitors caused memory impairments. Neuronal 5HT depletion from electrolytic and neurotoxic lesions have impaired, [9, 10] facilitated, [11, 12] or had little effect [13, 14] on memory processes. An array of ligands synthesized to function as agonists or antagonists at 5HT subtypes have been evaluated in recent years in an effort to resolve these controversies. However, due to the lack of adequate subtype specificity, in many cases the compounds

have only served as a further source of confusion. Nevertheless, there is now a credible body of evidence suggesting that several of the 5HT receptor subtypes could indeed serve as therapeutics targets for memory enhancement. The purpose of this chapter is to provide a brief overview of these therapeutic targets within the 5HT system and the pharmacologic approaches (with a few representative drug examples) designed to enhance memory function. For a more comprehensive overview of the role of 5HT and its receptors in memory function see reviews [3, 15].

5HT receptor subtypes as potential drug targets

The mammalian 5HT receptor superfamily currently consists of 14 cloned and sequenced receptors divided into seven classes designated as 5HT1–7 [16]. The following text provides a brief overview of the receptor subtypes that have been targeted specifically for memory enhancement based on their anatomical distribution and in some cases based on alterations known to occur with aging and Alzheimer's disease (AD).

Table 1. Serotonin receptors and drug development. Representative compounds with memory enhancing properties

Subtype	Regional distribution (Memory-related areas)	Effective ligands	Action
5HT1A	septum hippocampus entorhinal cortex cingulate cortex	WAY 1000635 MDL 73005	agonist agonist
5HT1 B	hippocampus subiculum striatum	GR 127935	antagonist
5HT2A	frontal cortex entorhinal cortex pyriform cortex claustrum nucleus accumbens hippocampus	MDL 100907 EMD 281014	antagonist antagonist
5HT3	hippocampus amygdala striatum cortex	ondansetron granisetron tropisetron RS 56812	antagonist antagonist antagonist antagonist
5HT4	frontal cortex hippocampus	RS 17017 SL 65.0155	agonist partial agonist
5HT6	nucleus accumbens cortex	Ro 04-6790 SB-271046-A	antagonist antagonist
5HT7	hippocampus thalamus	SB-357134-A SB-258741?	antagonist antagonist

5HT₁ receptors

With regard to memory-related brain area distribution, 5HT_{1A} receptors are concentrated in the limbic system, especially the hippocampus, lateral septum, cingulate, and entorhinal cortex. They are known to interact with a number of other neurotransmitter systems including noradrenergic, dopaminergic, GABAergic, and cholinergic systems. Their interactions with cholinergic systems may have particular relevance in dementing illnesses such as AD. For example, 5HT_{1A} receptors are expressed by cholinergic neurons in the medial septum and in the diagonal band of Broca [17]. They occur as somatodendritic receptors at high concentrations in the hippocampus [18], and are reduced in the *post mortem* brains of patients with AD-like dementia [19, 20]. Agonists of these receptors promote the growth and branching of cholinergic neurites in primary cultures of septal neurons [21], a finding that could have relevance to drug development strategies for AD. Further, 5HT_{1A} agonists MDL 73005, WAY 1000635, and WAY 1000135 prevent impairments induced by cholinergic antagonists [22]. However, the 5HT_{1A} agonists (8-OH-DPAT) and partial agonists (buspirone, tandospirone) disrupt passive avoidance, water maze spatial learning, and water avoidance learning tasks in rodents [23–26]. Therefore, at present it is unclear whether agonists or antagonists at this subtype offer the best approach to treating conditions such as AD.

5HT_{1B} receptors appear to be located in several important memory-related areas including the subiculum, hippocampus, and striatum. They are thought to reside on axon terminals [27] in the hippocampus where they may function to inhibit the release of ACh [28] and possibly the release of other neurotransmitters [29, 30]. The 5HT_{1B} agonist CP-93,129 was found to disrupt radial arm maze performance in rodents [30], whereas administration of the 5HT_{1B} antagonist, GR127935, at higher doses was found to increase the consolidation of learning [31]. Furthermore, 5HT_{1B} knock-out mice performed better in a spatial memory task compared to controls [32]. Collectively, the data summarized here support a therapeutic strategy of using functionally selective antagonists at 5HT_{1B} receptors in treating memory disorders.

5HT₂ receptors

The functional role of 5HT₂ receptors in the prefrontal cortex (PFC) and their contribution to working memory function and attention has received considerable attention recently. Preclinical as well as clinical evidence indicates that alterations in PFC function may at least partially underlie the cognitive and affective symptoms observed in depression and schizophrenia [33]. While it has been generally accepted that 5HT₂ antagonists have a potential to enhance memory function, the 5HT₂ subtype is now known to be more heterogeneous than originally thought, thus requiring further experimentation before firm conclusions can be made about the activity of specific ligands. The receptor is

now subclassified as 5HT_{2A}, _{2B}, or _{2C} and the compounds previously thought to be selective for the 5HT₂ subtype are now known to have various affinities for these (further classified) subtypes. The antagonists, ketanserin and ritanserin, are now designated as 5HT_{2A/2C} antagonists. Ketanserin improves memory of previously learned inhibitory responses and experimentally induced amnesia in rodents [34, 35], and enhances visual recognition memory in primates [36].

The 5HT_{2A} receptor subtype (specifically) has received considerable attention recently as a therapeutic target for the cognitive dysfunction associated with schizophrenia. The subtype is widely expressed in the prefrontal cortex and other memory-related brain regions including the entorhinal and pyriform cortex, claustrum, nucleus accumbens, and hippocampus. It has been shown in non-human primate studies to serve an important role in working memory function [37] and, further, antagonist activity at this subtype has been suggested to at least partially underlie the therapeutic advantages of atypical neuroleptics over typical agents. Support of this latter premise lies in the accumulating evidence that several atypical antipsychotics (which are known to antagonize 5HT_{2A} receptors) appear not only to attenuate positive symptoms of schizophrenia and to ameliorate negative symptoms, but also to enhance cognitive function in this patient population [38]. Accordingly, efforts to synthesize more selective 5HT_{2A} receptor antagonists are underway. MDL 100907, the first such agent introduced, is a 5HT_{2A} receptor antagonist with slightly greater than 100-fold selectivity for 5HT_{2A} receptors as compared to 5HT_{2C} receptors in radioligand binding studies [39]. The compound advanced from preclinical to clinical trials for schizophrenia [40], but has since been discontinued, possibly due to an inadequate separation between 5HT_{2A} and 5HT_{2C} receptors. In comparison to MDL 100907, the novel serotonin 5HT_{2A} ligand, EMD 281014, demonstrates a much higher selectivity for 5HT_{2A} *versus* 5HT_{2C} receptors with 5HT_{2A} antagonistic properties (Bartoszyk GD, personal communication, 2003). The effects of this more selective compound on memory function are unknown at present, but are currently the focus of behavioral studies.

5HT₃ receptors

5HT₃ receptors, the most extensively characterized of the serotonin receptor subtypes, are unique in that they are ligand-gated ion channels as opposed to second messenger-coupled receptors. They occur in relatively high concentrations in the amygdala and hippocampus (and to a lesser extent the cortex) of rodents and humans, and also appear to mediate inhibition of ACh release [41–44]. Considerable data now clearly support the assertion that antagonism of 5HT₃ receptors offers a rational approach to the therapy of some cognitive disorders. For example, whereas the 5HT₃ receptor agonist mCPBG impairs retention of an associative learning task in rats [45], ondansetron, a 5HT₃

receptor antagonist, improves performance in several rodent and primate memory tasks while lacking cholinergic side-effects [42, 46, 47]. Other selective 5HT₃ receptor antagonists, granisetron, tropisetron, and itasetron (DAU 6215), also improve memory in rodents [48–50].

Several years ago, we evaluated the (R) and (S) isomers of a potent serotonin (5HT₃) receptor ligand, RS-56812, to assess their effects on working memory performance in macaques trained to perform a delayed matching-to-sample (DMTS) task. A representative figure summarizing the results of these studies is presented in Figure 1A. While both isomers enhanced certain aspects of task performance, the (R) isomer produced more systematic and reproducible improvements [51] as indicated by the maintenance of improvements after repeated exposures to optimal doses. These results further support the potential therapeutic role for 5HT₃ receptor antagonists in disorders involving cognitive decline.

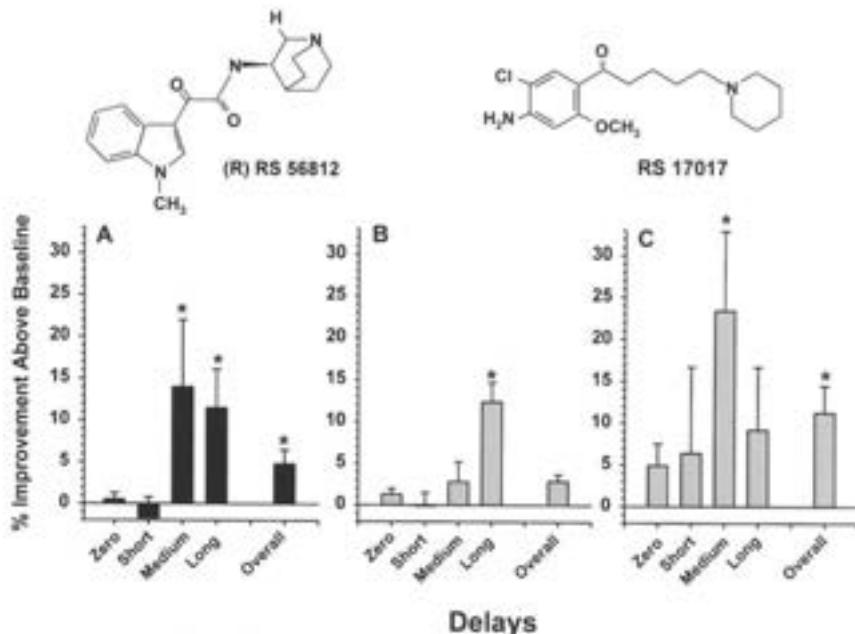


Figure 1. Improvements in accuracy above baseline levels of performance in a delayed matching-to-sample task (DMTS) task (employing 96 trials/session) by monkeys following administration of specific serotonin receptor ligands. (A) Performance associated with the (R) isomer of the 5HT₃ antagonist RS 56812 in young adult macaques. (B) DMTS performance associated with the 5HT₄ agonist RS 17017 in young adult macaques. (C) DMTS performance associated with the 5HT₄ agonist RS 17017 in aged macaques. The bars represent the mean of at least 2–4 replicates of the individualized optimal doses in percentage points above baseline \pm S.E.M. * = significantly different from placebo control value (Two-way repeated measures ANOVA, $p < 0.05$).

5HT₄ receptors

5HT₄ receptors are most dense in limbic system structures including the frontal cortex, hippocampus, and amygdala (areas which also contain a high density of cholinergic neurons or terminals) [52]. These receptors are markedly reduced in AD, and therefore have been identified as potential therapeutic targets for the disease. This hypothesis is supported by experiments in which activation of 5HT₄ receptors resulted in enhanced release of acetylcholine in rat frontal cortex [53], and in studies in which the non-selective 5HT₄ agonists BIMU-1, BIMU-8 and RS 66331 reversed experimentally induced amnesia in rodents. In addition, the selective 5HT₄ agonist RS 67333 improved atropine-impaired performance in spatial learning in rodents; and its cognitive enhancing effects were reversed by the selective 5HT₄ antagonist, RS 67532. [54]

Previously, experiments were conducted in our laboratory using a computer-assisted DMTS task to evaluate the potent and selective 5HT₄ agonist, RS 17017 [55], in younger and older macaques [56]. Significant improvements in DMTS accuracy were observed in both age groups after oral administration of the compound across a dose-effect series. Furthermore, repeated administration of optimal doses of RS 17017 to both cohorts resulted in sustained cognitive enhancement, indicating the reproducibility of the drug effect. Representative data from this study are provided in Figure 1 (B and C). As indicated in Figure 1C, RS 17017 enhanced performance of the DMTS in aged monkeys by more than 20 percent during trials of medium delay intervals. More recently, the partial 5HT₄ agonist, SL65.0155, was shown to enhance object recognition and water maze performance in young rats as well as linear maze performance in aged rats [57]. The compound was also found to produce a synergistic effect with the cholinesterase inhibitor, rivastigmine, in the object recognition task in young rats. The results of the studies cited above suggest that 5HT₄ receptor agonists and (partial agonists) offer a potential means of providing memory enhancement in disorders involving cognitive decline. Furthermore, the results are consistent with the hypothesis that central 5HT₄ receptors have a role in memory. The improvements observed in older animals might have particular implications for treating age-related conditions such as AD, while the positive results in the younger animals indicate that these compounds may have additional potential for treating memory disorders not necessarily associated with advanced age.

5HT₆ receptors

The recently characterized 5HT₆ receptor, subtype appears to be almost exclusively expressed in the brain. In regard to limbic and memory-related areas, the subtype appears to be expressed in relatively high levels in the nucleus accumbens, cerebral cortex, and subfields of the hippocampus [58]. While the func-

tion of this subtype is currently unknown, the distribution of this receptor, combined with its affinity for certain antipsychotic (e.g., clozapine, olanzapine), as well as antidepressant drugs (e.g., amoxipine, and amitriptyline), supports the hypothesis that $5HT_6$ ligands may have a therapeutic role in conditions such as schizophrenia and depression. Recent data also suggest that $5HT_6$ ligands may have memory enhancing capabilities. For example, the $5HT_6$ receptor selective antagonists AO and RO 04-6790, SB-271046-A, and SB-357134-A have all been associated with positive effects in rodent water maze tasks [59].

5HT₇ receptors

The $5HT_7$ subtype is the most recently cloned serotonin receptor, although functional responses now attributed to this receptor have been published for several years. The receptor has been identified as important in circadian rhythms and sleep and some recent electrophysiologic studies appear to suggest that the receptor could serve as a target for anticonvulsant drugs [16]. As in the case of the $5HT_6$ subtype, $5HT_7$ receptors also bind several antidepressants (e.g., mianserin, maprotiline) and antipsychotics (clozapine, risperidone) with high affinity, indicating that this receptor may represent a therapeutic target for schizophrenia and other psychiatric disorders. In fact, the selective $5HT_7$ antagonist SB-258741 is currently being evaluated in preclinical studies as a potential treatment for schizophrenia [60]. With regard to its CNS distribution in memory-related areas, the receptor is found in relatively high concentrations in the hippocampus and thalamus, with generally lower levels found in the cortex and amygdala. To date several reports and reviews [15] suggested (based on receptor distribution and preliminary pharmacologic analyses) that this receptor might represent another serotonergic target for memory enhancement. At present, this hypothesis awaits further experimentation.

Conclusion

While many aspects regarding the functional role of serotonin (and its complex array of receptor subtypes) are yet to be learned, few would argue against the importance of this neurotransmitter system in memory function. Through the combined use of modern molecular biology, transgenic animal models, and other more traditional research methods such as medicinal chemistry and classical pharmacology, a clearer picture of the role of serotonin and its receptors in mnemonic processes is likely to emerge in the near future. Considerable data now support the argument that selective ligands at $5HT$ receptor subtypes could indeed serve as therapeutic agents designed to enhance cognitive function in both age-related CNS diseases and in diseases encountered by younger patients.

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Drugs that target ionotropic excitatory amino acid receptors

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Introduction

The idea of glutamate as a neurotransmitter in the mammalian CNS emerged around the middle of the 20th century [1–3], and over the following years it became evident that multiple receptor subtypes were responsible for the physiological properties of glutamatergic neurotransmission [4]. Thanks to the combined efforts of physiology, molecular biology and pharmacology we gained a complex picture of the various types and flavours of ionotropic and metabotropic glutamate receptors. Today we possess the biological and chemical tools to characterize glutamate receptor function from the molecular level to the situation *in vivo*. Based on this knowledge, the clinical use of advanced glutamate receptor modulators for the treatment of e.g., cognition deficits comes into view. In this chapter I will focus on the two main subtypes of ionotropic glutamate receptors, namely those activated by the specific agonists AMPA and NMDA, and the pharmacological intervention which should improve cognition in man. For details about receptor subtype classification and structure the reader is referred to recent reviews (e.g., [5]).

Physiological functions of glutamate receptors

In the prototypic glutamatergic synapse AMPA- and NMDA-receptor gated ion channels act in concert: Upon the release from the presynaptic terminal glutamate reaches millimolar concentrations in the synaptic cleft and binds to both receptor types. NMDA receptor channels, however, are blocked by extracellular Mg²⁺ ions, and this voltage-dependent blockade must first be relieved by the post-synaptic depolarization caused by AMPA receptor channel activation. Thus, the post-synaptic potentials (as well as the underlying membrane currents) are biphasic, and the two components can be discriminated biophysically and pharmacologically [6]. Upon high-frequent presynaptic stimulation the influx of Ca²⁺ via NMDA receptor channels can trigger further downstream processes, which can result in plasticity processes like long-term poten-

tiation (LTP), which is regarded as prerequisite for memory formation [7, 8]. In recent years evidence has accumulated to further indicated that activation of AMPA receptor channels is important for synaptic bouton maintenance [9]; moreover the concentrations of neurotrophic factors in brain tissue are affected [10]. Based on these findings it is not surprising that drugs which target ionotropic glutamate receptors can have effects on cognition, memory formation and higher brain functions. The following sections will highlight some of the most relevant findings.

AMPA receptor modulators

AMPA receptor agonists and antagonists

Due to the physiological role of AMPA receptors it is not surprising that their ligands can affect cognition. Flood and co-workers [11] investigated the effects of agonists (quisqualate and kainate), as well as antagonists (GAMS and DNQX) of AMPA receptor channels on memory processing in a T-maze model in mice. Not unexpectedly, performance was improved by the agonists and worsened by the antagonists. The use of AMPA receptor agonists as medications in the treatment of disorders of cognition, however, is prevented by the neurotoxicity of such compounds. Domoic acid, for example, is an orally-active AMPA receptor agonist which can accumulate in sea mussels under certain environmental conditions. Consumption of these poisonous mussels can lead to intoxications resulting in seizures [12]. The administration of AMPA itself can be lethal, and a rodent assay based on this AMPA cytotoxicity is used as a simple *in vivo* test for the effects of potential neuroprotective drugs [13]. These non-physiological agonists can cause excitotoxicity, thereby mimicking elevated levels of glutamate which are known to occur in conditions of global or focal ischemia. Indeed, it has been postulated that excitotoxicity represents an underlying cause for the slow neurodegeneration that occurs in Alzheimer's disease [14].

Positive allosteric AMPA receptor modulators (PAARMs)

The prototype AMPA receptor channel modulator aniracetam was reported to improve performance in rodent models of learning and memory [15]. This compound had been used clinically for the treatment of cognitive dysfunction [16]. The mechanism of action of aniracetam was identified in the early 1990s, and it was shown that the drug reduced the desensitisation of AMPA receptor mediated membrane currents, it slowed the decay of excitatory synaptic signals, and it augmented LTP in hippocampal slices [17–22]. During subsequent years, a variety of new structures from different chemical classes having AMPA receptor modulating properties were identified (for review: [23]). The

most completely characterized of these are the "ampakines" (Cortex Pharmaceuticals), and IDRA 21 (Fidia-Georgetown Institute of the Neurosciences). The latter compound will be described in more detail.

IDRA 21 is structurally related to cyclothiazide, and it produced similar effects on AMPA receptors in thin slices of rat brain. Membrane currents in response to glutamate application were augmented, and the desensitisation of glutamate responses in excised neuronal membrane patches was removed [24]. Our own experiments using HEK 293 cells which recombinantly expressed human GluR1/2 receptor channels support these findings (Fig. 1). In electro-

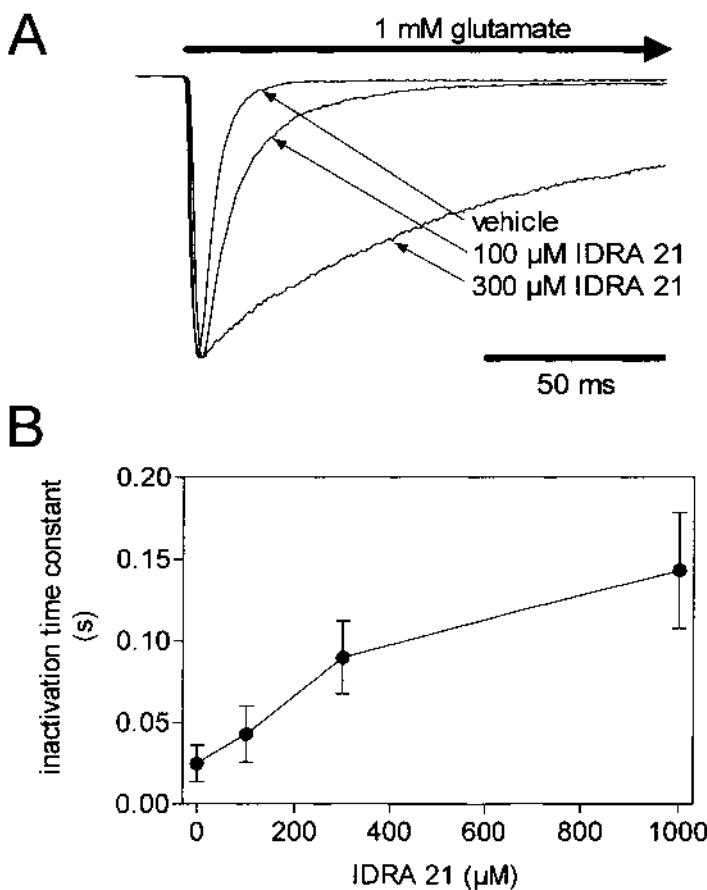


Figure 1. Effect of IDRA 21 on glutamate-induced ion currents in cells expressing recombinant human GluR1/2 receptors. Cells were investigated in the whole-cell, voltage-clamp configuration of the patch-clamp technique. (A) Original recordings from a cell to which 1 mM glutamate was applied together with vehicle, 100, or 300 μ M IDRA 21. Traces were normalized to the peak current of the vehicle response. IDRA 21 dose-dependently inhibited inactivation. Holding potential was -80 mV. (B) Concentration-dependent increase of the time constant of inactivation. Data are shown as the means of 4–13 cells per IDRA 21 concentration. Recording conditions were as in (A).

physiological whole-cell, voltage-clamp experiments IDRA 21 reduced desensitisation of membrane currents in response to the application of 1 mM glutamate (Fig. 1A), and this effect was concentration-dependent (Fig. 1B). Thus, IDRA 21 is also active on human AMPA receptors.

The duration of synaptic responses in hippocampal cultures was increased by IDRA 21, and the development of LTP was promoted [25, 26]. The overall potency *in vitro*, however, was relatively low, and the effective concentrations in the various models were between 200 and 1,000 µM. Nevertheless, IDRA 21 was effective *in vivo* at relatively low doses. In a passive avoidance test in rats, behavioural impairment induced by the administration of the GABA_A modulator alprazolam was reversed by 10 mg/kg IDRA 21 [27]. In the same study, the performance of rats in a water maze model was evaluated. Task performance (spatial memory) was dose-dependently inhibited by alprazolam, the AMPA receptor antagonist NBQX, or by the muscarinic antagonist scopolamine. With co-administration of IDRA 21, task proficiency was normalized. These data indicated that IDRA 21 was effective in enhancing cognition, and that this property was not dependent of the nature of the amnestic challenge. IDRA 21 also abated cognitive impairment in monkeys (induced by alprazolam [28]). The *in vivo* data shown so far, however, are hampered by the fact that amnestic drugs were used to decrease cognition. To obtain data in a model which is more relevant for the clinical situation, IDRA 21 was tested in aged monkeys in a delayed-match-to-sample task. The drug also was shown to effectively enhance task accuracy in this model [29].

The first experimental results from another class of PAARMs, the ampakines, provided further support for the validity of this pharmacological approach to treat cognition deficit disorders. The ampakines have proven efficacy *in vitro* and *in vivo* [30–33], and CX 516 improved memory in early clinical trials, although relatively high doses (up to 1,200 mg) were used [34–36]. Moreover, the plasma half-life of this compound is relatively short. Nevertheless, CX 516 is reported to be in development for use in Alzheimer's disease, schizophrenia, mild cognitive impairment, and attention deficit hyperactivity disorder [37]. Newer compounds with improved properties have been described (e.g., LY 404187, [38]; or S 18986, [39]), but no clinical data are thus far available. Taken together there exists compelling evidence that PAARMs could be a useful approach to treat disorders involving cognition deficits in man.

Open questions

One still unresolved issue is the large discrepancy between the concentrations of PAARMs which are active *in vitro*, and their plasma/brain concentrations *in vivo*. Concentrations of IDRA 21 to measurably affect AMPA receptor channel kinetics or synaptic transmission are in the range of 200 to 1,000 µM. Effective doses in animal experiments (~ 10 mg/kg) induce peak brain levels in the range

of 10 to 30 μM (K. Klinder, personal communication). Similarly, Lynch and co-workers reported plasma levels of up to 16 μM after the administration of 1,200 mg CX 516 to healthy volunteers [35], and it can be assumed that brain levels are within the same range since in rats, the plasma/brain concentration ratio is ~1 (K. Klinder, personal communication). Such low concentrations do not show any observable effect *in vitro*! Since there is no doubt that PAARMS do possess cognition-enhancing properties, it is likely that slight modulation of glutamatergic synapses can account for their robust effects observed *in vivo*. This situation renders the conclusion that the *in vitro* identification and characterization of PAARMS may only be achieved at high, physiologically irrelevant, concentrations.

Another issue concerns the possible neurotoxicity of AMPA receptor modulators. One could postulate that facilitation of glutamate receptor function could worsen the clinical outcome in excitotoxicity-related neurodegenerative disorders. Indeed it has been reported that both the ampakine 1-BCP, and IDRA 21 potentiate the toxicity to glutamate in a cell culture model [40, 41]. Moreover, IDRA 21 enhanced hippocampal damage *in vivo* in a model of global ischemia [40].

Conversely, PAARMS were also shown to be neuroprotective *in vivo*, and are discussed as medication to promote recovery after traumatic brain injury or stroke [42, 43]. Thus, future will have to tell whether or not, or under which conditions, PAARMS can be neurotoxic.

NMDA receptor agonists

Due to the prominent role of NMDA receptors in mediating CNS plasticity, NMDA receptor agonists have the potential to serve as cognition-enhancing drugs, and indeed this has been demonstrated in rodent experiments [11]. Any beneficial effects to be gained in the clinical situation, however, would likely be masked by the inherent toxicity of NMDA receptor agonists, which are potent excitotoxic compounds. Alternatively, the use of allosteric NMDA receptor channel modulators might reduce the risk of unwanted side-effects. This may be the case as Schwartz and co-workers [44] reported that administration of d-cycloserine (a partial agonist at the modulatory glycine site of the NMDA receptor) to Alzheimer's patients resulted in the augmentation of implicit memory.

NMDA receptor antagonists

NMDA receptor channel antagonists like phencyclidine or ketamine produce acquisition deficits in animal models [45] and they can mimic certain symptoms of schizophrenia in animals [46], as well as in man [47, 48]. Thus, it appears contra-intuitive that receptor blockers could also exert positive effects

on cognition. This very action was demonstrated, however, for the non-competitive NMDA receptor blocking drug memantine. This compound binds to the same binding site as phencyclidine or dizocilpine, but its affinity is lower, and the kinetics of binding and unbinding are much faster [49]. Surprisingly Frankiewicz and Parsons [50] reported that memantine increased LTP in a rodent hippocampus slice preparation at concentrations which were clinically relevant.

In Europe, memantine is approved for the treatment of Alzheimer's disease, and two mechanisms of action can be hypothesized. The drug can be postulated to reduce the progression of slow excitotoxicity-related neurodegeneration [51]. The second effect directly affects neurotransmission: In resting neurons, NMDA receptor channels are blocked by extracellular Mg^{2+} ions, which by a voltage-dependent process, occlude the channel pore. Upon depolarization (by the activation of AMPA receptors) this block is released, and downstream processes are initiated, which can culminate in LTP and memory formation. However, under conditions of chronic neurodegeneration and compromised energy metabolism, extracellular levels of glutamate should be slightly increased, and the neuronal membrane potential depolarized. This could lead to partial relief of the Mg^{2+} block of the NMDA receptor. As a consequence, the physiological processes underlying LTP would be functioning abnormally. Memantine might possibly replace the physiological function of Mg^{2+} , thus re-enabling LTP.

Conclusions

Ionotropic glutamate receptors are cornerstones of brain function, and biophysical and pharmacological investigations have left no doubt as to their importance for cognition. Future studies will determine whether it will be possible to develop potent glutamate receptor modulating drugs to treat cognitive disorders.

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Cognitive enhancing effects of drugs that target histamine receptors

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Introduction

While histamine effects have been studied for decades in many classical experiments of basic pharmacology, and histamine is often used as a tool to elucidate fundamental pharmacological principles of drug-receptor interactions, it is only within recent years that its importance as a neurotransmitter has been recognized. This is despite findings of the substance within the CNS in the early 1900s, with a differential distribution and enzymes necessary for synthesis (for review, see [1]). As will be described below, new findings suggest important therapeutic roles for histamine and therapeutic potential for drugs that interdict the histaminergic pathways in various neurological diseases, particularly those having a cognitive deficit that these drugs may be anticipated to ameliorate (see Fig. 1 for chemical structures of key compounds discussed).

Histamine as a neurotransmitter

Histamine, a key neurotransmitter in the CNS, is synthesized in hypothalamic neurons (for review see [1, 2]). These neurons are large, localized to the tuberomammillary nucleus and have been shown to have a regular rate of spontaneous firing [2, 3]. Nerve terminals from these cell bodies project mostly ipsilaterally throughout the CNS, especially to the hippocampus and cortex, areas important for memory and cognition, but also to other regions where histamine may play a key role in various physiological and homeostatic functions such as the hypothalamus, basal forebrain and amygdala [2–4]. The neuroanatomical architecture and electrophysiology of the histaminergic system, therefore, are much like those of other biogenic amine neurotransmitters such as dopamine, serotonin, or norepinephrine [2, 4].

Histamine is synthesized from L-histidine, and is degraded by histamine N-methyl transferase [1, 5]. However, the metabolic fate of histamine differs from dopamine, serotonin, or norepinephrine. These amines are normally

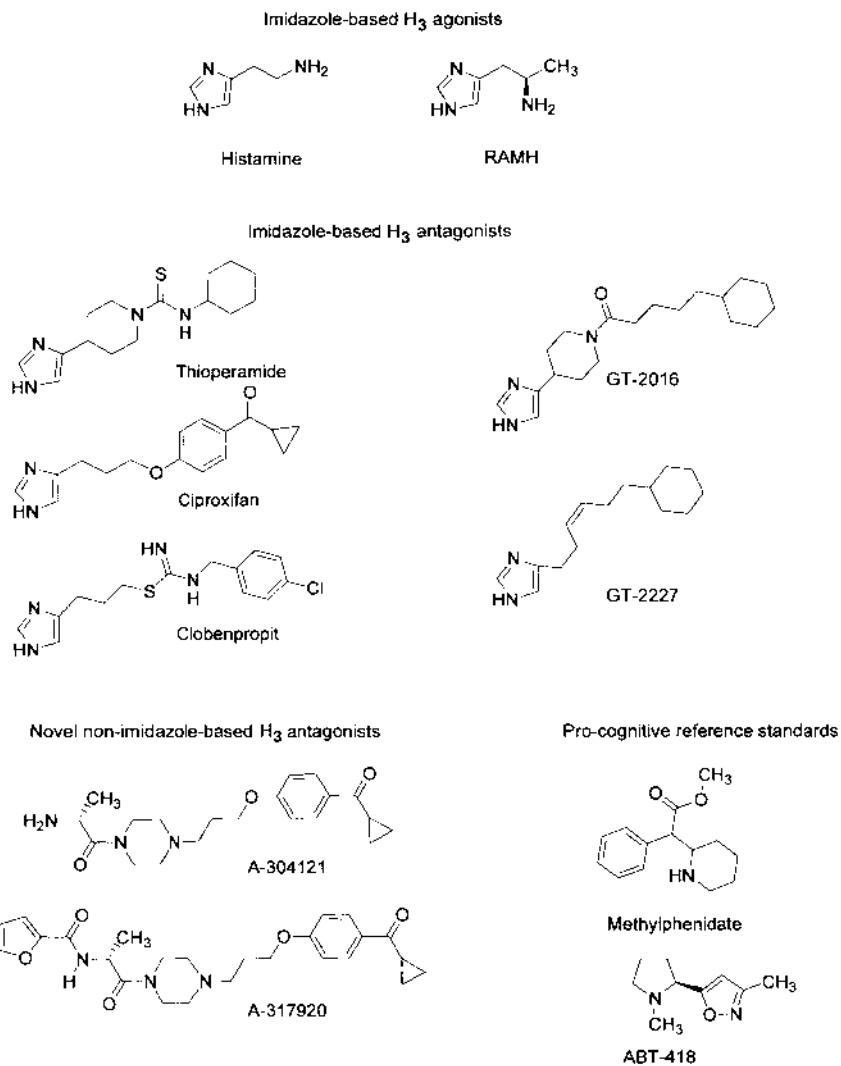


Figure 1. Chemical structures of H₃ receptor ligands and pro-cognitive reference standards discussed in the text.

removed from the synapse by very high affinity re-uptake pumps, sequestered in the presynaptic neuron where they may be repackaged for reuse and/or metabolized by inactivating enzymes such as monoamine oxidase. For histamine, there is no active uptake pump, and histamine is thought to diffuse from the synapse where it is inactivated by extrasynaptic histamine N-methyl transferase [1, 5].

Histaminergic receptor subtypes

Neuronally-released histamine can potentially interact with four subtypes of histaminergic receptors, H₁, H₂, H₃ and H₄, although there is little evidence for H₄ receptors within the CNS [6]. All four of these receptor types have been successfully cloned [7] and shown to belong to family 1 of the superfamily of G-protein coupled receptors (GPCRs), although they differ substantially in their protein sequences, their signal transduction pathways, their pharmacological characteristics and their anatomic distribution and function [7, 8]. H₁ receptors are known for their functional role in atopy and can be blocked by antiallergic "antihistamines". Their signal transduction pathway appears to be primarily the stimulation of calcium mobilization via a G_q protein [9]. H₂ receptors are important for acid secretion within the stomach and are blocked by antiulcer H₂ antagonists. These receptors preferentially couple to G_s to enhance cyclic AMP production [9], whereas H₃ and H₄ receptors appear to couple to G_i, the G-protein linked to inhibition of cyclic AMP production [7].

Within the CNS, H₁ receptors are chiefly neuronal and have a high density within the human neocortex and various limbic structures, although this is not the case in all species [1]. Administration of selective agonists and antagonists (and the well-known clinical somnolence of classical antiallergic antihistamines) have shown an important role for histaminergic stimulation of H₁ receptors in vigilance and alertness, most likely at sites within the ventrolateral posterior hypothalamus [1]. The autoradiographic analysis of H₁ receptor density in the rat brain also shows high densities in thalamic nuclei and the cerebellum [1].

CNS H₂ receptors are distributed in discrete regions, with highest densities in caudate, putamen, nucleus accumbens and cortical areas, with low levels in the hippocampus and globus pallidus [10]. Like H₁ receptors, H₂ receptors are primarily associated with neurons within the CNS [1]. Their functional role within the CNS is unclear. Early electrophysiological studies indicated that stimulation of H₂ receptors might inhibit firing rate in a number of brain regions, although the interpretation of that data has been questioned by the lack of specificity of some of the antagonists employed [1]. In comparison to functions known to be associated with H₁ receptor stimulation, H₂ receptor activation plays a lesser role, both in the number of functional behaviors linked to H₂ receptors, but also in the relative importance of H₂ compared to H₁ receptors in those functions where both receptors may be involved [3]. Knockout studies have similarly failed to elucidate an obvious role for H₂ receptors, since the behavioral phenotype of H₂ knockout animals (decreased spontaneous locomotor activity) is similar to that of H₁ receptor, H₃ receptor and histidine-decarboxylase knockout animals [11].

H₃ receptors have also a diverse CNS distribution pattern among species, as observed for the H₁ receptor [1]. Highest densities are observed in the cerebral cortex, with lesser amounts in the hippocampus, amygdala, anterior olfactory nuclei, nucleus accumbens, striatum and other regions [1]. A recent report has

shown similar distribution patterns of H₃ receptors in the rat brain based on either autoradiographic data or the analysis of mRNA levels [12]. H₃ receptors have also been detected by autoradiographic, *in situ* hybridization and functional activation assays in human brain [13].

H₁ and H₂ receptors appear to be localized primarily post-synaptically, where they respond to histamine as described above to enhance vigilance (H₁) or to inhibit neuronal firing (H₂). In contrast, H₃ receptors are located presynaptically as autoreceptors or heteroreceptors to modulate neurotransmitter release [1, 2, 5]. In their role as autoreceptors, H₃ receptor stimulation has been shown to inhibit the synthesis of histamine [2, 5]. In their role as heteroreceptors, H₃ receptor stimulation has also been shown to modulate the release of a variety of neurotransmitters such as acetylcholine, norepinephrine, dopamine, serotonin and gamma-amino butyric acid (GABA), [1, 2, 5].

H₃ receptors display a number of splice variants that differ between species [14]. The differences among the splice variants are located at regions of the receptor that are thought to be important for different signal transduction pathways [14] such that drug actions at different splice isoforms could have different pharmacological results. In either rat or human, the long form of the receptor is predominant in the hippocampus and cortex, areas important for cognition and learning, while the short form has a higher expression level in the hypothalamus [15–17]. In addition, there are minor differences between the amino acid sequences of H₃ receptors from various species [14, 18–20] that are responsible for substantial pharmacological differences between species. Such differences at the pharmacological level have not been noted for either H₁ or H₂ receptors.

Another complexity of histaminergic H₃ receptors is the finding that the H₃ receptor is constitutively active [21–23], meaning that even in the absence of exogenous histamine, the receptor is activated. In the case of an inhibitory receptor such as the H₃ receptor, this inherent activity causes activation of downstream signal transduction pathways leading to tonic inhibition of neurotransmitter release. Therefore, it may be found that a desirable property of any H₃ antagonist used clinically would be to act also as an inverse agonist to not only block the effects of exogenous histamine but to also shift the receptor from its constitutively active (inhibitory) state to a neutral state lacking the tonic inhibition of neurotransmitter release.

Histaminergic neurons in the tuberomammillary region of the hypothalamus receive afferent input from a number of prefrontal and infralimbic regions of the cortex, from all septal regions and from other areas of the hypothalamus, particularly the preoptic and anterior areas [4]. Such afferent input results in the release of a number of neurotransmitters that have the potential to alter histaminergic neuronal function, including orexin, glutamate, serotonin, acetylcholine, GABA, galanin, and histaminergic neurons also receive inputs mediated by NMDA (N-methyl-D-aspartic acid) and AMPA (γ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptors [4, 24].

Histaminergic drugs that influence cognitive processes

The central histaminergic system has been shown to play an important role preclinically in rodent cognitive processing. For example, early work demonstrated that histamine administered intracerebroventricularly (*icv*) enhanced consolidation of passive avoidance recall [25]. In contrast, interruption of histaminergic neurotransmission following *icv*, intravenous (*iv*) or oral (*po*) administration of the classic H₁ receptor antagonists promethazine, diphenhydramine and pyrilamine negatively impacted the acquisition and retention of active avoidance responding in rats, effects that were attenuated by activation of the histaminergic system following *icv* administration of histamine itself or, intriguingly, by acetylcholine [26, 27]. Similarly, *icv* injection of histamine has been shown to attenuate hippocampal lesion-induced deficits in passive and active avoidance responding in adult rats [28, 29] and improved performance of aged rats in an active avoidance task [30]. Intracerebroventricular administration of histidine or histamine was also shown to improve social recognition in adult rats [31]. These early experiments demonstrated that histaminergic neurotransmission is involved in higher learning and memory processing in the rat. Many subsequent studies by research groups working in this field have focused on indirectly facilitating the histaminergic system by blocking H₃ auto- and/or heteroreceptors. Consequently, much of the remainder of this section will focus on recent advances in elucidating the role of H₃ receptors in cognition and the identification and testing of new, highly selective and potent H₃ receptor blockers.

H₃ receptor antagonists and arousal/attention

Initial evidence that histamine neurotransmission is involved in arousal, vigilance and attention was derived from clinical observations that 'antihistamine' H₁ receptor antagonists prescribed for treatment of allergic disorders sometimes induced sleepiness and cognitive deficits [32, 33]. The severity of this impairment was found to be correlated with H₁ receptor occupancy in the human brain, as revealed by positron emission tomography (PET) [33]. Preclinically, the arousal effects of hypocretin/orexin were not observed in H₁ receptor knockout mice [34], suggesting the histaminergic system is mediating the arousal effects of orexin. Hypothalamic histaminergic neurons are also known to fire in a rhythmic, synchronous pacemaker-like fashion, a property intrinsic to individual neurons [35] and a circadian rhythm in the release of histamine from the anterior and posterior hypothalamus has been shown in rats [36, 37]. Further, histaminergic neuronal firing has been shown to be correlated with behavioral state in rats, cats and monkeys, with maximal firing corresponding to periods of wakefulness, and reduced activity corresponding to periods of sleep [38].

This association of histaminergic neuronal firing with behavior state has led to significant interest in developing drugs that might modulate attention, vigilance and arousal in humans, potentially without the liabilities of current, clinically-used medications such as stimulants for attention deficit hyperactivity disorder (ADHD). Indeed, the discovery of the selective H₃ receptor agonist (*R*)-α-methylhistamine and the relatively selective antagonists thioperamide, ciproxifan, GT-2016 and GT-2227 has allowed a better understanding of the role of histamine in attention and arousal. For example, during natural arousal in response to handling, H₃ autoreceptors have been shown to modulate histamine release in the rat frontal cortex, with microdialysis probe-infused (*R*)-α-methylhistamine suppressing endogenous histamine release and thioperamide potentiating release [39]. In separate studies in the conscious rat and cat, activation of H₃ receptors with (*R*)-α-methylhistamine increased slow wave EEG activity (indicative of a sleep-like pattern) while opposite effects with thioperamide were interpreted to enhance wakefulness [40, 41]. Similarly, the potent H₃ receptor antagonist ciproxifan significantly decreased slow wave cortical activity following oral administration in the conscious cat [42], which has also been demonstrated following *ip* administration in the conscious rat [43]. In these latter two studies, ciproxifan also improved response accuracy in an adult rat 5-choice serial reaction time task [42] or enhanced performance in a rat pup 5-trial inhibitory avoidance task (attenuated by (*R*)-α-methylhistamine), both of which model aspects of attention and impulsivity (Fig. 2).

Both GT-2016 and GT-2227 have also been shown to improve acquisition of a 10-trial inhibitory avoidance task [44]. A potential confound of some of these early data with antagonists is binding at additional receptors such as α2_c or at recently discovered H₄ receptors. Highly selective antagonists for H₃ receptors such as A-304121 and A-317920 were recently described by our laboratories; these have been found to be at least as efficacious as methylphenidate (Ritalin[®]) and ABT-418, both clinically effective for treating ADHD, in the rat pup 5-trial inhibitory avoidance task, offering improved therapeutic ratios over thioperamide and ciproxifan [43, 45, 46] (Fig. 3).

Interestingly, these pro-cognitive effects were observed at doses below the threshold for affecting slow wave activity, indicating that alteration of EEG patterns is not a prerequisite for efficacy in cognition models. In the recently described H₃ receptor knockout mouse [47] thioperamide had no effect on wakefulness, non-REM or REM sleep whereas thioperamide increased wakefulness and decreased non-REM sleep in wild type controls. Taken together, these data confirm the important role of the H₃ receptors as a mediator of wakefulness and support the idea that blockade of H₃ receptor function with highly selective compounds will promote wakefulness and improve attention *in vivo*.

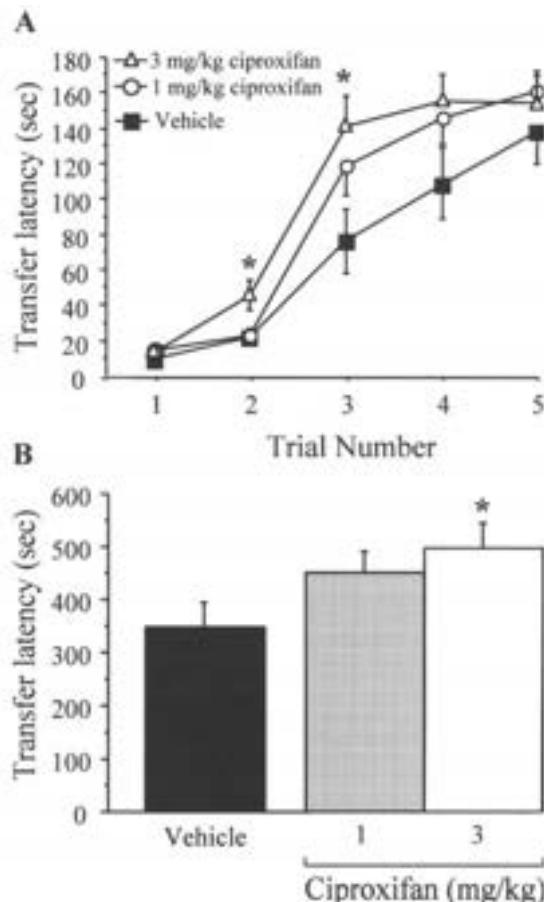


Figure 2. The H_3 receptor antagonist ciproxifan enhances performance of SHR pups in a 5-trial, repeated acquisition, inhibitory avoidance task, reaching significance (* $p < 0.05$ when compared with vehicle-treated controls) as early as the second trial (A). When data for trials 2–5 are summed and meaned, a significant improvement in performance over vehicle-treated controls was evident at 3 mg/kg (B). Rat pups were dosed with antagonist 30 min prior to the first trial. Data are represented by mean \pm S.E.M for clarity: statistical calculations used non-parametric analyses. Reproduced with permission from [45].

H_3 receptor antagonists and short-term/working memory

Clear evidence for the involvement of histaminergic neurotransmission in short-term and working memory was demonstrated in a number of early studies utilizing several different animal behavior models. Prast and colleagues [31] showed that *icv* injection of histamine facilitated short-term social memory in the adult rat. Further, histidine, a histamine precursor, significantly increased histamine levels in frontal cortex, hypothalamus and other brain

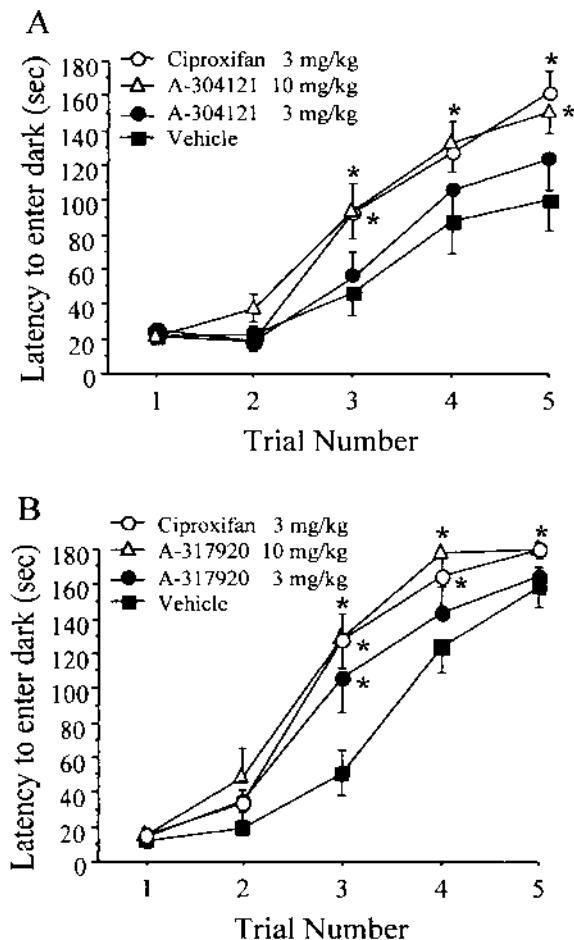


Figure 3. Enhancement of acquisition of a 5-trial inhibitory avoidance response in SHR pups with A-304121 (A) and A-317920 (B), with ciproxifan as a positive control in both instances (* $p < 0.05$ with respect to vehicle-treated controls). Rat pups were dosed with antagonist 30 min prior to the first trial. Data are represented by mean \pm S.E.M for clarity: statistical calculations used non-parametric analyses. Reproduced with permission from [43].

regions and enhanced social memory. Taken together, these data indicate that histamine release from histaminergic neurons is involved in this particular task. Also in these studies, social memory impairment was produced by inhibiting synthesis of histamine with *icv* administration of the histidine decarboxylase inhibitor α -fluoromethylhistidine (α -FMH), which also significantly decreased brain histamine levels. A similar impairment was found following *icv* administration of the H_3 receptor agonist immezipip, whereas the H_3 receptor antagonist, thioperamide, improved social memory, indicating that modulation of histamine release through blockade of the H_3 autoreceptor is an effec-

tive strategy for facilitating short-term memory in the rat. In the radial arm maze, Chen and colleagues [48] utilized a paradigm that addressed the effects of modulating histamine levels on spatial working and reference memory in adult rats. Significant performance deficits were observed following *icv* administration of α -FMH, with a strong correlation between total errors and decreased histamine levels in the cortex and hippocampus, regions of the brain known to be involved in performing this task. Histamine and thioperamide both attenuated the deficits induced by α -FMH in these studies. H_1 receptor antagonists have also been shown to impair radial arm maze performance [49]. In another model of short-term memory, Blandina and co-workers [50] reversed scopolamine-induced deficits in an object recognition task with thioperamide, but showed impairment in a similar task following *ip* administration of the H_3 receptor agonists imetit or (*R*)- α -methylhistamine [51]. Similarly, Orsetti et al. [52, 53] demonstrated enhanced short-term spatial recall in a hippocampus-dependent, two-trial, place recognition Y-maze task in rats following post acquisition intraperitoneal (*ip*) or intra-nucleus basalis injection of thioperamide. Even more recently, we have demonstrated significant enhancement of short-term memory in an adult rat social recognition model with methylphenidate and ciproxifan, as well as with the highly selective H_3 receptor antagonists A-304121 and A-317920 (Fig. 4) [43], a task that is also dependent on an intact hippocampus. As mentioned earlier, A-304121 and A-317920 were also effective in a five-trial inhibitory avoidance task that models aspects of attention and impulsivity in SHR pups, supporting a potential therapeutic role for specific H_3 receptor antagonism in neurological disorders such as attention deficit hyperactivity disorder. Interestingly, A-304121 and A-317920 did not exhibit any of the stimulant-like effects of methylphenidate on EEG or on locomotor activity in habituated or non-habituated mice [43].

H_3 receptor antagonists and spatial reference memory

Histaminergic neurotransmission has been shown to play an important role in delayed recall up to 24 h following acquisition in an inhibitory avoidance model in adult rats and in H_3 receptor knockout mice [47, 51] or 24 h following acquisition in a modified version of an elevated plus maze test in mice [54, 55]. Both of these tasks tap into a form of episodic memory that is dependent on intact frontal cortex and hippocampus. The effects of histamine and H_3 receptor ligands are less clear with respect to long-term, spatial reference memory, however. For example, there is a paucity of information on the effects of H_3 receptor ligands in the water maze, despite this model being perhaps the most commonly employed test of spatial learning and memory. An early report on the effects the agonist (*R*)- α -methylhistamine described, surprisingly, an attenuation of a scopolamine-induced deficit in a hidden platform version of the task [56]. The authors suggested a possible post-synaptic H_3 receptor

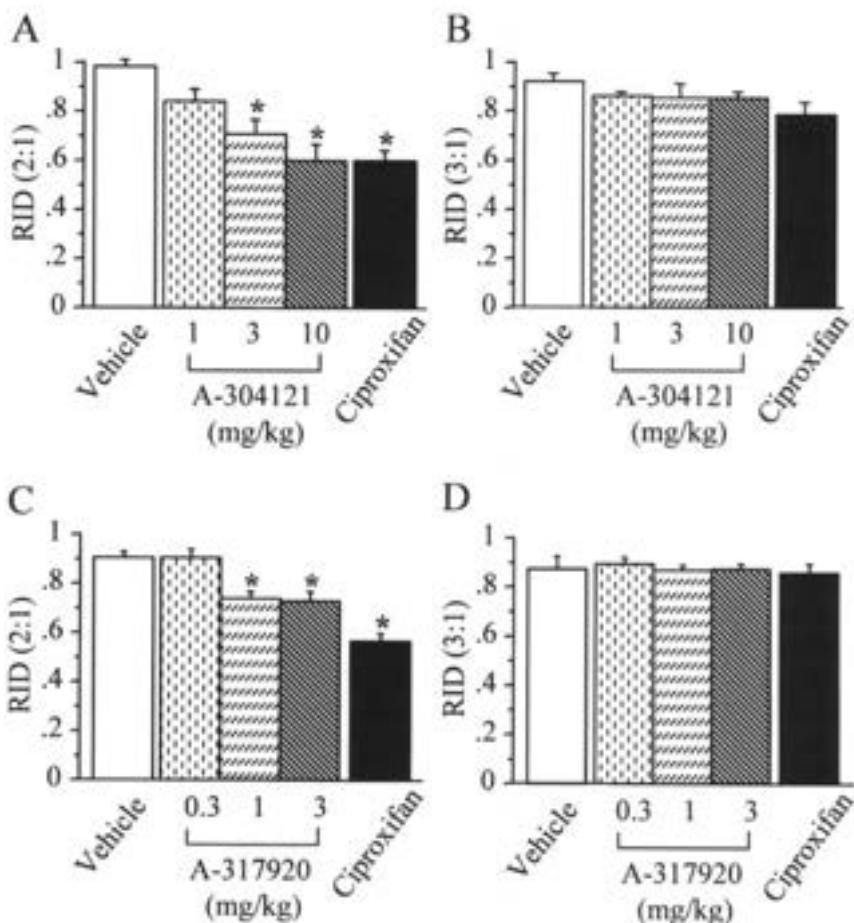


Figure 4. Enhancement of social memory in the adult rat with A-304121 (A) and A-317920 (C), with ciproxifan (1 mg/kg) as a positive control in both instances. Adult rats were dosed with antagonist immediately after a 5 min exposure to an unfamiliar juvenile. After a period of 120 min, the juvenile was reintroduced to the same adult for a second 5 min period and the ratio of investigation between the second and first exposure periods determined. Immediately after the second exposure, the adult was exposed for a further 5 min to an additional, novel juvenile, to control for non-specific effects of the antagonists (B, D). Data are represented by mean \pm S.E.M (* $p < 0.05$ with respect to vehicle-treated controls). Reproduced with permission from [43].

mechanism, but no evidence has ever been shown for this. Since blockade of H₃ receptors causes increased release of acetylcholine [57], models that specifically address the interactions of the histaminergic and cholinergic systems are of particular interest. We have developed a different version of the water maze task, a two platform, visual discrimination test in which an adult rat is required to distinguish, using extramaze spatial cues, a stable escape platform from a

inescapable, floating platform. Components of spatial reference and working memory can be assessed using this task, which is dependent on an intact cholinergic input to the hippocampus [58, 59]. In our hands, both thioperamide and ciproxifan reversed a scopolamine-induced deficit when administered *ip* once daily over the five testing days in this model (Fig. 5). This represents the first time, we believe, that procognitive effects of H_3 receptor blockade have been shown in the water maze. Further, we have also utilized the Barnes circular maze to evaluate the role of H_3 receptors in spatial reference memory. In this task, mice are trained to navigate a large, brightly lit circular plastic disk for a dark tunnel hidden below one of forty holes located around the perimeter of the maze. Unlike the water maze in which escape from water is the primary motivation to find the escape platform, in the Barnes maze the animal's motivation stems from an inherent survival instinct to avoid open, brightly lit spaces [60]. In the Barnes maze, ciproxifan (3 mg/kg *ip*) significantly attenuated a scopolamine-induced performance deficit in the acquisition of this task when administered to mice once daily for the four-day duration of the test. In addition, ciproxifan-treated mice that did not receive scopolamine exhibited a tendency toward improved acquisition of the Barnes maze task compared with vehicle controls. When these animals were reevaluated drug-free eight days after the last training session, a significant improvement in recall of the tunnel location was observed compared with animals that had previously received vehicle alone (Fig. 5).

H_3 receptor antagonists and sensory processing

Deficits in sensorimotor gating are frequently observed in patients presenting with Huntington's disease [61], Tourette's syndrome, obsessive-compulsive disorder [62], and schizophrenia (for review, see [63]). It is possible that deficits in attention and cognition also observed in these disorders may be related to impairments in sensorimotor processing. Recent evidence also suggests a role for the histaminergic system in psychosis. For example, overdose of first generation H_1 R antagonists produced toxic psychoses with hallucinations resembling those observed in schizophrenia [64]. Central histaminergic systems have also recently been implicated in the pathophysiology of schizophrenia [65], although the receptor subtype involved is not clear. Given that blockade of H_3 Rs consistently improves cognition, particularly with attention components, it seems plausible that H_3 Rs may play a role in sensorimotor processing. One way of assessing deficits in sensorimotor gating is to measure prepulse inhibition (PPI) of a startle response, a phenomenon observed across species in which a startle response to an acoustic tone is attenuated when preceded by a weaker stimulus tone [66]. Deficits in PPI are believed to reflect the deficient sensorimotor gating underlying the sensory flooding and disrupted cognition in schizophrenia [63]. We have recently evaluated the H_3 R antagonists ciproxifan and thioperamide in two mouse strains exhibiting a naturally

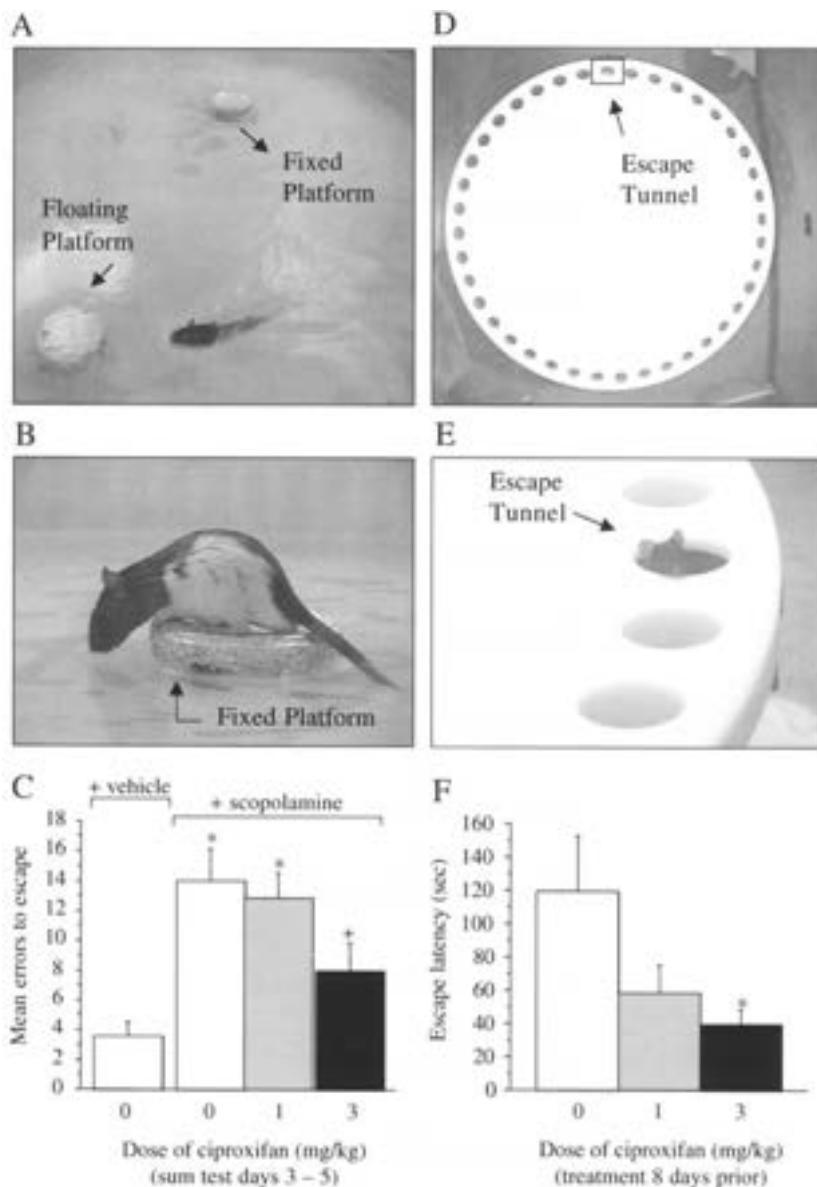


Figure 5. The rat water maze with two visible platforms, one stable and one fixed, is shown (A). Escape from the water is possible only for the fixed platform (B). Ciproxifan administered prior to acquisition sessions over five days attenuated scopolamine-induced increase in mean error number, as shown for data collapsed over days 3–5 (C). The mouse Barnes maze is also shown (D) with bright, overhead illumination, motivation for finding the darkened escape tunnel hidden beneath one of forty holes around the perimeter (E). Ciproxifan administered during acquisition sessions over four days enhanced recall for the tunnel location when the same mice were re-evaluated for recall in a drug-free state eight days after the last acquisition session (F). (* $p < 0.05$ with respect to vehicle-treated controls). Adapted from Society for Neuroscience (2003), abstract 938.3.

occurring deficit in PPI. Interestingly, both compounds enhanced PPI in DBA/2 mice, while thioperamide also enhanced PPI in C57Bl/6 mice to a degree comparable with the atypical antipsychotic risperidone (unpublished observations). Taken together, these preliminary data suggest that drugs targeting blockade of H₃Rs have additional potential in neurological disorders with deficits in sensorimotor processing.

H₃ receptor antagonists and contextual fear conditioning

Very recent evidence from the laboratory of Patrizio Blandina suggests that the histaminergic system and H₃Rs may play an important role in fear-associated memory. For example, direct injection of the H₃R antagonists ciproxifan, clobenpropit and thioperamide into the basolateral amygdala inhibited the release of acetylcholine and adversely affected memory for contextual fear conditioning [67], whereas direct injection of the H₃R agonists (R)- α -methylhistamine or immezipip produced the opposite effects [68]. These data are the first to suggest that modulation of cholinergic tone in the amygdala through an interaction with H₃Rs can affect the consolidation of memory associated with a fear response and indicate a potential role for H₃R ligands in treating disorders such as social phobias or generalized anxiety disorders.

Potential clinical utility of histaminergic-related cognition enhancers

To date, no drugs with specific effects on histaminergic systems and cognition enhancing effects are clinically available. Historically, drugs acting via histaminergic pathways have, if anything, had negative effects on CNS function in the context of soporific effects of classical H₁ receptor antagonists, although there has been the suggestion of a positive effect of the H₂ receptor antagonist, famotidine, in schizophrenia [65]. In addition, there is not a clear relationship between changes in histamine levels (either in the CNS or periphery) and cognitive disorders [65]. For example, in Alzheimer's disease (AD), various studies have reported either increased or diminished histamine content in the brains of patients with AD (for review, see [65]). On the other hand, schizophrenic patients have been reported to be hyporesponsive to histamine, to have elevated H₂ receptor density in areas implicated in their disease, to have a reduced number of H₁ receptors and to have elevated levels of the histamine metabolite, *tele*-methylhistamine (an indicator of CNS histaminergic activation), in their cerebrospinal fluid [65]. Finally, in an animal model of neurodegeneration [69], injections of β -amyloid reduced histamine levels in cortex and/or hypothalamus (but not hippocampus), and animals exhibited cognitive deficits, with reversal of both parameters by the procognitive compound S12024 [69]. These data are consistent with findings of disruption of histaminergic neurotransmission as a result of neurodegenerative pathology in Alzheimer's disease [70].

Lesion studies and/or chemical disruption of histamine synthesis have shown discrepant effects on cognitive behavior in a variety of animal models [71]. Linkage to H₃ receptors was suggested by the effects of several specific H₃ agonists that disrupted cognitive behavior in several animal models of learning and memory, while pro-cognitive effects have been observed in other animal models [71]. In addition, several studies have suggested a potential role for the histaminergic system to modulate a variety of neurotransmitters that may be linked to attention and vigilance and may be important components of the cognitive deficits found in patients with ADHD [71]. Moreover, data cited above shows a potential for H₃ antagonists to ameliorate cognitive and attentional deficits in animal models that may be suggestive for a role of these compounds in ADHD [44, 45, 71].

Looking toward the future, a number of compounds have been recently described that illustrate the potential for agents that interact with the histaminergic system of the brain. These come from a broad variety of antagonists and inverse agonists of the H₃ receptor that seem to offer the most promise as new agents to treat cognitive disorders. These compounds are emerging from a number of laboratories and chemical classes. While imidazole-based compounds were the first potent and selective ligands at H₃ receptors [42, 72–78], newer agents are becoming known from diverse chemical series that lack the imidazole moiety [79–89] known to be a liability for drug-drug interactions [90]. Thus, there exists the potential that compounds similar to those described above will have useful pharmacological and therapeutic activity in the treatment of the serious cognitive deficiencies in ADHD, AD and schizophrenia.

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Peptide and steroid hormone receptors as drug targets for enhancement of learning and memory performance

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Introduction

"Then Dr Strauss said Charlie even if this fales your making a grate contribyushun to sience. This experimint has been successful on lots of animals but its never bin tried on a humen being. You will be the first. After the operashun Im gonna try to be smart. Im gonna try awful hard." (sic) p. 8, Flowers For Angermon [1]

While therapeutic enhancement of cognitive function was once a topic for science fiction [1], the neuroscience and clinical literature of the past few decades provides ample documentation of the basic brain mechanisms for, and potential clinical utility of, neuroendocrine steroids and hormones tested in tasks sensitive to learning and memory performance [2–6]. Endocrine hormones represent a highly evolved messenger molecule system in vertebrates, and may guide and even be part of the normal machinery of a complex cognitive process such as learning and memory [7, 8]. Because there are many hormones that interact with neurotransmitters in order to influence learning and memory, Martinez and McGaugh (1981) proposed that these hormones be termed "learning modulatory hormones" [9].

The list of hormones that are reported to influence memory is impressively long and includes catecholamines, pituitary secretagogues, opioids and gut-brain peptides. The present review is by no means exhaustive and instead focuses upon a selected set of steroid/hormone mechanisms which share three attributes: 1) recent introduction or prominent representation in published literature over the past five years, 2) evidence from electrophysiological, genetic or other non-pharmacological dependent measures to validate the claim that changes in learning and memory performance are functionally specific, and 3) an indication that the therapeutic utility of the target can be critically examined in human patients suffering from some form of cognitive impairment or dementia.

The reader experienced in pre-clinical psychopharmacology will appreciate the conservative nature of the selection criteria listed above. These criteria serve to ascribe behavioral changes to underlying learning and memory processes only if alternative hypotheses related to non-specific general arousing or affective properties of experimental treatments, for example, can be convincingly discounted [10]. For instance, the putative learning modulatory actions of vasopressin, one of the peptide hormones to be discussed below, can be challenged based upon orthogonal affective and motor properties of peptide administration which exert generalized changes in arousal in a non-specific manner, or in peripheral organs entirely separate from the brain [11–13]. One way to effectively meet this challenge is to advance increasingly stringent criteria for documentation of cognitive enhancement which can be assumed *a priori* to be unproven [6, 12]. For example, information about how hormones actually modulate long-lasting synaptic connectivity and efficacy would be a welcome adjunct to functional testing methods [14]. Accordingly, recent advances in centrally acting neuropeptide and steroid hormone mechanisms relevant for animal and human learning and memory functions are presented in this review. In addition, we address specific examples of convergent electrophysiological [15] and behavioral genetic [16] methodologies which are likely to yield future advances in this field.

Pharmacological tools for learning and memory enhancement

Peptide hormone receptor targets

Classification of signaling molecules is somewhat arbitrary in that a single peptide can act as a classical neurotransmitter, as a neuromodulator, or serve neural and endocrine hormone functions simultaneously [8]. The common property shared by the present peptide hormone grouping is a chemical structure consisting of amino acids joined by peptide bonds. A great many putative peptide and hormone modulators of learning and memory performance are known at the present time (Tab. 1), although all require further characterization if we are to fully understand their role in learning and memory.

Neurohypophysial hormones

Vasopressin (AVP), sometimes labeled anti-diuretic hormone, is secreted by the hypothalamus and stored in and released from the posterior pituitary gland in order to increase blood pressure and the rate at which the kidneys absorb water. Extra-hypothalamic AVP pathways mediate learning and memory predominantly via receptors distributed in limbic structures such as the hippocampus, amygdala and septum. AVP can act centrally through AVP type 1 receptors, which activate protein kinase C and increase cytosolic Ca^{2+} , AVP

Table 1. Potential peptide and hormone targets for learning and memory performance enhancement

Peptide	Receptor	Species	Main findings	Reference
Growth hormone releasing hormone	GHRHR	Rat	Chronic GHRH improves age-related spatial memory impairments, and this corresponds to an increase in insulin-like growth factor 1 release in old age.	[243]
Leptin	OB	Rat	Leptin facilitates the induction of hippocampal synaptic plasticity, by enhancing NMDA-receptor mediated Ca^{2+} influx.	[244]
Nociceptin/Orphanin FQ	ORL1	Mouse	Leptin receptor deficient mice exhibit impaired LTP and spatial memory.	[245]
Orexin A	Orexin A	Mouse	At low doses (10–100 fmol), nociceptin reduces scopolamine-induced deficits in passive avoidance and spontaneous alternations in a Y-maze.	[246]
	Orexin A	Rat	Orexin A facilitates learning, consolidation and retrieval processes in passive avoidance.	[247]
Substance P		Mouse	Orexin A improves retention in T-maze footshock avoidance and step-down passive avoidance tests in CD-1 mice. In young and aged SAMP8 mice, which overproduce amyloid <i>Beta</i> , Orexin A similarly improves retention.	[248]
Neurotrophin-3	TrkB	Rat	Substance P (10 ng, but not 100 ng) infusion into the basolateral amygdala post-shock in a passive avoidance paradigm enhances learning.	[249]
Insulin-like growth factor-1	NMDAR2A and R2B	Rat	Both systemic administration of SP and injection into the ventral pallidum improve memory, and in the latter case also result in the release of acetylcholine in the frontal cortex.	[250]
			NT-3 reverses cholinergic deficits and performance of a spatial memory task in aged rats when administered over a period of four weeks by iCV injection.	[36]
			IGF-1 reverses age-related deficits in working memory in a repeated acquisition task and an object recognition task, and reference memory in a place discrimination task.	[251]

type 2 receptors, which stimulate adenylate cyclase, and oxytocin receptors. Central vasopressinergic neural systems are widely distributed in many parts of the brain where they and their breakdown products have neuromodulatory effects completely independent of the neurohypophysis [17, 18].

AVP and certain AVP analogues that have no anti-diuretic action may improve long-term memory in a variety of different tasks [19]. Intra-cerebral administration of AVP restores learning and memory function in rats with *diabetes insipidus* and anti-AVP antiserum impairs learning in the rat [20]. An AVP analogue that is devoid of hormonal activity, AVP (4–9), enhances radial maze performance in rats, producing a faster rate of acquisition of reference and working memory. Likewise, AVP (4–9) inhibits scopolamine-induced memory deficits in the radial maze in rats [21, 22]. This enhancement is likely mediated by AVP type 1 receptors, whose activity stimulates acetylcholine release in hippocampal slices, while AVP type 2 receptors do not appear to be involved [23]. A second AVP analogue, AVP (4–8), improves concept learning in a win-stay/loose-shift paradigm in rats with hippocampal damage but not in rats with prefrontal cortex lesions [24]. Another AVP derivative, NC-1900, enhances place learning in rats with cycloheximide-induced hippocampal lesions [25]. Taken together, these findings suggest that increased signaling via AVP type 1 receptors is capable of counteracting pharmacologically-induced and tissue damage-related learning impairments in rodents via a mechanism which is dissociable from the classical hormonal (pressor) actions of AVP [17]. AVP has also been reported to have clinical efficacy following human administration in alleviating post-traumatic amnesia [26].

Oxytocin (OXT) is a peptide hormone that is produced by the posterior lobe of the pituitary gland and induces contraction of the smooth muscle of the uterus and myoepithelial cells of the mammary gland. In contrast to AVP, OXT impairs memory retention in an inhibitory avoidance paradigm, and this also occurs via modulation of the cholinergic system [27]. Both of these peptides influence social recognition, but different effects have been noted across different rodent species, different genders within the same species, and different brain regions of interest [28, 29]. For example, although oxytocin (OXT) has complex effects on social memory in rats, mice with a null mutation of the OXT gene are completely socially amnestic without other cognitive deficits [30]. As OXT given centrally before, but not after, the initial encounter restores social recognition in these mutant mice, the neuropeptide appears critical for the acquisition rather than the consolidation phase of memory. These findings support the hypothesis that OXT is essential for social memory, although it may modulate different mnemonic processes and different neural systems depending on the organism.

Trophic factors

Nerve Growth Factor (NGF) is a multimeric protein, the beta subunit of which is required for the proper development and maintenance of the sensory neurons

of the dorsal root ganglion and of the post-ganglionic sympathetic neurons. NGF has been implicated in several forms of learning and memory. In aged rats, chronic, four week intracerebroventricular infusion of exogenous NGF improved memory performance in a delayed non-matching-to-position task, and this restoration of memory performance persisted for at least four-weeks after NGF infusions ceased [31, 32]. In a water maze spatial memory task, exogenous NGF improved performance in aged rats [33] as well as in rats withdrawn from chronic ethanol exposure [34]. In the latter case, improved water maze performance was accompanied by a restoration of septohippocampal cholinergic projections [34], while in the former case, the number of synaptophysin immunoreactive pre-synaptic terminals increased in the frontal cortex [33]. The link between NGF and cholinergic activity is further supported by the observation that the effects of NGF on recent memory in the delayed non-matching-to-position task correlate with changes in the cholinergic system, including increased size of cholinergic neurons, and a change in the terminal fields of these same neurons [35]. Additionally, the characteristic cholinergic atrophy observed in aged rats can be reversed by chronic, four-week infusion of NGF, and a concurrent improvement in spatial memory performance is observed [36].

The generality of NGF-induced facilitation of learning and memory performance is supported by efficacy of NGF treatment in a variety of other animal species and testing contexts. In a classical fear conditioning paradigm, endogenous NGF is reported to increase one week after training (i.e., during the consolidation phase), but infusion of tyrosine kinase A (TrkA) antisense into the hippocampus one week post-training can block this effect, impairing contextual retention [37]. In a simple passive avoidance paradigm, combined central administration of NGF and epidermal growth factor resulted in improved learning in aged mice, but had no effect in normal adult mice [38]. Neither trophic factor administered alone had any effect on learning in this paradigm. In developing CD-1 mice, a single intracerebroventricular administration of NGF at post-natal day 15 resulted in adult-like spatial novelty discrimination in males but not females tested at post-natal day 18, although increased choline acetyltransferase activity was observed in both sexes as a result of NGF treatment [39]. By post-natal day 28, no behavioral or neurochemical effects of NGF administration are observed. Importantly, NGF administration in infrahuman primates is reported to attenuate age and lesion-induced forebrain cholinergic degeneration [40, 41]. Taken together, these results suggest that central administration of exogenous NGF can facilitate learning performance and ameliorate age and pharmacologically-induced learning impairment while remodeling cholinergic brain areas thought to subserve learning and memory functions.

Brain-derived neurotrophic factor (BDNF), a NGF-related neurotrophin with high affinity for the TrkB receptor, is known to have numerous roles in learning and memory, and contributes to the process of hippocampal long-term potentiation. Two excellent reviews provide a very comprehensive

review of our understanding of the role of BDNF in learning and memory [42, 43]. BDNF contributes to the functional decline that occurs with aging [44, 45]. Mnemonic effects of BDNF are found in rodents, and in primates, where the peptide is up-regulated in the inferior temporal cortex during visual pair-association learning [46], and in day-old chicks, where anti-sense administration impairs memory consolidation in a one-trial inhibitory avoidance paradigm [47].

The research highlighted above provides evidence that NGF and BDNF, in addition to their effects on neuronal survival and differentiation, enhance learning and memory processes in a number of different species. Both trophic factors can influence the cholinergic system [48], and may represent potential clinical therapies.

Hypothalamo-pituitary-adrenal axis peptides

It is widely documented in both animal and clinical studies that certain neuropathological states, including human and animal models of dementia, are accompanied by an altered endocrine stress axis. The endocrine stress axis can be defined as the biological interface for neural and humoral communication between the central nervous system and peripheral glands or organs responsible for mobilizing the stress response. Largely correlational results link some measure of cognitive capacity in man or indices of information plasticity in animal studies with a circulating marker of stress-like activation such as plasma glucocorticoid levels. These findings can now be critically examined due to the availability of a variety of targeted mutant mice in which specific components of the biological stress axis are neutralized or made constitutively active (Tab. 2). These new research tools provide leverage in clarifying a long-standing issue of causality: are endocrine markers of stress merely diagnostic of dementia-like disorders or do they instead constitute the primary defect of homeostasis which provides a mechanism for increased vulnerability to cognitive decline?

Stress and behavioral plasticity are interrelated; levels of alertness correspond to success in performance of a learning task in what can be described as

Table 2. Learning task performance of CRF system and glucocorticoid receptor gene knockdown and mutant mice

Targeted gene	Knockdown	Knockout	Over-expression
CRF	Impairment [252]	No effect [253]	Impairment [254]
CRF-R1	No effect [255]	Impairment [256]	ND
GR	Impairment [257]	Impairment [258]	ND

CRF – Corticotropin-releasing factor; CRF-R1 – Type I CRF receptor; GR – Glucocorticoid receptor;
ND – not yet determined

the stress-cognition axis [49]. Stressors, neuroendocrine peptides activated by stressors, and circulating stress-related hormones all modulate learning and memory storage processes. For example, brief, mild activation of brain, autonomic or endocrine stress systems coincident with an emotional experience as well as long-term or intense activation of the hypothalamo-pituitary-adrenocortical (HPA) axis, both alter learning and memory capacity [50]. It is important to note that an inverted U-shaped function governs the relationship between HPA axis stimulation and cognitive function. In particular, elevating stress hormone dose levels, either acutely or chronically, or prolonging the duration of exposure to low or high stress hormone levels would be expected either to enhance or impair learning and memory functions in both animal models [51, 52] and man [53].

Many HPA axis neuropeptides which act as neurotransmitters within the central nervous system, including corticotropin-releasing factor (CRF), adrenocorticotropin (ACTH), β -endorphin, and α -melanocyte-stimulating hormone can modulate learning and memory through central, extrahypophysiotropic mechanisms [54]. For example, rodent pharmacological studies of learned avoidance behavior employing administration of CRF itself [55] the pituitary product of CRF secretagogue action, ACTH [56], and the adrenocortical product of ACTH secretagogue action, corticosterone (or cortisol in humans) [57], into either brain or periphery consistently reveal modification of learned performance when the hormone/steroid is administered prior to training, immediately following training or prior to retention testing [56]. Depending on the interaction between the intensity of training stimulus employed and the dose of steroid or neuropeptide administered, either enhancement or impairment of learned behavior is observed [56]. An extensive literature implicates deficiency in ACTH-related peptides in defects in the acquisition of and the more rapid extinction of learned behaviors [58]. Similar to the results described for AVP above, central administration of ACTH or ACTH analogues that have no adrenal cortex-stimulating activity restores learning ability in rats, whereas the results of studies of the effects of ACTH-related peptides on human memory are conflicting. A typical study reports that human administration of an ACTH analogue devoid of endocrine effects prior to completing a range of performance tests including a complicated serial reaction task, running memory span, verbal learning and non-verbal mental ability tests, increases sustained attention [59]. The important implication of these findings is that an optimal level of performance associated with a moderate, fine-tuned degree of activation is modulated by deviations in HPA activity.

Corticotropin-releasing factor (CRF) is widely recognized as part of a neuropeptide system whose activation is a necessary component of the biological response to stressor exposure [60]. Characteristic features of brain CRF system activation include behavioral vigilance, suspension of appetite and pituitary-adrenocortical stimulation [61]. Previous research suggests that the consequences of stress neuropeptide activation in an information processing context can be predicted according to an arousal/performance heuristic [62, 63].

One of the hallmarks of the arousal/performance interaction is that the direction of change produced by a particular treatment in a learning task depends on the exact context in which the treatment is administered [5]. For example, whereas acute stress neuropeptide activation facilitates active avoidance performance, chronic activation has an impairing effect [64]. The bimodal effects of CRF administration in an inhibitory avoidance task also suggest that mild arousal facilitates performance whereas over-arousal engenders memory impairment [62]. This conclusion is supported by the finding that many other hypophysiotropic neuropeptides of the pituitary gland, such as ACTH, are also capable of exerting bimodal impairing and enhancing effects on learning and memory performance [65]. The multiple endocrine, autonomic and behavioral actions of CRF and urocortin systems complicate the task of identifying the exact mechanism underlying stress-related memory modulation [66]. However, emerging evidence suggests that central actions of brain stress neuropeptide systems provide an autonomous mechanism for exerting memory modulatory consequences of stress-related activation [67, 68].

Several lines of evidence support the present identification of a physiological role for CRF systems in information processing functions of the central nervous system. First, steady-state levels of endogenous CRF family neuropeptide receptor agonists appear sufficient to modulate learning and memory functions since pharmacological dissociation of CRF and a related neuropeptide, urocortin, from their binding protein enhances performance in appetitive- and aversively-motivated memory tasks [69–72]. Second, central CRF administration exerts electrophysiological and neurochemical activation of hippocampal circuits relevant for learning and memory processes in several species [73–75]. Finally, brain and cortical CRF levels are significantly reduced in patients with both mild and severe dementia [76]. Moreover, cerebrospinal fluid levels of CRF correlate with degrees of cognitive impairment in dementia sufferers [77]. Thus, CRF decrements may serve as a potential neurochemical marker of early dementia and possibly early Alzheimer's disease [76].

Homeostatic peptides

The following homeostatic neuropeptides/hormones are considered in some depth because plausible overlap between their brain substrates of action and brain learning and memory mechanisms is articulated. Note that some of the following memory modulatory neuropeptides/hormones, angiotensin, cholecystokinin, galanin and neuropeptide Y, are known primarily for their prominent role in homeostatic regulatory systems, controlling eating and drinking behaviors. This least-common functional denominator may be relevant for generating hypotheses to explain effects of these peptides in learning and memory contexts as discussed below.

The angiotensins are a group of blood pressure-regulating peptides that act as powerful vasopressors and stimulators of aldosterone secretion by the adrenal cortex. At least four angiotensin receptor subtypes subserve a variety of different physiological functions [78]. Angiotensin IV (Ang IV) binds specifically to AT4 receptors in the brain, which are not activated by Ang I-III, although the AT4 receptor can be activated by certain fragments including Ang II (2-7) and Ang I (3-10). Full-length Ang II binds to AT1 and AT2 receptor subtypes.

Ang IV administration improves recall in a passive avoidance task in a dose-dependent manner [79]. Similarly, Ang II (2-7) and Ang I (3-10) also improve performance in both active and passive avoidance tasks [80-82]. Intracerebroventricular injection of an Ang IV agonist increases the rate of acquisition in the Morris water maze, while an Ang IV receptor antagonist impairs the rate of acquisition in the same task [83]. Ang IV, and LVV-hemorphin-7, an AT4 receptor ligand, both potentiate depolarization-induced acetylcholine release from rat hippocampal slices in a concentration-dependent manner [84]. This effect is blocked by an AT4 receptor antagonist, but not by AT1 or AT2 receptor antagonists. The distribution of Ang IV binding sites in the human brain is similar to that found in other species, with a high density of AT4 receptors found in numerous regions including the hippocampus, entorhinal, prefrontal and cingulate cortices, and some thalamic nuclei. This distribution supports multiple roles for AT4 binding sites in the central nervous system, including facilitation of memory retention and retrieval [85].

Ang II inhibits potassium-induced release of acetylcholine from fresh slices of human temporal cortex obtained at surgery, but this effect is blocked by an Ang II receptor antagonist [86]. Ang II inhibition of potassium-evoked release of acetylcholine occurs in a concentration-dependent manner in slices of rat entorhinal cortex [87]. It is hypothesized that the potential cognitive enhancing effects of acetylcholinesterase inhibitors are modulated through the removal of the Ang II-mediated inhibition of cholinergic function. In general, it appears that Ang II facilitates learning and memory in conditioned avoidance and inhibitory avoidance tasks, whatever role it may have in other learning and memory tasks [80].

Cholecystokinin (CCK) is a polypeptide hormone of the duodenum released in response to increased amounts of free fatty acids in the intestinal lumen in order to promote contraction of the gallbladder and secretion of pancreatic enzymes. CCK-8 (an octapeptide derivative of CCK) is the most abundant form in the brain and it activates both CCK-A and CCK-B receptors, although only the latter are widely distributed in the CNS. Systemic CCK-8 injections help prevent memory deficits induced by age [88], electroconvulsive shock [89], NMDA receptor antagonists [90], scopolamine [91], or protein kinase inhibitors [92] in the inhibitory avoidance test. There is evidence to suggest a functional balance between CCK-A and CCK-B receptors in the mediation of memory tasks. CCK-A-specific antagonists are detrimental to memory processes [93], and rats lacking CCK-A receptors exhibit learning and memo-

ry impairments in a radial maze [94]. In contrast, the role of CCK-B receptors in learning and memory processes has not been unequivocally shown. For instance, two selective CCK-B agonists (BC264 and BC197) produce opposite effects on working memory in a Y-maze paradigm [95], an effect that appears to be modulated by differential dopamine activation [96]. Others have also reported either enhanced [97–99] or impaired [100] learning with other CCK-B receptor agonists. More evidence is needed to clearly elucidate the complete role of CCK in learning and memory, especially for effects mediated by CCK-B receptors.

CCK-8 administration induces a dose- and time-dependent increase in NGF levels in the hypothalamus, pituitary and hippocampus. Pre-treatment with a selective CCK-A receptor antagonist blocks the increase in NGF in the hypothalamus and pituitary but not in the hippocampus, while pre-treatment with a selective CCK-B receptor antagonist blocks the increase in NGF in the hippocampus only [101]. In unlesioned mice, CCK-8 injections increase choline acetyltransferase activity in the forebrain, while in fimbria-formix lesioned mice, which exhibit reduced levels of choline acetyltransferase in the septohippocampal circuit, CCK-8 injections counteract the choline acetyltransferase deficits. This effect on cholinergic cells is mediated through the synthesis and release of NGF [102]. Similarly, continuous intracerebroventricular infusion of CCK-8 prevents the degeneration of cortical cholinergic neurons following basal forebrain lesions in rats [103]. Taken together, these results suggest that the cognitive enhancement resulting from CCK-8 exposure may be partially mediated by effects on the cholinergic system.

The neuropeptide galanin enjoys widespread expression in the nervous and endocrine systems and is reported to alter performance in learning and memory tasks. There is now a substantial body of work to indicate that galanin plays an important biological role as a regulator of neurotransmitter and hormone release in the adult organism. Studies demonstrate that galanin acts as a developmental and trophic factor to subsets of neurons in the nervous and neuroendocrine systems [111]. Galanin has been shown to impair learning and memory in a variety of paradigms [104–106]. However, a dual nature of galanin is evident as infusions into the ventral hippocampus produce bi-phasic, dose-dependent effects on spatial learning in rats [107]. The learning and memory effects of galanin are predominantly mediated through changes in cholinergic systems, as galanin administration decreases acetylcholine release in the ventral hippocampus and cerebral cortex [106, 107], but increases release in the dorsal hippocampus and striatum [108]. Thus, galanin's functional role appears to depend on the brain region in question. Further, galanin co-localizes with choline acetyltransferase in a subset of cholinergic neurons in the rodent basal forebrain.

Although the role of galanin in Alzheimer's disease is controversial, galanin expression is up-regulated in forebrain nuclei in Alzheimer's tissue and innervation of the remaining basal forebrain cholinergic neurons is likewise augmented [116]. Thus, it has been postulated that this galanin hyper-innervation

results in depression of the septo-hippocampal circuits involved in learning and memory [117]. However, evidence suggests that galanin may compensate for the loss of cholinergic neurons in this region through an excitatory action on the remaining cells, thus augmenting the release of acetylcholine, and ultimately leading to a delay in the progression of Alzheimer's disease [118].

Neuropeptide Y (NPY) is one the most abundant neuropeptides in the mammalian brain where it exerts control over endocrine hypothalamic and pituitary functions, hypothalamic control of food intake and circadian rhythm, and limbic emotional integration [109]. Within the mammalian brain, Y2 is the predominant NPY receptor and can be found in the hippocampus, hypothalamus, thalamus, amygdala, and brainstem as well as in other regions, although species differences do exist in Y2 receptor localization and quantity [110–112]. Activation of the Y2 receptor, a G-protein coupled receptor, leads to inhibition of adenylyl cyclase. NPY modulates memory retention in both inhibitory and active avoidance tasks, where regional injections of NPY into the rostral portion of the hippocampus and septum enhance memory retention while injections into the caudal hippocampus and amygdala impair retention [113]. NPY injections alleviate MK-801 induced learning impairments in a step-down inhibitory avoidance task [114].

While there is great interest in developing NPY receptor antagonists for the treatment of obesity-related disorders [115], application in dementing disorders can also be considered. Significant decreases in NPY immunoreactivity occur in conjunction with Alzheimer's disease, and it is thought these may be related to the cholinergic deficits that are observed in the same individuals [116]. Activation of the Y2 receptor may stimulate the release of AVP and OXT [117], thereby providing one potential indirect mechanism for cognitive improvement. A review of NPY receptors with a focus on their potential as therapeutic drug targets for cognitive dysfunction is available [118].

Opioid peptides

Opioid peptides in the neocortex, hippocampus, and amygdala play an important role in learning and memory. Derived from the proteolytic cleavage of the precursor proteins preproenkephalin, preprodynorphin, proopiomelanocortin (POMC), they produce enkephalins, dynorphins (A and B), and β -endorphins, respectively [119–121]. Binding of opioid peptides to μ , δ , and κ opioid receptors results in varied behavioral and physiological responses.

Enkephalins are neuronally produced and released. They are also released in conjunction with adrenaline in response to stress. β -endorphin and ACTH are also released from the anterior pituitary in response to stress. Numerous studies have outlined the hormonal/neuromodulatory actions of enkephalins and β -endorphins on learning and memory. Most frequently, β -endorphins and enkephalins are associated with impairments in acquisition and retention of learning tasks, although some studies report memory enhancement [122].

Peripheral administration of enkephalins results in impairments in inhibitory avoidance retention in rodents [123, 124]. Similar impairments result from systemic injection of β -endorphin. Thus, it seems that enkephalins and endorphins share a modulatory role in learning and memory-related events.

The dynorphins also affect memory formation. For example, injection of dynorphin A enhances rodent retention of avoidance responses, and impairs aversive and appetitive learning in chicks [125, 126]. Hippocampal injections with dynorphin impair spatial learning/water maze performance, and working memory in the radial arm maze [127]. Spatial memory deficits observed in aged rats are partly attributed to elevated levels of dynorphin A [128]. The existing evidence suggests that opioid peptides can enhance or inhibit memory formation. This modulation is dependent on the strength of training, and peripheral *versus* central administration. Systemic administration results in enkephalin, endorphin, or dynorphin action on opioid receptors located outside of the blood-brain barrier in order to alter learning and memory mechanisms. Central administration, on the contrary, results in direct effect of the opioid peptides on central nervous system activity. These results indicate that opioid peptides might influence the strength of a memory via parallel central and peripheral mechanisms.

Steroid hormone receptor targets

The steroid hormones are synthesized from cholesterol, predominantly in the gonads in the case of estrogen and progesterone, or in the adrenal cortex in the case of glucocorticoids. Although classical steroid hormone actions are mediated predominantly through nuclear receptors, many steroid hormone effects on learning and memory are exerted via membrane receptors that regulate ion channel function.

Estrogen

The estrogens are a family of steroid hormones that regulate and sustain female sexual development and reproductive function. Besides affecting the hypothalamus and other brain areas related to reproduction, ovarian steroids have widespread effects throughout the brain, on serotonin pathways, catecholaminergic neurons, the basal forebrain cholinergic system, and the hippocampal formation [130]. In rats, intrahippocampal infusions of estradiol potentiate acetylcholine- and glutamate-mediated memory retention in an avoidance learning task [129]. Estrogen is also important for performance during acquisition training, as ovariectomized females exhibit a slower rate of acquisition in a delayed matching-to-position task and this deficit can be overcome by chronic administration of estrogen [130]. In intact male and female mice, as well as ovariectomized females, chronic estrogen treatment improves radial arm maze work-

ing memory performance [131]. Additionally estrogen-mediated improvement in radial maze working memory is dependent on acetylcholine acting through M2 muscarinic receptors to increase N-methyl-D-aspartate (NMDA) receptor binding in the hippocampus [132]. Preliminary evidence suggests that mice deficient in either estrogen receptor subtype, ER α or ER β , show impaired learning, which indicates that both estrogen receptor subtypes are important for normal cognitive functioning [133, 134].

Ovarian hormones regulate synapse turnover in the CA1 region of the hippocampus during the four- to five-day estrous cycle of the female rat [135]. Formation of new excitatory synapses is induced by estradiol, involves NMDA receptors and is mediated by acetylcholine [132]. Although NMDA receptor activation is required for synapse formation, inhibitory interneurons may play a pivotal role as they express nuclear ER α . It is also likely that estrogens may locally regulate events at the sites of synaptic contact in the excitatory pyramidal neurons where the synapses form [136]. Estrogen interacts with the rat cholinergic system in numerous ways, such as enhancing cortical cholinergic innervation and preserving synaptic density following excitotoxic lesions in the basal forebrain [137]. Estrogen replacement in aged female mice similarly increases hippocampal synaptophysin immunoreactivity, a marker for synaptic density, and this effect correlates with an increase in spatial reference memory [138].

Ovarian steroids have measurable effects on affective state as well as cognition, with implications for dementia [136]. In particular, replacement of estrogen in post-menopausal women protects against cognitive effects of aging assessed using several measures of cognitive function. In a large sample of non-demented post-menopausal women assessed longitudinally either with or without estrogen and/or progestin replacement therapy, hormone supplementation reduced the risk of contracting Alzheimer's disease and protected against a decline in verbal memory task performance [139]. One meta-analysis of 42 studies examined the effects of estrogen replacement therapy (ERT) on memory and cognition in non-demented post-menopausal women [140]. While some studies report no performance benefit of ERT, the preponderance of significant findings favor efficacy of ERT as evidenced, for example, by a consistent beneficial effect of ERT on verbal memory [140]. The observational studies suggest that there may be a long-lasting effect of continued ERT on cognitive functioning. ERT is associated with a decreased risk for dementia, but there is little evidence for a positive effect of estrogen therapy on cognition in women with Alzheimer's disease [140].

Neurosteroids

Neurosteroids, synthesized in the central and peripheral nervous systems from cholesterol or steroid precursors [141], can rapidly alter neuronal excitability by non-genomic mechanisms such as GABA-A, NMDA, and sigma 1 receptors [142]. Pregnenolone sulfate (PREGS) and dehydroepiandrosterone (DHEAS)

act as antagonists at GABA-A receptors, and positively modulate NMDA receptor responses. DHEAS also acts through sigma receptors [143, 144].

Recent evidence suggests a role for PREGS and DHEAS in improving hippocampally-mediated memory tasks such as spatial recognition [145, 146], Y-maze [147, 148], visual discrimination go/no-go [149], and motivated lever-press learning [148]. PREGS and DHEAS are both effective at increasing learning and retention when administered pre- and post-training, but not when administered just prior to retention testing in a passive avoidance paradigm [150]. In addition, PREGS blocks memory impairments induced by scopolamine [149] and D-2-amino-5-phosphonovalerate [148] while DHEAS blocks dizocilpine-induced learning deficits [144]. In aged rats, PREGS levels correlate with performance in the Morris water maze and in a Y-maze task, such that low levels were indicative of poor task performance [151]. In addition, the deficits of aged rats were transiently ameliorated by either intraperitoneal or bilateral intra-hippocampal injections of PREGS, which was shown to stimulate acetylcholine release in the hippocampus [145, 151, 152]. Central PREGS administration up-regulates neurogenesis in the dentate gyrus of adult and aged rats, which occurs via blockade of the GABA-A receptor [152]. These findings suggest that PREGS and DHEAS play a role in preserving or enhancing cognitive abilities, and that this may occur, at least in part, through modulation of the cholinergic system [153].

There are several mechanisms that could contribute to the promnestic actions of PREGS. One possibility is that PREGS enhances central cholinergic function, a major system involved in attention and memory processing. This concept is supported by the observation that administration of PREGS in the nucleus basalis magnocellularis, the main source of cortical cholinergic innervation, improves memory performance of young rats [146]. Additionally, central administration of PREGS increases extracellular acetylcholine concentrations in the hippocampus [145, 151].

PREGS modulates several ligand-gated ion channels, with NMDA and GABA-A receptors being the most potently affected (Fig. 1). PREGS enhances NMDA-activated currents and inhibits GABA-mediated currents in cultured rat hippocampal neurons [154]. These *in vitro* results are consistent with neuronal excitatory and convulsant effects of PREGS *in vivo* [155]. In addition, PREGS could influence NMDA and GABA-A receptor functions by a non-specific action such as altering membrane fluidity [156].

Glucocorticoids

The glucocorticoids are a group of adrenocortical steroid hormones whose metabolic effects include stimulation of gluconeogenesis, increased catabolism of proteins, mobilization of free fatty acids and potent inhibition of the inflammatory response. In addition, the effect on learning capacity of chronic activation of the hypothalamo-pituitary-adrenal axis has been characterized

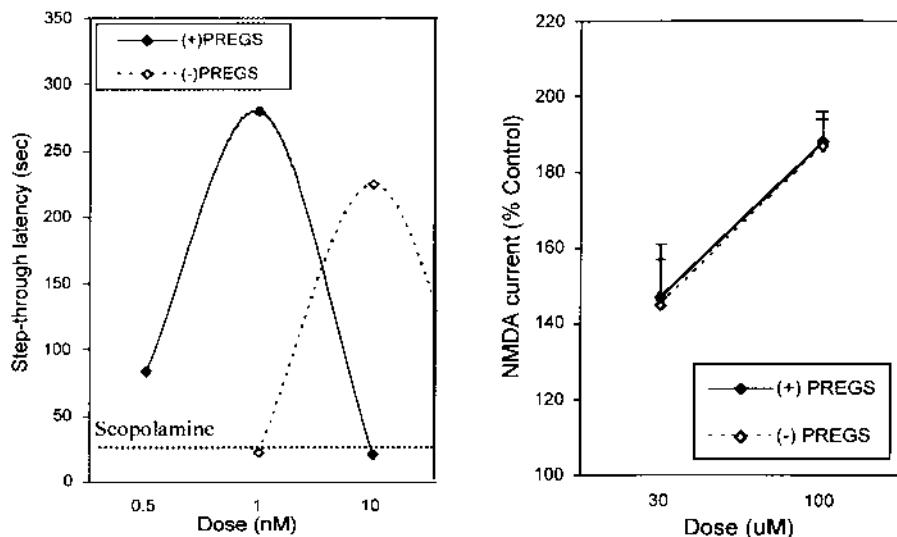


Figure 1. The effects of pre-training injection of two pregnenolone sulfate (PREGS) enantiomers in combination with scopolamine on retention performance after 24 hours in a passive avoidance paradigm is depicted in the left panel. Performance was differentially affected in an inverted U-shape dose-dependent manner for each of the enantiomers, with a peak in step-through latency (best performance) for (+) PREGS occurring at a dose of 1 nmol and for (-) PREGS occurring at a dose of 10 nmol. The step-through latency after scopolamine administration, but in the absence of either PREGS enantiomer is denoted by the dotted line. The same PREGS enantiomers were tested for their ability to potentiate NMDA currents in hippocampal neurons, summarized in the right panel. Both enantiomers modulated the NMDA current to the same extent regardless of dose. These results indicate that the cognitive effects of PREGS are not mediated through an effect on NMDA currents. (Modified from [259])

using long-term peripheral administration of glucocorticoids in mice, rats, monkeys and man [157]. Administration of these stress hormones alters acquisition of a previously unlearned task in a dose-related, inverted U-shaped fashion [158]. Brains from chronic corticosterone-treated animals reveal morphological changes, usually cell loss, in hippocampal areas of the brain believed to subserve learning and memory [159]. Note that while a significant literature appears to exclude corticosteroids from the set of stress hormones which act to modulate retrieval processes [160, 161], other evidence suggests that corticosterone administration prior to retention testing can indeed alter retrieval of spatial memory [162]. One prediction from this correlational link between the level of arousal and performance of learned behaviors is that intrinsic overactivation and/or long-term stimulation of neurobiological and endocrine substrates of the stress response would have the effect of producing learning and memory deficits. In contrast, short-term exposure to physiological levels of exogenous glucocorticoids could be expected to enhance performance in a learning and memory context and this hypothesis is supported by animal and human clinical studies [163, 164].

One objective, multi-species index of brain integrity within pathways thought to mediate learning and memory is provided by *post mortem* and imaging studies of the hippocampus. These studies follow logically from results suggesting that sustained stressor exposure or corticosterone administration in rats atrophies hippocampal neurons and impairs spatial learning [165]. Nuclear magnetic resonance imaging studies in man suggest that glucocorticoid levels in plasma do not correlate overall with hippocampal volume in longitudinal studies, but that individuals which manifest dementia-like loss of short-term memory capacity exhibit high glucocorticoid levels and reduced hippocampal volume [166]. For example, aged humans with significant and prolonged elevations in basal cortisol levels show reduced hippocampal volume and deficits in hippocampus-dependent memory tasks compared to controls with normal cortisol levels.

In concert with the demonstration of central actions of exogenous CRF administration in animal models of learning and memory [167, 168] the above results suggest that CRF over-expression affects learning task performance via direct neurotransmitter-like actions rather than accumulated changes in gross neuropathology. This conclusion is supported by results indicating that CRF, ACTH and corticosterone continue to be significant modulators of learning and memory processes when either the organism or the treatment itself is rendered incapable of HPA activation [64].

Neurophysiological approach to developing novel targets

The previous sections outlined important hormones and their respective targets. In this section, we will discuss neurophysiological approaches used to determine such targets, and we will address the role that hormones play in the modulation of learning and memory-related processes. We know that the brain can communicate with, and be influenced by, other systems, particularly endocrine systems. Hormone-triggered responses can serve to strengthen memories for particular events by providing input to the memory trace through hormonal pathways (Fig. 2) [169].

Hormones that modulate learning and memory, for instance through hormone-neurotransmitter interactions, also play an important role in the modulation of long-term potentiation (LTP). It is believed that modifications at synaptic connections within the nervous system may underlie memory [170]. LTP refers to a relatively long-lasting change in synaptic strength that remains as an ideal candidate model for the cellular mechanisms that underlie learning and memory [15]. LTP is expressed in multiple forms by neurons in the hippocampus, a brain structure that has been widely implicated in long-term memory processes [170]. Because LTP is typically recognized as a model for memory storage, vast research has focused on the role that either endogenous or exogenous compounds exert on its induction and maintenance.

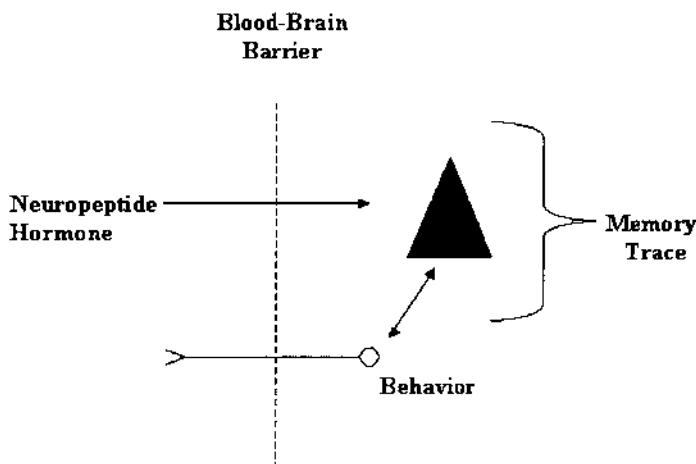


Figure 2. Diagram depicts a model of how neuropeptides are thought to modulate the memory trace. (Modified from [5]).

As noted in a previous section, the neurons of the posterior pituitary produce oxytocin (OXT) and arginine vasopressin (AVP). Co-localization of these hormones with other neuropeptides is typical, and they are released selectively during stress [171]. Because OXT and AVP can act as both hormones and neurotransmitters, it is not surprising that they mediate fast neuronal responses by altering the permeability of both pre- and post-synaptic ion channels [172, 173]. AVP and AVP derivatives such as AVP(4–9) facilitate LTP *in vitro* [174] and *in vivo* [175]. There is evidence to suggest that AVP potentiates LTP via intracellular mechanisms [176]. Further work is in progress to clearly identify the effect that AVP has on cell membranes to facilitate this potentiation.

Oxytocin, typically known as an essential hormone during mammalian labor and lactation, is also involved in the development of social recognition. There is evidence to suggest that pregnancy, birth, and lactation improve spatial memory in the female rat [177]. This type of memory becomes essential when the mother rat wanders to find food and water, yet must rely on spatial memory to remember the location of her offspring. OXT results in hippocampal plasticity and LTP, and this may account for the observed memory enhancements during motherhood, which occur as a result of activation of the mitogen-activated protein (MAP) kinase cascade and CREB phosphorylation [178]. It follows that OXT-induced memory improvement is regulated by the action of OXT as a neuromodulator, and not by the induction of receptor expression. Although current research is still on-going to confirm the role of OXT in memory, this information has provided insight into the development of therapies to selectively transduce OXT into the brain, as OXT or OXT agonists may be useful in the therapeutic treatment of memory deficits.

Neurotrophins (NTs) also play an important modulatory role in neuronal plasticity in the developing and adult brain. Brain-derived neurotrophic factor (BDNF) is the most widely distributed NT in the brain. BDNF-knockout mice have shown LTP impairments in the hippocampus [179, 180], and these impairments can be rescued by local administration of BDNF [181]. Both LTP and spatial learning are associated with increased phosphorylation of TrkB (BDNF receptor) and extracellular signal-regulated kinase (ERK) in the dentate gyrus following administration of BDNF [181]. Although it is still unclear whether BDNF and other NTs exert housekeeping functions to maintain neuronal functioning, BDNF appears to play an important role in LTP induction and modulation [182, 183]. The specific mechanism of BDNF-mediated LTP, which is induced post-synaptically [183], suggests that BDNF interacts directly with NMDA receptors to increase their activity [184]. Whatever the case, the modulatory role of BDNF and other NTs on neuronal plasticity seems to depend on the individual NTs, their respective receptors, and appears to be mediated by local increases in Ca^{2+} .

CRF modulates learning, food intake, arousal, and fear responses [185–187]. More recently, it has been implicated as an enhancer of synaptic efficacy in the rodent hippocampus *in vivo* [188, 189]. In contrast, sustained CRF administration actually blocks hippocampal LTP in a dose-dependent manner [190]. CRF produces a protein synthesis-dependent LTP in the dentate gyrus [188], and improves retention in various memory tasks, making the peptide a potentially important player in the mechanisms underlying stress and cognition. CRF's actions are mediated by activation of Ca^{2+} /calmodulin-dependent kinase II, and this may represent an essential mechanism by which CRF contributes to memory storage [191].

The angiotensin system, widely implicated in neural plasticity and memory, facilitates LTP, spatial, and associative memory. In particular, agonists of the AT₄ receptor augment CA1 LTP *in vitro* [174, 192], and *in vivo* [193]. Ang I is correlated with increases in hippocampal MMP-9 levels. MMP-9 is a gelatinase that serves as a plasminogen activator. Previous literature indicates that the tissue plasminogen activator (tPA) is a serine protease that plays an important role in tissue remodeling and LTP. The existing literature indicates that tPA serves as an immediate-early gene and is induced in the hippocampus during seizures, kindling, and LTP [194]. tPA knockout mice show a decrease in late-phase LTP [195], and show deficits in two-way avoidance tasks. Additionally, over-expression of tPA results in enhanced CA1 LTP and learning [196]. To date, the role of tPA on hippocampal function is not clear. One possibility is that tPA converts plasminogen, which is the enzyme's main substrate and is known to be found in the hippocampus, to the protease plasmin, which in turn can cleave many other extracellular substrates (for example, laminin) to result in alterations of hippocampal structure and function [197]. Other studies have found binding of tPA to the low-density lipoprotein receptor-related protein in hippocampal neurons enhances the activity of cAMP-dependent protein kinase, a key molecule in LTP [198]. Overall, the

angiotensin-tPA interaction appears to play an important role in the successful acquisition of a new memory and needs to be further examined [199].

Alterations in estrogen levels influence many behaviors, even those not necessarily linked to sexual behavior. Among these are memory-related alterations, which are not clearly understood. Research indicates that the administration of estradiol to ovariectomized rats results in synaptic modifications in the hypothalamus [200]. Additional work indicates that hippocampal LTP is facilitated by increased levels of circulating estrogen, as evidenced by the finding that cyclical changes in endogenous estrogen levels can augment LTP [201, 202]. These studies support the hypothesis that estrogen is a regulator of learning-related mechanisms, although some contradictory findings exist. For instance, estrogen administration results in improved performance in avoidance tasks but not in the Morris water maze [203]. However, a different study indicates that estrogen can improve performance in the water maze task [204]. LTP enhancement by estrogen is mediated by both mitogen-activated protein kinase-dependent and independent components [205]. Further, evidence suggests that estrogen increases NMDA receptor activity, and this is likely a further mechanism through which it enhances LTP [206]. Further studies are needed to elucidate the exact mechanism through which estrogen modulates hippocampal LTP and learning and memory.

Neurogenomic techniques in the development of novel targets

It is widely thought that long-term alterations of cell function may mediate learning and memory in the brain. These long-term changes must involve gene expression and resultant protein production. Thus, for every sustained memory there is likely a chain of events leading from the initiation of activity at a synaptic receptor, to the activity of second messenger systems, to intermediate early gene induction, and to secondary gene induction in every cell that participates in the memory network. The same is likely true for LTP [207].

A number of research groups are endeavoring to trace the chain of cellular events that underlie induction and maintenance of LTP [208]. In these studies single genes, controlling what are hoped to be specific events within cells, can be eliminated and the resultant effect can be studied simultaneously in whole animals minus one gene, so-called knockouts. In this method the gene of interest, usually a well-characterized gene, is cloned and in most cases altered so that important regulatory regions of the gene are non-functional.

One reason to target genes is that these genetic procedures have the potential to overcome the current limitations of pharmacology. Numerous studies have evaluated hormone involvement in learning and memory by genetic manipulations. For instance, OXT knockout mice show complete social amnesia, but exhibit no other cognitive deficits [28]. Research using CRF peptide, CRF post-synaptic receptor and CRF binding-protein transgenic and knockout mouse models allows for critical analysis of hypotheses relating HPA axis tone

and brain CRF system activation in animal models to a variety of clinical psychopathologies. While the putative role of brain CRF in affective disorders has been the primary focus of research, learning and memory capacities of CRF over-expressing and knockout mice and CRF₁ receptor knockdown and knockout mice can now be assessed in these animals, which exhibit targeted defects in homeostasis. In Table 2, results from antisense oligonucleotide studies are tabulated alongside gene targeting studies since gene knockdown via translational arrest could be expected to produce functional, albeit transient, consequences similar to a null mutation.

Galanin knockout and over-expressing transgenic mice have recently been generated to facilitate understanding of galanin activity in basal forebrain function. Galanin knockout mice have fewer cholinergic basal forebrain neurons and show behavioral memory deficits [209]. On the other hand, mice over-expressing galanin exhibit hyper-enervation of the basal forebrain, but still have functional memory deficits [210]. These data highlight the need to explore the putative mechanisms by which galanin signaling might be beneficial or deleterious to cholinergic cell survival and activity within the basal forebrain [119]. Mice carrying a targeted loss-of-function mutation in the galanin gene exhibit age-dependent deficits in stimulated acetylcholine release, water maze performance, and induction of long-term potentiation in the CA1 region of the hippocampus, as well as a decrease in the number of cholinergic neurons in the medial septum and vertical limb diagonal band [112]. This suggests galanin plays a trophic role in the development and function of septo-hippocampal cholinergic neurons. The following sections will outline new techniques, other than knockout technology, currently used to identify genes that play an important role in the modulation of memory and cognition.

As mentioned previously, the hippocampus is widely recognized as an important structure involved in learning and memory. The hippocampus displays two forms of LTP: an NMDA receptor-dependent form [211], and an NMDA receptor-independent form [211–216]. Of particular interest to this section is the mossy fiber projection from granule cells in the dentate gyrus to the *stratum lucidum* layer of area CA3 of the hippocampus, which displays the NMDA-independent form of LTP, and is dependent on the activation of opioid receptors. This activation is thought to be facilitated by opioid peptides contained in, and released by, the mossy fibers and has been confirmed both *in vivo* and *in vitro* [212–218].

A number of mechanisms are implicated in LTP induction and maintenance [219]. Studies indicate that an influx in Ca^{2+} activates a series of second messenger cascades, including the calcium/calmodulin-dependent adenylyl cyclase pathway [220], results in an increase in cAMP, and activates the cAMP-dependent protein kinase [219]. Additional work has shown that protein synthesis inhibition blocks the induction of LTP *in vivo* in the mossy fiber-CA3 pathway of the hippocampus, which has been previously implicated in spatial memory [221]. Despite the mounting evidence supporting NMDA-receptor independent LTP in the mossy fiber-CA3 pathway, the specific mech-

anisms by which opioid peptides facilitate LTP in this region have not yet been elucidated. Alterations in gene expression are important for LTP induction and maintenance in multiple hippocampal pathways. Because relatively little is known regarding the actual mechanisms involved in LTP in the mossy fiber-CA3 pathway, one laboratory examined the regulatory processes underlying LTP induction in this pathway by focusing on alterations in gene expression after LTP induction using Affymetrix microarray technology [222].

Microarrays allow one to monitor the expression patterns of numerous genes simultaneously. DNA oligonucleotides are synthesized directly onto the array by means of photolithography. A given gene is represented by 15–20 different 25mer oligonucleotides that serve as unique, sequence-specific detectors. An additional control element on these arrays is the use of mis-match control oligonucleotides that are identical to their perfect match patterns except for a single base difference in a central position. The presence of the mismatched oligonucleotide allows cross-hybridization and local background to be estimated and subtracted from the perfect match signal. Hybridized probes are detected by incorporated fluorescent nucleotide analogs. Thus the DNA Microarray is very suitable to study global gene expression profiles. Thompson et al. (2003) used Affymetrix oligonucleotide arrays that contained probe sets corresponding to 1,200 genes (Rat Neurobiology Array, RN-U34) to identify changes in hippocampal gene expression associated with LTP induction in the mossy fiber-CA3 pathway. They found that genes involved in synaptic plasticity, neurotransmission, transcription factors, cell survival, trafficking, and ion channels are altered in the hippocampus following LTP induction in the mossy fiber-CA3 pathway (Tab. 3).

Opioid-related genes altered in the LTP group are known to be involved in enhancing neurotransmission. For example, proenkephalin was found to be up-regulated in the LTP group and this corresponds with previous literature showing that enkephalin peptides released from hippocampal mossy fibers lower the threshold for induction of LTP at mossy fiber synapses [223]. Neuropeptide Y (NPY), which was also up-regulated in our LTP animals, has been previously linked to inhibition of glutamate release and LTP in the dentate gyrus [224]. tPA, which interacts with the angiotensin system and is known to play an important role in synaptic remodeling, was similarly up-regulated following LTP induction in the MF-CA3 pathway.

Growth factor changes were also noted. BDNF, for example, was up-regulated in the group in which LTP was blocked. Previously linked to LTP induction in the hippocampus, BDNF is thought to trigger long-lasting synaptic strengthening through MEK/ERK [219]. Other growth factors such as VGF are also up-regulated in both the LTP and non-LTP groups, suggesting a possible role in synaptic modification. One possibility is that the up-regulated growth factors interact with down-regulated IGF-1 and endothelin receptor to regulate cell survival during synaptic alterations.

Microarray technology is still in its infancy and, therefore, it is essential to verify findings using other mechanisms, such as Real-time PCR (polymerase

Table 3 - List of significantly altered (up or down-regulated) genes in the rodent hippocampus following LTP induction in the MF-CA3 pathway (see Thompson et al. [222] for details)

Accession number	P value	Direction	Description	Function
AJ006710_at	0.007	Down	<i>Rattus norvegicus</i> mRNA for IP3-kinase	Signal transduction
L39018_at	0.048	Down	<i>Rattus norvegicus</i> sodium channel protein 6 (SCP6) mRNA	Ion channel
S65355_g_at	0.02	Down	non-selective endothelin receptor	Neuronal survival
L36884_at	0.02	Down	<i>Rattus norvegicus</i> protein tyrosine phosphatase (OST-PTP) mRNA	Phosphorylation
AJ001641_at	0.01	Down	<i>Rattus norvegicus</i> mRNA for Brain-1 (Brn-1) protein	Transcription factor
AJ007632_s_at	0.008	Down	<i>Rattus norvegicus</i> mRNA for ELK channel 3, partial	Transcription factor
M10244_at	0.049	Down	Rat tyrosine hydroxylase mRNA	Signal transduction
L13040_s_at	0.048	Up	<i>Rattus norvegicus</i> calcitonin receptor C1b mRNA	Signal transduction
X07729exon#5	0.044	Up	<i>R. norvegicus</i> gene encoding neuron-specific enolase	Neuronal marker
K02248cds_s_at	0.032	Up	Rat somatostatin-14 gene, complete cds	Synaptic transmission
S49491_s_at	0.02	Up	Proenkephalin	Synaptic transmission
X06655_at	0.018	Up	Rat mRNA for p38	Signaling
M15880_at	0.017	Up	Rat Neuropeptide Y	Inhibit glutamate release and LTP
M74223_at	0.015	Up	Rat VGF mRNA	Synaptic transmission

chain reaction). The findings to date provide new information regarding gene alterations that occur in the hippocampus following stimulation of the mossy fiber-CA3 pathway, thus enhancing knowledge of the potential mechanisms underlying opioid-dependent hippocampally-dependent learning.

Conclusions and future directions

An increasing number of structurally heterogeneous compounds, which may act via very different neuronal mechanisms, have been proposed to facilitate

attention and acquisition, storage and retrieval of information, and/or to attenuate the impairments of such cognitive functions associated with age or dementia [225]. It should be noted that while the present review describes many elaborate neurobiological mechanisms for enhancing performance in animals and humans tested in learning and memory contexts, very simple approaches already exist that are capable of achieving the same feat. For example, glucose administration regulates many neural and behavioral processes in rodents, including learning and memory [226]. Glucose ingestion can improve memory in highly functioning populations [227] and glucose restriction can have an impairing effect on cognitive performance [228]. Similarly, glucose enhances performance on specific measures in an elderly population, particularly on those tasks where mild age-related deficits appear (e.g., verbal declarative memory) [226, 229]. Thus, one could rightly question whether direct neuropharmacological actions of peptide or steroid hormones which regulate energy balance, such as neuropeptide Y and adrenal steroids, exert their long-term effects on cognitive function by indirect stimulation of glucose availability [230]. The often cited link in the present review between facilitated learning performance on the one hand and enhanced cholinergic neurotransmission on the other, is another double-edged sword which does suggest favorable correlates of functional efficacy for a particular peptide/steroid hormone in a behavior assay while rendering the actual mechanism of action more ambiguous and imprecise from one hormone to the next. This problem of specificity has been called one of the most difficult in learning and memory research [10] and can be pursued in the future by defining the number and identity of unique mechanisms for optimizing learning and memory capability [231].

The search for drugs that enhance cognition requires the development of behavioral tests for animals [232]. These tests must be able to identify potentially therapeutic drugs and reject ineffective drugs. Therefore, a coherent conceptual and experimental framework is needed to organize future research in this area. Unfortunately, previous pre-clinical research strategies appear to have focused on the demonstration of drug effects in a wide variety of tests of uncertain validity, rather than on determination of the specific psychological and neurobiological processes affected by putative cognition enhancers. For example, some sort of noxious stimulus, such as electric shock delivered unexpectedly to rodent paw pads, is typically used to motivate learning in the widely-used avoidance conditioning context in spite of the fact that shock exposure produces an unconditioned affective arousal state which confounds interpretation of learning performance in the task [2]. A further disincentive for employing alarming and traumatic stimuli in the conditioning environment is provided by behavioral and cognitive neuroscience studies demonstrating that the affective salience of stimuli can bias encoding and retrieval of learned information in an automatic manner [233, 234]. Thus, future efforts require explicit identification of the goals of the research, the cognitive process, the neural systems and cellular gene products involved in the process, the selectivity and sensitivity of tasks that measure the process and the validity of the behavioral tasks as a

model to predict the effects of the drug in humans. For example, a hierarchical, multi-task approach employed to phenotype learning and memory capabilities in mutant mice appears to be suitable for wider adoption [235, 236].

The combined use of genetic tools, such as genetically engineered mice and microarray technology, together with more classical pharmacological methods can greatly enhance our ability to detect novel therapeutic targets. For instance, peptides such as proenkephalin and neuropeptide Y, which are known to play a role in learning and memory, are up-regulated after LTP induction [222]. Moreover, genes identified in other studies of learning and memory-related genetic regulation could provide unforeseen, previously unidentified drug targets for cognitive enhancement. Pharmacological and behavioral characterization of these targets will confirm or refute their ability to modulate learning and memory processes, and ultimately their therapeutic potential in clinical populations.

The conceptual foundations of research aimed at the determination of potential neuronal, neuropharmacological, genetic, and behavioral/cognitive mechanisms mediating drug-induced cognition enhancement require definition [237]. For example, peptides and steroid hormones of the HPA axis such as CRF and glucocorticoids are presumed to be the neurochemical mediators of enhanced long-term memory for stressful or emotionally arousing experiences [238]. The pharmacological [239], neurobiological [240] and clinical [241] evidence necessary to support this claim convincingly is only now being assembled. In particular, the behavioral phenotype of learning and memory impairments described in the present review for CRF and glucocorticoid receptor mutant mice supports the view that pharmacological normalization of HPA axis tone would have therapeutic benefits for dementing disorders [242]. Nonetheless, the systematic development of a psychopharmacology of cognition enhancement may require concerted application of behavioral, genetic, cognitive, pharmacological and electrophysiological techniques.

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Natural products as cognition enhancing agents

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Introduction

Natural products have a widespread public appeal that appears only to be growing. The fastest growth is in the western world although in lesser economically developed countries the demand remains as strong as ever. This appeal is aided by the almost universal, though completely irrational assumption, that if a product is natural it must be safe. This chapter will consider those naturally-occurring substances that are believed to beneficially affect cognitive function.

The definition of cognitive enhancement adopted for this chapter is an improvement in core aspects of cognitive function, which is crucial to the conduct of the activities of daily living. For normal volunteers, this represents identifying an improvement on a valid measure of cognitive function known to be important for everyday behaviour. The same is true for a patient population known to have a cognitive impairment, the point simply being that for enhancement to have taken place, cognitive function needs to be measurably better than it was before treatment. The improvements must be in domains of cognitive function which are widely recognised to be important to everyday behaviour, such as attention and memory. Further, the improvements must be assessed by objective tests that are appropriate, valid and sensitive instruments for assessing the areas of function of interest. Finally, the study design must reflect the current gold standard in psychopharmacology, i.e., be at the minimum randomised, double-blind and placebo controlled,

Not surprisingly there is considerable interest in natural products that may enhance human cognitive function. A thorough review of the field would fill this and probably several more volumes, even if restricted to human studies. The 'natural product' field is widely characterised as being an area of 'soft science', where trials are rarely conducted with the rigour required for publication in leading peer reviewed human cognitive psychopharmacology journals. Often claims are based on anecdotal evidence, uncontrolled trials or work with animals. While such evidence is helpful in helping to select a substance to study, this evidence cannot form part of the 'core' evidence to support a particular claim for its cognition-enhancing potential.

This chapter will consider a number of substances that have been the subject of repeated scientific scrutiny and which have shown cognition enhancing properties. The substances described will either have a clear mechanism of action for which the chemical structure of the active ingredient is known, or will be available in standardised preparations in which the amounts and ratios of the various ingredients can be guaranteed to be present within acceptable limits. Other promising substances with little experimental evidence to date are covered in Table 1. Trials of compounds for which such standardisation is not possible, at least for the known active ingredients, will not be covered, as such trials cannot be replicated using comparable formulations.

Ginseng

'Ginseng' is generally taken to refer to the dried root of several species in the plant genus *Panax* (Araliaceae family). The most widely used family member is *Panax ginseng*, which is indigenous to the Far East (most notably China and Korea). It was first cultivated around 11 BC, and has a medical history (as a wild herb) stretching back more than 5000 years [1]. Other members of the genus include *Panax quinquefolius* (American), *Panax notoginseng* and *Panax japonicus*. Given that ginseng has been estimated to have the second highest financial turnover of any herb (after *Ginkgo biloba*) in the US marketplace [2] and enjoys ubiquitous and undocumented use throughout a number of societies and traditional medicinal systems, it is potentially the most widely taken herbal product in the world. While ginseng is taken both as a general tonic and prophylactic agent it is also widely taken in western markets to ameliorate 'memory loss' and 'absentmindedness' [3].

The major active constituents of the *Panax genus* are thought to be triterpenoid glycosides or saponins, also known as ginsenosides, of which over 30 individual examples, many of which exist only in minute amounts, have been identified [4]. The ginsenoside content of ginseng extracts can vary depending on the species, the age and part of the plant, the preservation method, the season of harvest, and the extraction method [5–7] also notes that no herb is more subject to adulteration and misrepresentation. Currently, the only standardised extract that has attracted widespread research attention is G115 (Pharmaton SA) which is standardised to an invariable 4% of ginsenosides.

A number of *in vitro* and *in vivo* properties potentially relevant to the modulation of cognitive performance have been attributed to single and multiple ginsenosides and whole extracts of ginseng. These include; effects on vasoconstriction, [8, 9], a beneficial influence on blood flow through modulation of platelet aggregation [10, 11], roles in both cardio-protection [12–14] and neuroprotection following a number of insults [15–18], shifting of the hormonal balance of the hypothalamic-pituitary-adrenal system [19–21], modulation of a number of neurotransmitter systems [22–25], and modulation of blood glucose levels in both diabetic [26–28], and non-diabetic humans [27, 29].

Table 1. Summary of promising cognition-enhancing substances not as completely investigated as those discussed in the text

Name	Anecdotal use	Alleged actions	Mechanism of action	- Drug interactions - Side effects - Precautions	Usual dosage or content	The science
<i>Bacopa monnieri</i> (Water yssop, Brahmi)	In Ayurveda, Brahmi is described as a brain tonic - and alleviation of the symptoms of anxiety and convulsive disorders. Powerful antioxidant actions are also attributed to <i>Bacopa</i> .	Facilitation of memory retention and promotes mental functioning and has general rejuvenative effects.	A number of biologically active compounds are present in the plant including alkaloids, saponins and sterols, however those responsible for the memory enhancing effects are triterpenoid saponins called "bacosides".	May interact with CNS active drugs. Some aminoglycoside antibiotics such as clindamycin may enhance the neuromuscular relaxing action of Brahmi. No adverse effects are reported from clinical trials.	2-6 g per day.	<i>Bacopa</i> has demonstrated antioxidant properties [188] and the protection of mental function in epilepsy when drug phenytoin is taken [189]. <i>Bacopa</i> administration improved learning [190] and had antidepressant activity [191] in rats. Recently <i>bacopa</i> improved learning and memory in healthy humans with maximal effects evident after 12 weeks [192].
Fava beans (<i>Vicia faba</i>)	A vegetable that is widely cultivated in China and the Mediterranean region.	The L-dopa in Fava and <i>Mucuna pruriens</i> facilitates memory, energy, sense of well-being, sex drive, and helps to control the symptoms of Parkinson's Disease.	Fava and <i>Mucuna pruriens</i> beans (and the entire fava plant) have high concentrations of L-dopa, an amino acid that is enzymatically converted to the neurotransmitter dopamine.	Taken in combination with monoamine oxidase inhibitors (MAOIs), blood pressure can increase dangerously. Nausea, dyskinesia and allergic reactions have been reported. People with Favism can develop a serious condition called hemolytic anemia [193].	L-dopa in <i>Mucuna pruriens</i> is from 3 to 7%, in Fava beans is below 0.5%.	<i>Mucuna</i> seed powder (HP-200) was given to 60 Parkinson's patients for 12 weeks, whilst a control group of 26 patients were given synthetic treatments. The Unified Parkinson's Disease Rating Scale showed that <i>Mucuna</i> was effective with minor adverse consequences (HP-200). Similar results were obtained with fava beans [194, 195].
<i>Mucuna pruriens</i>	In the Ayurvedic system, <i>Mucuna</i> powder is used for the treatment of Parkinson's disease					

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Table 1. (Continued)

Name	Anecdotal use	Alleged actions	Mechanism of action	- Drug interactions - Side effects - Precautions	Usual dosage or content	The science
Ashwaganda (<i>Withania somnifera</i>)	Ashwagandha is a bush cultivated in India and North America. It has been used for thousands of years by Ayurvedic practitioners.	Ashwagandha is supposed to have anti-inflammatory, antitumor, anti-stress, antioxidant, mind-boosting, and rejuvenating properties.	Ashwagandha contains flavonoids and active ingredients of the withanolide class. Ashwagandha has GABA-like activity (anxiolytic activity) and can increase acetylcholine level in brain (cognition enhancement).	Should not be used in combination with sedative drugs. Potential source of hypoglycemic, diuretic and hypcholesterolemic agents.	100–200 mg standardised extract daily.	A research indicates that ashwagandha stimulates the growth of axons and dendrites and levels of antioxidants [196]. Several studies in rodents showed Ashwagandha had memory boosting, anxiety, anti-depression and testosterone-like effects [197–199].
<i>Celastrus paniculatus</i> (Jyotishmati)	Ayurvedic medicine in India considers <i>Celastrus paniculatus</i> (CP) as a brain tonic and aphrodisiac.	Cognitive enhancing effect and powerful antioxidant activities are attributed to extracts of CP	Some data suggested that active ingredients of CP (Bacosides A and B) cause an overall decrease in the turnover of norepinephrine, dopamine and serotonin [200] but the mechanism underlying the cognitive effect of CP is not yet very clear.	Reversible arrest of spermatogenesis and reversible liver necrosis (lesions were absent after 45 days post treatment) were found after chronic administration of CP in rats [201].	10 drops of the oil extracted from seeds.	Seeds of CP administered chronically in rats selectively reversed the impairment in spatial memory produced by scopolamine [202]. Strong antioxidant effects in rats brain was also demonstrated [203].

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Table 1. (Continued)

Name	Anecdotal use	Alleged actions	Mechanism of action	- Drug interactions - Side effects - Precautions	Usual dosage or content	The science
<i>Origanum majorana</i>	Traditionally used as an anti-microbial remedy. The Greeks used it as a remedy for narcoleptic poisoning, convulsions and dropsy.	Natural and synthetic Acetylcholinesterase inhibitors (AChEi) increase the cholinergic tone in brain and are useful in Alzheimer's disease and for cognition enhancement.	Ursolic acid, one of the active components of <i>origanum majorana</i> (OM) and other herbs from the Labiate family, is a potent natural AChE inhibitor [294].	No evidence of drug interactions with OM has been found in the medical literature. OM should not be used during pregnancy as it stimulates menstruation and may cause miscarriage.	Dose for cognition enhancement is not established.	Ursolic acid demonstrated antioxidant action, reducing Abeta neurotoxicity in rat's brain [205].
Alpha lipoic acid (ALPA, thioctic acid)	This water and fat-soluble antioxidant has been suggested for liver health and liver intoxication, for diabetes-related neuropathy, as a possible HIV inhibitor and as an antioxidant.	Improves memory by reversing the damage that had been induced by oxidative stress to neurons and preventing them from lack of oxygen and poor blood supply.	Raises glutathione, vitamin C and F levels and decreases concentrations of reactive oxidants. It also lowers glucose levels and preserves the mitochondria [206].	Drugs that influence blood-sugar level may require a change in dosage if taken with ALPA.	200–600 mg a day.	Double blind placebo controlled trials support the antioxidant effect in various kind of poisoning [207], aging [208] and diabetes [209] damages. In animal studies, ALPA taken alone [210, 211] or with acetyl-L-carnitine [212] was able to reverse memory impairment and oxidative stress in aged mice.

This plethora of potential mechanisms is unmatched by solid evidence of efficacy, including that pertaining to cognitive function following chronic regimens of ginseng extracts administered to humans. However, to a great extent, the equivocal nature of this area can be attributed to the methodological limitations of the extant literature [3, 30–32].

Whilst human chronic dosage research has almost exclusively been conducted by carrying out testing only at the end of the dosing period, usually of 8 to 12 weeks, a series of recent studies suggests that single doses of a standardised *Panax ginseng* extract (G115) can modulate cognitive performance [33–35].

These studies shared the same randomised, double-blind, placebo-controlled, balanced-cross-over design, with 20 participants receiving three different single doses of the relevant extract and an identical placebo on separate occasions seven days apart. In the initial study [33], which utilised a computerised cognitive assessment system (the CDR system), all three doses of ginseng (200 mg, 400 mg, 600 mg) were associated with improvements on a factor analysis derived 'Secondary Memory' measure (comprised of % accuracy scores from four secondary memory tasks). However, these improvements were most pronounced for the middle (400 mg) dose. In contrast to these improvements, both of the less mnemonically active doses (200 mg and 600 mg) were associated at the later testing sessions with slowed performance on a 'Power of Attention' factor (comprising reaction times on three attention tasks). This improved secondary memory performance following 400 mg of G115 was replicated in the second, methodologically similar, study again using the CDR system which compared the effects of single doses of *Ginkgo biloba*, ginseng G115, and their combination against a placebo in a single cohort [34]. A further experiment also assessed the effects of the same doses of G115 on Serial subtraction mental arithmetic tasks. On the most demanding (Serial 7 s) task the 400 mg dose proved beneficial, with increased accuracy, but once again the 200 mg dose led to decrements, this time in terms of the number of responses made. A trial which has just been completed studied the effects of G115 in combination with 22 minerals and vitamins upon fatigue and cognitive decline induced by shift-work [36]. It was found that compared to nurses who took placebo over 12 weeks, nurses who took the active treatment showed smaller shift-work induced fatigue, mood changes and cognitive deficits. The clearest cognitive effect was seen on the CDR Quality of Memory factor.

The CNS effects of *Panax ginseng* have also been assessed in a double-blind, placebo controlled, balanced cross-over, topographic Electroencephalograph (EEG) experiment comparing the effects of both 360 mg of *Ginkgo biloba*, and 200 mg of *Panax ginseng* G115 in 15 healthy volunteers [37]. The results suggested that there were similarities in the topographic EEG effects elicited by both extracts (in comparison to placebo) with reduction in the power of 'eyes closed' frontal theta and beta wavebands. However, these effects were more marked for ginseng, and were accompanied by reductions in frontal alpha waveband activity and decreased latency of the P300 component of the auditory evoked potential.

Ginkgo biloba

The *Ginkgo biloba* tree is one of the oldest surviving tree species on earth [38] and has probably existed in its current form for up to 200 million years, leading to its description by Darwin as a "living fossil". The Ginkgo tree has a lifespan of up to a thousand years [39]. Extracts and infusions made from Ginkgo leaves have been used in traditional Chinese medicine for at least 5,000 years. Currently it is sold either as an 'over the counter' food supplement, or as a prescription medicine throughout the western world, and ranks as the best-selling herbal medication in western marketplaces [40, 41]. The popularity of Ginkgo can be attributed in some measure to the development in the mid 1960s of manufacturing processes capable of producing high quality standardised extracts. These extracts, derived by a complex drying process, are concentrated in a ratio of approximately 1 part extract to 50 part dried leaves, and are generally standardised to a content of 24%–25% flavonoids and 6% terpenoids (e.g., GK501, Egb761, LI 1370).

The most important active substances in ginkgo are thought to be the flavonoids – ginkgo-flavone glycosides of kaempferol, quercitin and isohamnetin, and the terpenoids – bilobalide and ginkgolides A, B,C and J [42]. Whole extracts and the active components have been attributed with a number of physiological effects potentially relevant to the enhancement of cognition. These include: specific antagonism of platelet activating factor [43–45], scavenging and inhibition of free radicals [46–48], modulation of a number of neurotransmitter systems [49–52], beneficial effects on blood circulation [53–57], both *in vitro* and *in vivo* protection against hypoxic challenges [58–60] and *in vivo* neuro-protective properties [61–64].

Accumulating evidence suggests that Ginkgo may be effective in its prescribed role in the amelioration of cognitive decline. As an example, a recent Cochrane review [65] meta-analysed the 33 extant studies involving cohorts suffering from dementia or age-related cognitive impairment that met their inclusion criteria. The results across the studies suggest improvements in cognitive performance following Ginkgo at all dose levels (< and > 200 mg/day) and time points (12, 24 and 52 weeks). The authors, while allowing the possibility that the overwhelmingly positive literature may reflect a publication bias, conclude both that Ginkgo is not associated with any more adverse events than placebo, and that "Overall there is promising evidence of improvement in cognition and function associated with Ginkgo".

There is also evidence of cognitive enhancement in healthy 'cognitively intact' populations following chronic administration of Ginkgo. In older adults Mix and Crews [66] reported improved speed of performance on a timed Stroop task, and trends towards increased speed of performance on three further tasks following six weeks administration of 180 mg/day Egb 761 or placebo to 40 healthy participants. Similarly, Crews and Mix [66] assessed the effects of six weeks administration of 180 mg/day of Egb 761 or placebo to 249 healthy older individuals and demonstrated superior self-rated memory

performance and objectively assessed long-term recognition and recall. However, in contrast to these studies, Solomon and co-workers [67] administered 120 mg/day of GK501 or placebo for six weeks to 219 healthy elderly participants and found no treatment-related differences on any cognitive measure.

In healthy 'non elderly' adults Stough and co-workers [68] reported Ginkgo-related improvements on Digit Span Backwards, and speed on working memory and delayed auditory verbal learning tasks following 30 days administration of Ginkgo or placebo to 50 participants. In contrast, Moulton and co-workers [69] found no interpretable significant differences on a range of cognitive tasks in 60 healthy young males administered of 120 mg LI1370 or placebo for five days in a double-blind, between subjects experiment.

A number of studies have also assessed the effects of acute dosage of Ginkgo in healthy adults utilising a double-blind, counterbalanced cross-over design. In the earliest of these studies Hindmarch [70] reported shortened reaction times on a Sternberg short-term memory scanning task following the highest (600 mg) of three single doses of Ginkgo taken by a small cohort (8) of healthy females. Warot and co-workers [71] failed to replicate this effect on the Sternberg task but did generate an improvement in free recall score for one 600 mg dose of standardised extract. In a more adequately powered study, Rigney and co-workers [72] examined the effects of one and two day's administration regimens of four doses (120 to 300 mg) of ginkgo in 31 participants. Performance was only significantly improved on reaction times for the Sternberg numeric working memory task. Utilising the methodology described in relation to ginseng above, Kennedy and co-workers [73] demonstrated dose-dependent increases in speed across three attention tasks in 20 young participants administered single doses of 120 mg, 240 mg and 360 mg of GK 501. There was also evidence of improved secondary memory performance following the lowest dose. In a partial replication of this study [34] a different cohort of 20 young participants ingested single doses of Ginkgo GK501, ginseng (G115) and their combination. Whilst the speed of performing attention tasks was unaffected the results suggested that 360 mg of Ginkgo improved secondary memory performance.

Ginkgo biloba/Panax ginseng combination

A number of studies have shown improved cognitive performance following administration of a 60:100 combination of *Ginkgo biloba* GK501 and *Panax ginseng* G115.

In the first of two double-blind, placebo-controlled, chronic dosage studies utilising the CDR system, Wesnes and co-workers [74] assessed the effects of 80 mg, 160 mg and 320 mg or placebo, administered in two daily doses to 64 participants who satisfied the criteria for neurasthenia, an age related condition with a possible cerebro-vascular aetiology. Results showed significant

improvements for all three doses at one hour past the morning dose or at least two of the three (1, 30 and 90 days) assessments on a 'Quality of Memory' measure (comprising scores from six memory tasks). However, occasional impairments were seen on the same measure after the second lunchtime dose. The second study [75] utilised the same measures and involved a cohort of 256 healthy middle-aged participants who received 320 mg of the combination or placebo. Testing took place four times daily (1 hour pre-dose and 1, 3, and 6 hours after the first dose) at pre-commencement of treatment and at 4, 8, 12 and 14 weeks post-commencement of treatment. Results showed improvements on the same memory measure across the study at the 1 and 6 hour post-dose testing sessions. The overall improvement in the Quality of Memory measure was 7.5%, which sets a minimum standard for compounds aimed at improving memory in healthy middle-aged volunteers to achieve.

Improvements in memory performance have also been shown in groups of 20 healthy young adults following single doses of the combination in two studies utilising the CDR system in the study paradigm described above [73, 33]. In the first [76] the pattern of results following the combination was similar to that following ginseng alone, with improved Quality of Memory for the highest dose (960 mg), but decrements in the speed of performing attention tasks for the mnemonically-inactive lowest dose (320 mg). In the second study [34], comparing single doses of Ginkgo, ginseng and the combination to placebo, the improvement in memory performance was confirmed for the 960 mg dose.

Salvia officinalis/Lavandulaefolia and Melissa officinalis

Several recent strands of evidence suggest that members of the Labiate family may enhance cognitive performance. The potential utility of these plants was first suggested by a retrospective examination of historic pharmacopoeias and herbal texts with subsequent *in vitro* analysis of the plants that have had a historical role in improving mental function [77, 78]. This historical and *in vitro* analysis suggested that the two most promising candidates were the *Salvias* '*officinalis*' and '*lavandulaefolia*' (Sage) and *Melissa officinalis* (Lemon Balm). In the case of the former this was predicated on demonstrations of acetylcholinesterase inhibition in brain tissue both *in vitro* [78, 79] and *in vivo* [80], and in the case of the latter both nicotinic [78, 81] and muscarinic [81] cholinergic receptor binding in human brain tissue. Both plants also exhibited substantial anti-oxidant properties [82–84].

A series of double-blind, placebo-controlled, cross-over studies using the CDR battery have assessed the nootropic potential of these plants. Evidence from two studies suggested that single doses of *Salvia lavandulaefolia* could improve word recall in 20 healthy young participants [85], and that single doses of *Salvia officinalis* could improve the secondary memory and attention task performance of 20 elderly participants (mean age 72.9 years) [86].

With regards *Melissa officinalis*, findings of dose-dependent decrements in timed memory task performance along with increased calmness for a low dose (300 mg) and reduced alertness for a high dose (900 mg) of a concentrated manufactured extract administered to 20 healthy adults were broadly in line with current usage as a mild sedative and sleep aid. However, retrospective *in vitro* analysis showed that the extract lacked the expected substantial cholinergic binding properties [87]. In a subsequent study [37], a number of acquisitions of dried *Melissa officinalis* leaf were first assessed for cholinergic binding, with the cognitive and mood effects of single doses of the leaf with the most promising profile being investigated in 20 healthy adults. Once again decrements were seen on the timed memory tasks, but in this instance as dose increased (600 mg, 1000 mg, 1600 mg), these decrements decreased, with the highest dose engendering improved memory performance and increased calmness at all post-dose time points (1, 3 and 6 hours).

Vinpocetine

Vinpocetine is derived from the alkaloid vincamine found in the Lesser Periwinkle plant, *Vinca minor*. Vinpocetine is believed to be a promising treatment for cerebral vascular insufficiencies and may have clinical utility in stroke consequences. Vinpocetine increases cerebral metabolism and raises ATP levels in nerve cells; is a highly potent and safe vasodilator, acting by direct relaxation of the vascular smooth muscle; enhances cerebral blood flow and also has a great capacity to lower the viscosity of the blood [87a]. Vinpocetine is safer than other vasodilators because it selectively increases cerebral blood flow without a "stealing" effect, i.e., without removing blood from underperfused zones of ischemic damage, possibly due to its ability to lower blood viscosity. It has anticonvulsant actions related to its ability to maintain brain cell electrical conductivity and to protect against damage caused by excessive intracellular release of calcium. Vinpocetine also partially blocks hypoxic damage to brain tissue, and is a good scavenger of hydroxyl radicals [87b, 87c].

Other properties are its metal-chelating capacity [88], the enhancement of retinal microcirculation [89] and of the circulation of the inner ear [90]. Vinpocetine shares this latter property with *Ginkgo biloba*, thus also being a possible treatment for tinnitus. In animal models of anoxia, vinpocetine reduced cerebral oedema and prolonged survival [91].

Vinpocetine has cognitive-enhancing activities in an animal model of memory retrieval [92]. In double-blind clinical trials conducted with patients suffering from mild-to-moderate vascular dementia, vinpocetine enhanced memory and learning as well as clinical global measures of cognitive performance [93–95]. However, in one open-label trial with 15 Alzheimer's patients with doses increasing from 30 mg to 60 mg a day for a year there was no sign of improvement [96]. Clear evidence of the real benefits of this substance is still

not consistently documented; however, vinpocetine appears safe and potentially helpful for cognitive improvement.

Acetyl-L-carnitine

Acetyl-L-carnitine (ALC) is a transport molecule that occurs naturally in the brain, liver, and kidney which plays a role in mitochondrial energy production. Levels of Acetyl-L-carnitine diminish with age. The acetyl group that is part of acetyl-L-carnitine contributes to the production of the neurotransmitter acetylcholine. ALC may increase B-endorphin levels and may increase nerve growth factor production within the hippocampal region of the brain [97]. ALC improves age-related changes in intracellular membranes and in dopamine, NMDA and glucocorticoid receptors; sustains mitochondrial metabolism and cholinergic neurotransmission, improving mental function [98]. Trials on acetylcholine deficit correction in rodents showed benefits [99], and protection from beta-amyloid damaging effects was also demonstrated in the rat cortex [100]. Memory in aging rats improved when they received a combination of ALC and lipoic acid [101].

Meta-analysis [102] of double-blind randomised controlled clinical trials of ALC *versus* placebo in the treatment of mild cognitive impairment and mild Alzheimer's disease supports a significant advantage for ALC compared to placebo on both the clinical scales and the psychometric tests.

Phosphatidylserine

Phosphatidylserine (PS) is a naturally-occurring phospholipid which maintains neuronal structure and helps the neuronal membrane to maintain its charged state. It is believed to increase the receptor number and promote dendritic branching. It is also thought to activate protein-chinase-C within the neuronal membrane, a substance involved in the regulation of major neurotransmitters (e.g., acetylcholine, dopamine and norepinephrine) and in the activation of memory-specific genes. Generally speaking, PS should allow neurons to communicate within each other more efficiently, thus improving mental functioning.

In animal studies PS has been shown to enhance cognitive function [103]. Properly controlled clinical studies have also shown benefits of PS [104–106]. Reviews [107] have supported the case for PS: preventing and even reversing memory loss associated with aging and dementia; enhancing memory in normal people, and relieving symptoms of anxiety and stress [108]. It must be noted that these studies have evaluated the role of Bovine Cortex phosphatidylserine administration. Because of potential viruses present in cow brain extracts, PS is now obtained from soy. As the PS in soy is chemically different than that found in the cow brain, it is possible the effects of soy-based

PS could be different. However, a recent open trial with plant-source derived phosphatidylserine [109] showed encouraging results, although these of course need to be confirmed with double-blind, placebo controlled studies.

Huperzine A

Huperzine A (HupA) is an alkaloid extracted from a club moss (*Huperzia serrata*). The average content of HupA in plants is 0.011%. Huperzine A, in the form of a remedy called Qian Ceng Ta, has been used for centuries in Chinese folk medicine to treat fever, blood disorders, swelling and schizophrenia [110]. It acts as a potent, highly-specific and reversible inhibitor of the enzyme acetylcholinesterase (AChE) over butyrylcholinesterase, which crosses the blood-brain barrier [111]. HupA fits into the active site of AChE where acetylcholine is broken down, and binds onto this site. This binding closes off the enzyme's "cutting" machinery and keeps acetylcholine in circulation cycle, as do other AChE inhibitors (donepezil, galanthamine, tacrine) approved for Alzheimer therapy. HupA also has an antagonistic effect on N-methyl-D-aspartate (NMDA) receptors, and increases the flux of calcium ions into the neurons [112].

Huperzine A has shown both neuroactive and neuroprotective effects in animals and humans. It has been demonstrated to be a safe and effective substance for improving cognitive function and quality of life in patients suffering from varying degrees of dementia, and is used as an over-the-counter cognitive enhancer for healthy people complaining of memory problems. Its clinical evaluation for Alzheimer's disease is now in Phase IV.

One major theory [113] proposes that memory loss and other cognitive deficits in Alzheimer's patients result from degeneration of the nerve cells that release the chemical messenger acetylcholine (ACh). HupA increases the level of the neurotransmitter ACh (particularly in the cerebral cortex), but also of norepinephrine and dopamine, suggesting action on different systems [114]. It has little effect on nicotinic and muscarinic receptors, but huprine X (a hybrid between HupA and tacrine) exhibited more activity at muscarinic receptors [115], promising a superior therapeutic advantages in dementia therapy.

Glutamate activates NMDA receptors and increases the flux of calcium ions in the neurons. Calcium at toxic levels can disrupt normal cognitive processes and kill the cells [116]. HupA non-competitively inhibits the passage of calcium ions through NMDA ion channels, protecting primary neuronal cells against the toxic consequences of the excitatory aminoacid (EAA)-induced over stimulation, which is implicated in a variety of acute and chronic neurodegenerative disorders [117]. As a pre-treatment, HupA may act as a prophylactic drug against soman and other nerve gas poisoning used as chemical weapons, protecting cerebral ACh from soman inhibition, and interfering with EAA-induced toxicity [118]. HupA could be used as a preventive agent for AD having similar properties to Memantidine, a new drug that protects the brain

against the excess of glutamate observed in AD. HupA can also decrease the damage induced by free radicals [119], oxidative stress and by amyloid-beta-peptide (Abeta) [120]. The deposition of Abeta creates the plaques observed in the brains of AD patients.

In vitro experiments demonstrated that HupA had more power in the inhibition of ACh than tacrine and galantamine, but less than donepezil, with a pattern of inhibition of the mixed competitive type. Inhibition of BuChE revealed a different pattern of actions: HupA inhibited BuChE at a higher concentration than needed for AChE compared with donepezil [121]. This selectivity for AChE as opposed to BuChE may suggest a safer profile of side-effects [122] but a stronger inhibition of BuChE seems to be more useful in the later stage of AD [123]. *In vivo* tests revealed that the relative inhibitory activity of oral HupA on AChE was about 24-fold and 180-fold more potent, on an equimolar basis, than donepezil and tacrine respectively [124]. Maximal AChE inhibition in rat cortex and whole brain was reached 30–60 minutes following oral administration, and was maintained for 360 minutes [125]. After repeated doses of HupA there were no signs of declined AChE inhibition compared to a single dose, demonstrating that no tolerance to this alkaloid occurred [126].

HupA has been tested in various animal models of experimental cognitive impairment, showing memory-enhancing activities with a superior safety/efficacy ratio when compared with other AChEIs like physostigmine, galantamine and tacrine [127].

Its evaluation is now in Phase IV of clinical trials (AD), and approximately 800 AD patients have participated in studies with HupA, including double-blind and placebo-controlled studies [119, 128–130] with daily doses ranging from 0.05 to 0.10 mg. All trials found significant improvements in cognitive and behavioural function compared both to placebo and baseline. A study conducted using the Cognitive Drug Research computerised tests battery in 10 healthy elderly volunteers suggested that ZT-1, a HupA derivative, had the ability to partially reverse the cognitive impairment caused by scopolamine [131]. The longest open-label study with HupA (involving 33 patients) indicated a 66.7% improvement ($p < 0.05$) on cognitive and behavioural measures after the 48 week-long trial [132]. A study conducted with a non-patient population (68 secondary school students) suggested a potential use of HupA for cognitive-enhancing purposes [133]. The dose of HupA administered in this study was 0.05 mg twice daily and taken orally. Human testing at daily dosages ranging from 100 to 600 mcg for periods varying from 2 to 48 weeks showed no statistically significant side-effects with HupA. Following a supratherapeutic oral dose of 0.99 mg peak serum concentrations were reached in 79 minutes and half-life was 288 minutes; there were no side-effects with doses between 0.18 and 0.54 mg [134].

It is important to remember that the three compounds currently registered for the symptomatic treatment of AD (tacrine, galantamine and donepezil) also prevent the breakdown of ACh, so it would be advisable to avoid the use of HupA in combination with these drugs.

Glucose

In recent years there has been increasing focus on the potential for nutritional interventions to improve cognitive function. It remains to be established whether such agents have differential effects with respect to macronutrient composition, as opposed to being a function simply of calorific intake and absorption (see [135, 136] for reviews). Nevertheless, a starting point for such studies, and one which has attracted a good deal of attention, is to examine the effects of a simple carbohydrate such as glucose on cognitive function. The general finding is that ingestion of a glucose load (generally 25 or 50 g) can improve aspects of cognitive function in the minutes and hours following ingestions of the drink. Most work in this area has focused upon memory. Indeed it has been postulated that the effect may differentially affect memory processes (especially long-term episodic memory function). While there is reasonable evidence to support this contention, it is probably true to say that studies which have attempted to assess the impact of a glucose load on non-mnemonic processes have also found positive effects. An alternative hypothesis has been put forward which suggests that glucose has more profound effects upon more effortful cognitive processes and it remains to be established whether the glucose enhancement of cognition effect is domain- or demand-specific.

The background to this work is partly grounded in the assumption that brain possesses negligible stores of glycogen (which, in other tissue can be broken down to provide glucose to meet on-going energy demands). It should be noted that recent findings have challenged this assumption. For example the widely-held belief that astrocytes contain negligible, rapidly-depleted, static glycogen stores may not be true. It has recently been suggested that the astrocyte glycogen store is in a dynamic state of turnover may buffer glucose levels in response to on-going neural energy demands [137].

Nevertheless, current models of energetic brain function are based on the assumption that neural energy requirements are met almost exclusively by the oxidative breakdown of blood-borne glucose [138]. This model suggests that glucose is transported across the blood-brain barrier at a rate dictated by capillary surface area and changes in local metabolism [139]. Additionally it is assumed that there is a fairly tightly-coupled equilibrium between blood and brain glucose levels. It follows that any fluctuations in the availability of blood-borne metabolic substrates (i.e., glucose and oxygen) may modulate brain metabolism and consequently cognitive function. This contention is supported by evidence of transient cognitive impairment during hypoglycaemia [140, 141] and lowered by supra-hypoglycaemic glucose levels [142, 143]. Similarly, hypoxia is associated with cognitive impairment [144, 145] where such deficits at altitude can be reversed by supplemental oxygen breathing [146]. A number of studies have demonstrated that oxygen administration can improve cognitive performance in healthy volunteers [147–151]. Such improvement occurred only when concurrently measured hyperoxia coincided

with encoding of target material in memory tasks, or with performance of a reaction time task [149, 150]. It should be noted that at least one study has failed to replicate these effects [152]. Nevertheless the overwhelmingly positive results are consistent with the notion that there is a strong relationship between the availability of metabolic substrates and cognitive enhancement during periods of cognitive demand.

Enhanced cognitive performance on a number of memory tasks results from the administration of glucose, including in the elderly [153, 154] and in young adults [155–158]. Some authors have proposed that there is a fractionation of the memory-enhancing effects of glucose, with tests evaluating long-term declarative memory showing the greatest facilitation [159–162]. However not all studies examining declarative memory have found a glucose enhancement effect [35, 163, 164]. Additionally certain tests of working memory appear to be reliably enhanced by glucose [35, 162, 165, 166] as does kinaesthetic memory [167]. Furthermore, there is an association between glucose and performance on a number of non-mnemonic cognitive measures including choice reaction time tasks [168], the Stroop paradigm [156, 157] vigilance tasks [169], and rapid information processing [156].

With regards to non-memory tasks, glucose tends to facilitate only those tasks, or parts of tasks, that would appear to require a relatively high cognitive load [35, 166]. Indeed in a paradigm where robust enhancement of verbal memory been reliably, during word presentation, participants were required to simultaneously perform self-generated alternating hand movement sequences reported [159–162]. When this additional load was removed, so was the glucose enhancement effect [161].

Where no direct group effects have been found there is nevertheless a relationship between changing blood glucose level and improved performance. The issue is not straightforward; falling blood glucose levels predicted performance on tasks of memory and reaction time [159] and a dichotic listening task [170], while an increase in blood glucose was associated with better memory [155]. On the other hand Kennedy and Scholey [73] found that falling blood glucose levels during task performance were correlated with performance of a heavily-loaded working memory/executive task in a glucose group only, whereas baseline glucose levels predicted performance in both groups. The reason for the disparity between these studies is not clear but may be attributable to the level of cognitive processing required of participants either immediately following a glucose load [171] or while blood glucose levels are raised [35, 166]. Moreover it has been demonstrated that heavily-loaded cognitive processing itself results in a measurable drop in blood glucose levels [35, 171]. In the latter case the task was differentially-enhanced by a glucose drink.

These observations support the suggestion, not only that effortful cognitive processes may be “fuel limited” and therefore augmented by the simple provision of supplemental metabolic substrates. This is supported to some degree by the fact that the ability to efficiently utilise excess blood glucose predicts cog-

nitive performance. This contention is supported by the consistent finding that poorer regulators of blood glucose perform worse on memory tasks even without a glucose load [153]. Indeed there is some evidence that glucose may merely reverse memorial deficits in poor glucoregulators [172].

In a similar vein, Gold [173] has suggested that age-related memory deficits reflect "sub-optimal neuroendocrine regulation of memory storage". Wenk [174] also argues that many cognition-enhancing drugs work via the glyco-genic action of adrenaline. The putative role of adrenal activation in cognitive functioning is also supported by studies demonstrating an arousal-induced facilitation of memory, and its subsequent attenuation by the administration of adrenergic antagonists, both in animals [175] and humans [176, 177]. The mechanisms underlying glucose-related enhancement of cognition are not presently known. One possibility is that insulin released in response to a glucose load promotes glucose uptake in specific brain areas including the hippocampus (which is differentially rich in insulin receptors). Another suggestion is that glucose breakdown promotes the synthesis of acetylcholine, since acetyl CoA, formed as a by product of glycolysis, is rate-limiting in the cholinergic synthetic pathway. However, neither of these proposals adequately explains the pattern of cognitive enhancement seen following a glucose load and it is possible that both of these, in combination with glucose's role as a metabolic fuel, act in conjunction to aid cognitive processing.

A relatively high cognitive load has itself been shown to elicit physiological arousal. For instance, Fibiger and co-workers [178] reported increased levels of salivary cortisol and urinary catecholamines following a mental arithmetic task, with adrenaline levels differentially affected by task difficulty. Similarly, there appear to be increases in cardiac output above somatic requirements as a consequence of cognitive demand. Cardiovascular responses have also been found to be sensitive to levels of difficulty on a number of cognitive tasks, including mental arithmetic, Raven's matrices and sentence comprehension [179, 180]. Similarly heart rate, general metabolic rate and cerebral glucose utilisation increase with greater cognitive load of a working memory task [181, 182]. In some ways this mirrors both previous findings with regards glucose regulation, and also the results of Positron Emission Tomography (PET) studies which have demonstrated negative correlations between absolute cortical metabolic rate and performance on Raven's Matrices [183] and a Word Retrieval (Verbal Fluency) task [184]. Such findings suggest that there may be physiological individual differences which contribute to cognitive performance and, specifically, that a relationship may exist between physiological and cognitive efficiency.

From the above, it seems plausible to suggest that glucose regulation and utilisation, and physiological arousal due to cognitive demand are linked. The aforementioned autonomic responses to cognitive demand may reflect motivational changes, however it also seems feasible that certain processes associated with physiological arousal may serve as mechanisms to increase the delivery of metabolic substrates to the brain. On the other hand, a different inter-

pretation has recently been forwarded by Gibson. In this model, cognitive testing acts as a psychosocial stressor, mobilising cortisol and inhibiting the uptake and disposal of circulating glucose. Thus poorer regulators of glucose will not benefit from a glucose load [136]. While this model is attractive and consistent with much of the data in this area, it has not been tested directly. It is also difficult to reconcile with recent findings of retrograde enhancement of memory function, which occurs even when task demands appear minimal [167].

Discussion

This chapter has confirmed that natural products can enhance cognitive function even in healthy young volunteers. Improvements have been seen in important domains such as attention and memory with a variety of substances having a diverse range of potential mechanisms. Having established that such effects are possible, for each substance attention should in future be focussed upon the more important questions, i.e., the domains of function which can be improved, the extent of improvement possible, the time-course of such effects, and the optimal populations for improvement.

Detecting enhancements to cognitive function is not as easy as detecting impairments. In normal populations, there is a much smaller window of opportunity to enhance function than there is to impair it. As we have seen in the preceding pages, and elsewhere in this book, improvements in normals are rarely greater than 15%, whereas, of course, impairments can be as large as 100%. The signal is therefore weaker in such trials, and if enhancements are to be detected, it is necessary to do everything reasonably possible to ensure that the power of the trial is maximised.

There are a number of simple steps which can be taken to maximise power in human cognitive psychopharmacology. The first is to reduce the variability due to inter-individual differences. There are basically two types of trials which can be conducted in this field, single-dose acute studies or studies which involve multiple dosing. To minimise inter-volunteer differences, cross-over designs using the volunteer as his or her own control are an ideal way to enhance the sensitivity of the study. In single dose trials, the sessions can be separated by a week or more, to avoid any problems with carry-over effects, and such cross-over designs are the current gold standard for single dose trials in this field. A number of examples of this design was seen in this chapter, and a body of consistent results has emerged from this work. Cross-over trials are also possible in multiple dosing trials, though the length of the dosing period is an important practical issue. If the dosing periods are relatively short, say two to four weeks, then at least one cross-over arm is possible. A washout period needs to be inserted between the two dosing periods, and for example with two successive four-week dosing periods separated by a two-week washout, the study will last 10 weeks. With shorter dosing periods (two weeks for exam-

ple), two doses and a placebo could be tested and with two-week washouts, the study would again last for 10 weeks. This is not an unusual time to keep a volunteer or patient in a trial and such cross-overs are clearly possible. However, with dosing periods longer than a month it is rarely practical to have a cross-over, and most trials of this nature employ parallel group designs.

Whether in a cross-over or parallel group design, an important technique to further reduce the variability due to individual differences is to make repeated assessments of cognitive function. At the absolute minimum, each volunteer or patient should be assessed before treatment as well as after, and ideally such testing should be repeated to reflect the opportunity of the compound to enhance function. In acute trials, repeated testing over the hours immediately following administration can identify clear time-based profiles of effects as has been seen in this chapter [73]. In longer trials, testing can be repeated at various stages, e.g., monthly. A further enhancement to this design was seen in a trial in which testing was not only repeated regularly throughout a 14-week period, but was tested at 07:30, 09:30, 11:30 and 14:30 on each study day [185]. Improvements were only detected at two of the testing times but these effects were seen consistently throughout the study. This raises the stakes in trials in this field, as simply employing a single post-dosing test session in a trial lasting weeks or months may miss effects by testing at an inappropriate time (e.g., [67]).

Another technique for maximising the power in such work is to properly train the volunteers or patients on the cognitive testing procedures prior to the start of the trial. It has long been known that performance improves with repeated testing on cognitive tests [186]. These improvements can occur at all ages and over extended periods. A recent trial in an elderly population in which various pencil and paper cognitive tests were administered annually has identified practice effects to persist until at least the third year [187]. Trials must have pre-study training on the tests employed to overcome such effects, otherwise training effects may obscure any treatment effects, or make the interpretation of any changes identified extremely difficult. Most long-term training effects are due to procedural learning, and as this type of learning is fairly robust over the age-span, it is rarely the target for enhancement. If improvements in active are seen which are greater than those on placebo, the active improvements could be interpreted to be the results of improvements to procedural learning, as the study will have shown the presence of it by the placebo enhancement. [185]. Such problems need to be avoided in this field. Having parallel forms of tests (i.e., differing lists of words etc.) for each successive session is an absolute minimum requirement to prevent training effects. Over and above this, pre-study training on the tasks should be mandatory to minimise any practise. Finally, tests or test systems which have known training profiles or, better still, no training effects will be more useful in this area of research.

Reviews in cognitive psychopharmacology consistently comment upon the lack of standardisation of testing used in different laboratories making precise comparisons between trials difficult. Certainly there are advantages to having

standardised procedures in trials, and fifteen of the studies covered in this chapter have used the same computerised test system, which has enabled some general findings to emerge. Another useful technique in this field is to subject test batteries to factor analysis to identify the interrelationships between the various measures. This has been done for the CDR system and a number of factor scores have been derived. Some of these have shown great sensitivity to the effects of natural substances, for example the Quality of Memory Score, which combines the scores of the ability to retain and retrieve information from two working memory (spatial and articulatory) and four episodic secondary (verbal and pictorial) memory tasks into a single measure. Such measures become more powerful as they combine scores from a number of assessments, but also make trials more powerful as they can avoid the statistical problems involved in assessing multiple endpoints.

Overall, it is clear that some natural products can enhance cognitive function. The Quality of Memory factor from the CDR system has consistently been shown to respond positively to particular extracts of Ginkgo and ginseng, whether dosed individually or together. Such effects have occurred in four acute volunteer trials [33, 34, 73] and in multiple-dose trials in volunteers with neurasthenia [74], middle-aged volunteers [75] and nurses working night-shifts [36]. The sites for these trials have been located in the UK, Holland, Sweden and France. This replication in seven studies is one of the most consistent findings in the field of natural products. While further work is required to identify the precise roles of the two extracts in these benefits and the nature of any synergy between them, the consistency of the effects is striking. Besides using standardised extracts, the common features for all trials are the use of a widely-used and extensively-validated computerized cognitive test system, pre-study training on the tests, and repeated testing – both over the study day and for multiple dosing trials, during the dosing period as well. All trials were randomised, double-blind and placebo controlled. Such methodologies for acute cross-over trials and parallel group designs are clearly effective and should set the standard for future work in this area.

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Drugs with multiple CNS targets

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Diversity of neural systems important for cognition

A wide variety of clinical syndromes can manifest cognitive or memory dysfunction. These include head trauma, cerebrovascular accidents, convulsive disorders, nutritional deficits, drug-associated toxicity, etc. Although collectively these entities contribute significantly to the overall amalgamation of known memory disorders, by far the primary disease entity targeted by pharmaceutical research is Alzheimer's disease (AD). AD, which represents the most common form of dementia among individuals over 65 years of age, is now the third most expensive health care problem in the U.S. exceeded only by cancer and cardiovascular disease. It currently affects approximately 4 million Americans and imposes an annual economic burden estimated at between \$80 and \$100 billion. This devastating degenerative condition also inflicts an enormous emotional toll on patients, family members, and caregivers. As the geriatric population inexorably increases, the numbers of AD patients may increase to epidemic numbers (i.e., in excess of 9 million) by the middle of the twenty first century.

An additional concern of older adults is the perception that memory loss occurs as a natural result of aging. This apprehension has contributed at least in part to the enormous increase in sales of over the counter remedies and homeopathic products with claims of memory-enhancing properties. This demand from a large and ever increasing elderly population has also provided the basis for a rising interest in the development of pharmacological agents, not only for the treatment of AD, but also for the much more common, mild cognitive decline associated with normal, non-disease aging [1]. A measurable (albeit mild) decline in cognitive function can occur as a part of healthy aging in humans which begins at some point after the fifth decade of life. The changes observed in typical (non-pathologic) aging are manifested primarily as mild deficits in declarative memory which are thought to result as a consequence of a reduction in the speed of central processing necessary for encoding and retrieval of information [2]. Mild memory deficits that exceed those

associated with normal aging, but that do not meet the (DSM IV) criteria for a diagnosis of dementia, have been referred to as "benign senescent forgetfulness" and "age associated memory impairment" (AAMI). Recent evidence suggests that patients with AAMI have an increased risk of developing dementia [1], a finding that has generated considerable concern and provided the impetus for rigorous investigation.

The study of AD, particularly of the neurochemistry of the post-mortem AD brain, has provided perhaps a greater level of indirect evidence for important components of cognitive and mnemonic pathways than has the study of the "normal" aging brain. Indeed, the well-known selective vulnerability of basal forebrain acetylcholine-containing neurons in AD has underscored the importance of this neurotransmitter system in memory and perhaps in other behavioral and cognitive functions affected by the disease (see first Chapter by J.J. Buccafusco). Among the host of degenerative processes occurring in AD, reproducible cholinergic deficits are consistently reported; they appear early in the disease process, and correlate well with the degree of dementia (for review, [3]). Moreover, abnormalities in cholinergic function are frequently reported in other degenerative conditions such as Parkinson's disease (PD), diffuse Lewy body dementia and Huntington's disease. As in AD, such cholinergic deficits often correlate with memory decline and dementia. Extensive AD-related neuropathology is also commonly found in areas normally rich in norepinephrine (locus ceruleus) and serotonin (dorsal raphe nucleus), particularly in the later stages of the disease [4]. Findings regarding the loss of glutamatergic and certain peptidergic pathways in the AD brain are also extant in the literature [4, 5]. These findings have prompted studies designed to reveal the potential for targeting these depleted neurotransmitter systems with respective receptor agonists or other synaptic signal-strengthening drugs.

The diversity of neurotransmitter substances involved in cognition is perhaps not too surprising, since it has been well-known for many years that memory is represented by several distinct processes, and different types of memory are relegated to different (but sometimes overlapping) brain regions. For example components of the hippocampal formation have been implicated in mediating or processing spatial, declarative, and episodic types of memory in humans, primates, and rodents (e.g., [6–10]). A reasonable argument has been made for the possibility that the hippocampus does not play as important a role in semantic memory [11, 12], with habit learning more dependent upon the striatum [13, 14]. Also, emotional or conditioning learning processes appear to reside within the amygdala [15]. Even within what has been termed working memory or episodic memory, there appears to exist separable and interacting components that may include acquisition (attention), consolidation, and retention (short and long-term); alternatively, encoding, retrieval, storage, and consolidation (see [7]). Certain amnestic agents, such as scopolamine, appear predominantly to affect the acquisition of new learning (e.g., [16]). Selectivity of action with regard to the components of memory has also been attributed to certain memory-enabling drugs, even within a pharmacological

class [17]. Thus, it seems reasonable to conclude that there are several, if not numerous, potential targets for the pharmacological treatment of memory disorders, and that drugs that promote activity within different, but interacting components of cognitive function may be expected to act additively, if not synergistically, when administered together.

Potential multiple synergistic targets for memory enhancement

In recent years, much attention has been focused on the design of palliative agents (cholinergics, nootropics, etc.) that have the ability to offer subtle cognitive improvement. There has been much discussion as to the reason for the limitations in therapeutic efficacy noted for these classes of compounds. For cholinergic compounds demonstrated to improve the performance of cognitive tasks in animals, the potential effectiveness offered by them (cholinesterase inhibitors and direct cholinergic receptor agonists) in humans can be limited by the appearance of central and peripheral side-effects. The premise that high selectivity and high potency are the most desirable properties for a new therapeutic agent may not be the case for many drugs designed to treat brain disorders. For example, in PD activation of both D1 and D2 striatal dopaminergic neurons may be necessary for maximal drug efficacy in reducing motor symptoms. Also, in the treatment of major psychoses the most useful classes of agents are proving to be those 'atypical' drugs that often exhibit low potency and little selectivity. Similar pharmacological opportunities are available for AD drugs as well. As discussed in the preceding paragraphs, multiple neurotransmitter systems are affected to varying degrees in AD. Both noradrenergic neurons and cholinergic neurons have been shown to play a role in different components of learning and memory in rats. It may require combined therapy with adrenergic agonists such as clonidine and cholinergic agonists such as acetylcholinesterase (AChE) inhibitors to fully reverse the cognitive defects resulting from combined lesions of adrenergic and cholinergic neuronal pathways [18]. One other example of the concept of synergistic actions of different drug classes on memory-related task performance is a report in which the muscarinic M1-preferring receptor agonist, milameline, was shown to augment the ability of the AChE inhibitor tacrine to reverse a scopolamine-induced decrement in efficiency of maintaining a continuous performance task by Rhesus monkeys [19]. More recently it has been reported that the cognitive enhancement produced by cholinergic muscarinic agonists may involve septohippocampal GABAergic and hippocampal glutamatergic neurons [20]. The potential for combining drugs acting on the acetylcholine and glutamate systems is enhanced with the advent of the low affinity NMDA receptor antagonist memantine. This compound may prevent the excitatory amino acid neurotoxicity suggested to accompany AD without interfering with the actions of glutamate required for learning and memory [21]. Recent clinical trials have indicated that memantine may improve cognition and result in the early improvement in behavior in AD [22].

As described throughout this book, many drugs and other natural substances derived from a wide variety of chemical and pharmacological classes have been shown to improve memory-related task performance in animals and humans. The clinical use of AChE inhibitors is likely to continue for some time into the future, and this pharmacological class continues to represent one of the most effective drugs tested in animal models [23]; and the use of AChE inhibitors may benefit quite dramatically from the addition of other pharmacological classes of cognitive-enhancing drugs. Our first indication that combinations of drugs might prove useful as a therapeutic approach to improving memory-related task performance was derived from one of our earlier studies in which we attempted to block the improvement in delayed matching-to-sample (DMTS) task efficiency to nicotine with the antagonist mecamylamine [24]. Generally, responsiveness to nicotine is relegated to one or two doses in a series of less than two log units. The highly individualized response to memory-enhancing drugs led us [25] to suggest that the effectiveness of a drug could mainly be determined by performing a dose-response series, but then selecting the individualized optimum dose or 'Best Dose'. Bartus used this approach to help identify non-responders. We have used the Best Dose as a means of comparison of drug effectiveness (see: [26]). However, the question remains as to the mechanism(s) contributing to the inverted-U-dose-response relationship. For nicotine-induced cognitive enhancement, higher doses may be associated with side-effects that could interfere with task motivation. In our initial study with mecamylamine (which was used to confirm that central nicotinic receptors mediated the positive mnemonic response to nicotine) we used the quaternary nicotinic antagonist hexamethonium to control for the potential peripheral actions (mainly ganglionic blockade) of mecamylamine on subjects performing the delayed matching-to-sample task. When the monkeys were pre-treated with hexamethonium, the nicotine-induced improvement in average task efficiency was further enhanced across all delays (although the effect was not statistically significant). Thus, it may be possible to expand the therapeutic window of certain agents like nicotine by preventing peripheral side-effects with the use of low levels of peripheral nicotinic receptor blockade. Along these lines, it is somewhat perplexing as to why low doses of selective peripherally-acting muscarinic antagonists such as methylatropine or glycopyrrolate have not been used in combination with cholinesterase inhibitors to help limit the latter drugs' side-effects.

Targeting brain AChE and α_2 -adrenergic receptors

In addition to this heuristic approach to combination therapy, we also considered the possibility of pharmacologically exploiting at least two targets, central α_2 -adrenergic receptors (with clonidine) and AChE with physostigmine [27]. Clonidine may target α_2 -adrenergic receptors in the prefrontal cortex to evoke a moderate level of task improvement [28] (and see Chapter by Edward

D. Levin). From a different perspective there may be another rationale for considering that combined therapy may be superior to monotherapy. We have reported that clonidine is a potent inhibitor of the biosynthesis and the release of acetylcholine within specific brain regions (particularly in hypothalamic and hindbrain regions) in the rat and that the drug can inhibit the expression of cholinergic signs of toxicity to physostigmine and other cholinesterase inhibitors (see [29]). However, clonidine is only weakly effective in inhibiting cholinergic function within higher brain regions, presumably containing sites more relevant to the cognitive enhancing actions of AChE inhibitors [30]. We tested the possibility that combined treatment with clonidine and physostigmine could result in enhanced effects on DMTS accuracy by mature adult and aged macaques.

One of the most obvious effects of adding 0.5 µg/kg clonidine to the physostigmine regimen was that the animals were able to tolerate much higher doses of physostigmine. The individualized optimal ("Best") dose of physostigmine was determined for each animal as that dose which provided the greatest improvement in task accuracy averaged over the entire 96-trial session. The Best Doses determined for physostigmine alone ranged from 5–40 µg/kg (mean of 21.4 ± 4.5 µg/kg). Best Doses determined for physostigmine in the presence of 0.5 µg/kg clonidine ranged from 10–60 µg/kg (mean of 40.0 ± 6.9 µg/kg) – almost a two-fold increase. Despite the fact that doses used for physostigmine were maximal for each animal, when the two drugs were combined, a further improvement in performance was obtained. In fact, for the combination regimen, on the day after administration, performance accuracy continued to be elevated relative to baseline. Therefore, in this example the following factors appear to contribute to the enhancement of task performance in the combination: 1) a widening of the therapeutic window, most likely reflecting a reduction in AChE inhibitor-associated side-effects; 2) the targeting of separate neural substrates that each play a role in cognitive function; 3) the addition of clonidine extended the regimen's overall duration of action possibly through a unique pharmacodynamic action. In this particular study we used a fixed dose of clonidine previously determined to be optimal when used alone. It is possible that additional improvement might be obtained with further optimization of the regimen.

Targeting both brain AChE and nicotinic receptors

MHP-133 (3-(N,N-dimethylcarbamoyl)hydroxy-1-methyl-2-[[N-phenyl-amino-carbonyl]hydrazono]methyl]pyridinium chloride) is the lead compound of a novel series of analogs designed to target multiple brain substrates expected to have synergistic actions in the treatment of human cognitive disorders such as AD [31, 32]. MHP-133 was designed to target components of acetylcholine neurons that would act synergistically to enhance cholinergic function, including the stimulation of cholinergic receptors and the inhibition of AChE.

The strategy was to develop compounds with multiple targets relevant for enhancing cognition and memory, but avoiding the serious side-effects attributed to high potency cholinergic agonists. A preliminary assessment of the neurochemical properties exhibited by MHP-133 suggested that the drug may indeed fit this profile. For example, MHP-133 was shown in ligand binding studies to interact with subtypes of cholinergic (nicotinic and muscarinic, M1 and M2) receptors and to serve as a weak AChE inhibitor. Most importantly, however, MHP-133 was shown to enhance the performance of young and aged animals trained to perform memory-related tasks. Our study group of young and aged macaques was well-trained in the performance of an automated DMTS task. The monkeys were represented by individuals from three species, both genders, and two age groups, mature and aged. As indicated in Figure 1, MHP-133 significantly enhanced performance of all groups in the computer-assisted DMTS over a relatively wide range of doses. For the animals' individualized Best Dose (30–100 µg/kg, i.m.), performance of trials associated with long delay intervals increased on average by 32.2% over baseline performance for the entire group. Preliminary studies in a group of five aged rhesus monkeys demonstrated that MHP-133 was also effective at improving task performance after oral administration (data not shown).

MHP-133 has the potential to produce clinical improvement of the cognitive symptoms associated with AD and related disorders, and to inhibit the progress of the disease. The drug may offer multiple synergistic mechanisms

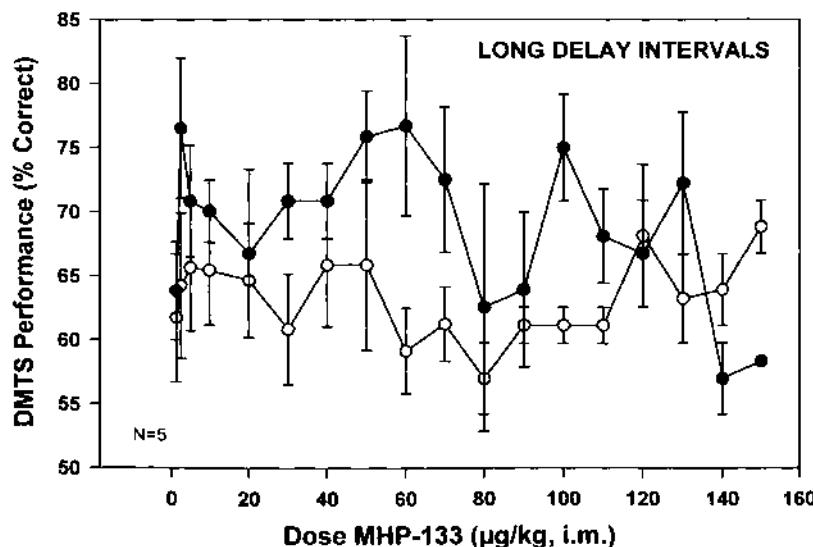


Figure 1. Effect of MHP-133 (filled circles) on DMTS performance efficiency by adult monkeys as a function of dose. Data are presented for trials associated with long delay intervals. Note the wide dose window for task improvement provided by the drug. Open circles = vehicle baseline values.

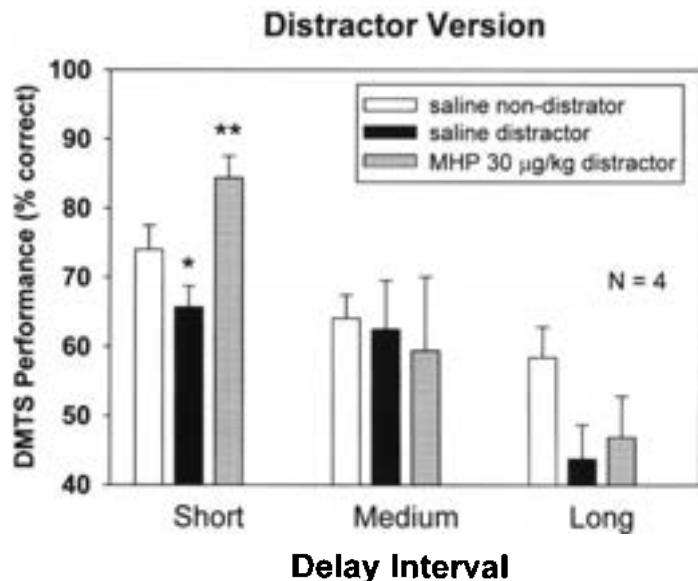


Figure 2. Mature (non-aged) Rhesus monkeys performed a modified DMTS task that included a randomly presented (20% of trials) distractor (flashing panel lights). Distractor trials were performed with significantly reduced efficiencies for short delay intervals. MHP-133 completely reversed the effect of the distractor, and further improved task performance above control (non-distractor levels).

of actions in this regard. This synergy of therapeutic actions may underlie both the marked effectiveness of the drug on memory, as well as the lowered potential for producing side-effects. As with nicotine ([33] and see Chapter by Andrea Wevers and Diana S. Woodruff-Pak), MHP-133 reversed distractor-induced (interference trials) performance decrements suggesting that part of its positive mnemonic action included improved levels of attention (Fig. 2). Also, like nicotine [34, 35], the drug enhanced nerve growth factor (NGF) – TrkA receptor expression in a neuronal cell line (Fig. 3A) indicating the potential for neuro-protective effects (Fig. 3B). MHP-133 and JWB-I-68-13 (a derivative of MHP-133) were also examined for their ability to alter the levels of amyloid precursor protein (APP) secreted by cultured astrocytes into the media during a 1 hr treatment with the cells. APP was measured by using a monoclonal antibody directed against the N-terminus of APP. Both MHP-133 and JWB-I-68-13 significantly increased the levels of secreted APP from cultured astrocytes over the concentration range of 10–100 µM. In general, the levels of secreted APP with MHP-133 or JWB-I-68-13 treatment were about 1.2–1.6-fold compared to the level of untreated control cells. This increase in APP secretion caused by MHP-133 and JWB-I-68-13 might be expected to decrease the cellular levels of amyloidogenic holoprotein. This possibility is in keeping with preliminary results showing that both compounds were associated with small

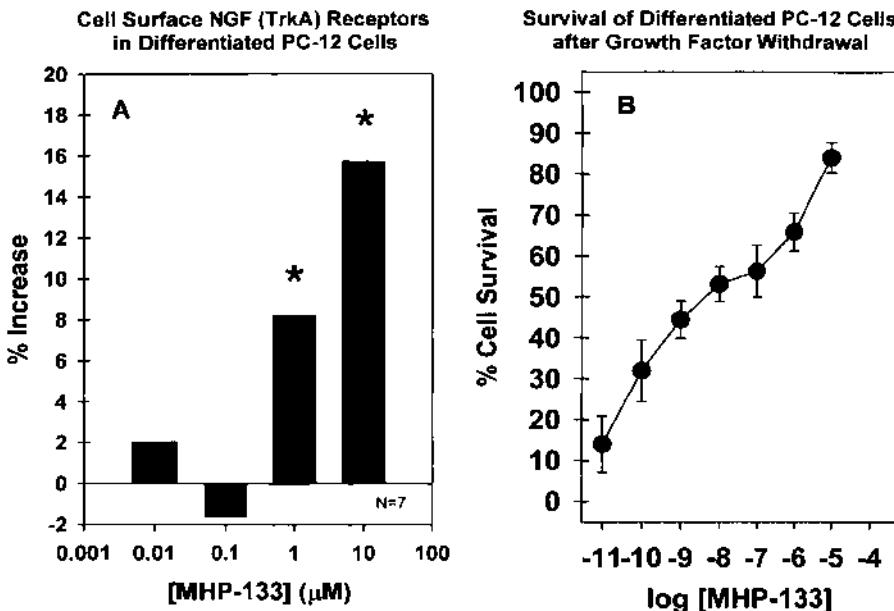


Figure 3. (A) cell-based ELISA was used to measure TrkA receptors on differentiated PC12 cells. Incubation of these cells for 24 hr with MHP-133 produced a significant concentration-dependent increase in TrkA receptor expression. * $p < 0.05$ compared to untreated control cells. (B) The ability of MHP-133 to protect differentiated PC12 cells from the cytotoxicity induced by (24 hr) NGF and serum (trophic factor) deprivation. Various concentrations of MHP-133 were incubated for 24 hr prior to trophic factor withdrawal. MHP-133 pretreatment produced a significant increase in cell viability compared with untreated control values (not shown), $p < 0.001$.

decreases in cell-associated APP holoprotein in cultured astrocytes and neurons (data not shown).

Despite the promise of MHP-133 and its analogs as potential therapeutic entities for AD and related disorders, these compounds are not yet optimized regarding the important pharmacological actions described above. Also, the concentrations required for neuroprotection and altered amyloid metabolism represent *in vivo* dose-ranges greater than that used for memory enhancement. Part of the challenge to developing bi- or multi-functional molecules is the ability to address the various targets with equivalent efficacies.

Targeting brain AChE and M2 muscarinic receptors

JWS-USC-75IX is a relatively potent AChE inhibitor (much more so than MHP-133), but it also exhibits high affinity antagonism for the muscarinic M2 muscarinic cholinergic receptor [36]. As AChE inhibitors have the potential of limiting their own actions through acetylcholine-induced feedback inhibition

(mediated *via* activation of presynaptic M₂ receptors), it was reasoned that M₂ receptor antagonism could result both in the enhanced release of acetylcholine, and mitigation of the AChE inhibitor-induced feedback inhibition. JWS-USC-75IX was shown to improve the performance of rats in three different memory-related tasks, and in one of these, a delayed discrimination task, the drug was shown to exhibit repeatable improvements without the development of tolerance. The task was developed so that we could employ an operant paradigm (not unlike the primate DMTS) in rats. JWS-USC-75IX also exhibited a marked safety profile relative to drugs acting only to inhibit AChE [36].

At this point it is appropriate to point out that all efforts to combine multiple actions in one molecule have not met with success. An example is the compound RS66331 which (neurochemically) exhibits the properties of a 5HT₄ agonist and a 5HT₃ antagonist. Both properties have been associated with enhanced release of brain acetylcholine [37]. We studied this compound in aged rhesus monkeys and compared its effectiveness with that produced by individual administration of a 5HT₄ agonist and a 5HT₃ antagonist, both of which were demonstrated previously to enhance task performance in the same subjects. Rather than this combination of properties imbuing RS66331 with augmented memory-enhancing action, the effectiveness of the drug proved to be similar to that produced by the 5HT₃ antagonist RS56812, but it was considerably reduced in effectiveness compared with the 5HT₄ agonist RS17017 [23]. However, RS66331 was developed prior to our work with the individual compounds. There are many reasons for the failure of compounds to achieve expectations in memory paradigms, however, this may be one case wherein the information derived from the combined administration of various dose-regimens of RS56812 and RS17017 may have alerted us to the possibility that this is not a useful neural target combination, or to the possibility that different proportions of relative receptor activity were needed as compared to that inherent in RS66331.

Targeting brain monoamine oxidase (MAO) and cholinesterase

Relatively little attention has been paid to development of neuroprotective drugs for the treatment of AD. In some respects there is a better understanding of nigro-striatal dopaminergic neurodegeneration and neuroprotection mechanisms in PD because of the availability of relatively appropriate models. The mechanisms that may be involved in the process of neurodegeneration, particularly in AD, include oxidative stress, inflammatory processes, and accumulation of iron at the site of neurodegeneration. As such, antioxidants, MAO-B inhibitors, non-steroidal anti-inflammatory drugs and iron chelators have been suggested to exert neuroprotective actions, and they have been incompletely examined in AD. The MAO-B inhibitors selegiline and rasagiline [38] are anti-Parkinson's (anti-PD) drugs that have warranted more scrutiny as a conse-

quence of their neuroprotective activity *in vitro* (neuronal cell cultures) and in animal studies. The established co-morbidities of (1) AD with extrapyramidal features; (2) PD with dementia; and (3) both AD and PD with depression stimulated the development of a series of novel bi-functional drugs possessing the MAO inhibitory activity and neuroprotective activity exhibited by anti-Parkinson drugs selegiline or rasagiline [39–41] along with the ability to inhibit AChE [42]. For these studies, the structural requirements for anti-PD activity as exhibited by rasagiline and selegiline, and the structural requirements for cholinesterase inhibition exhibited by rivastigmine, was initially considered.

Introduction of a carbamate moiety into the rasagiline molecule (Fig. 4) resulted in almost complete loss of brain MAO-B inhibitory activity for TV3326 and TV3279 as determined *in vitro*. However, chronic oral administration (12.5–76 mg/kg) in rats, mice and rabbits showed that TV3326, but not TV3279, is a CNS-selective inhibitor of MAO-A and MAO-B, with little inhibition of liver and small intestine MAO [43–45]. The possibility that TV3326 was serving as a prodrug in the brain to generate an active MAO inhibitory metabolite(s) is supported by the results of animal studies where, in contrast to the first order kinetic recovery of MAO activity from inhibition by rasagiline, TV3326 exhibited a biphasic action [45]. Also, an active metabolite of TV3294 has been identified. Both TV3326 and TV3279 inhibit AChE and butyrylcholinesterase, but with a slower time course and with slightly reduced effectiveness compared with rivastigmine. TV3326 is 100 times more potent against butyrylcholinesterase than it is against AChE. The cholinesterase inhibitory activity of these drugs is consistent with their dose-related

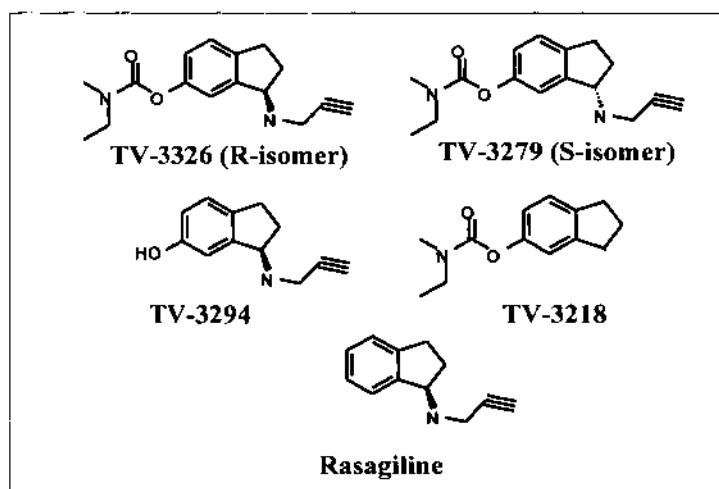


Figure 4. Structures of cholinesterase-monoamine oxidase inhibitors. TV3326 and TV3279 derived from rasagiline and rivastigmine. TV3294 and TV3218 are metabolites of TV3326 which respectively monoamine oxidase and cholinesterase inhibitors.

(12–26 mg/kg) antagonism of the spatial memory deficits induced by scopolamine in rats, indicating that they were able to increase brain acetylcholine levels sufficiently to compete with scopolamine for muscarinic receptors subserving memory [44].

Neuroprotective –antiapoptotic action of TV3326

The primary rationale for developing this class of bi-functional drugs was to combine the dopaminergic (anti-PD) and cholinergic (anti-AD) activities associated respectively with rasagiline and rivastigmine, with the neuroprotective effectiveness of rasagiline in a single molecule [46]. TV3326 exhibits both MAO inhibitory and anticholinesterase activities, although the drug's optical S-isomer TV3279 possesses only anticholinesterase activity. The profile of the neuroprotective activity of TV3326 as compared with rasagiline (Tab. 1) indicates that, to a large extent, TV3326 mimics the established neuroprotective

Table 1. Neuroprotective properties of rasagiline, TVP1022, TV3326 and TV3279

Increases SOD, catalase and BCL-2 activities by transcriptional and translational mechanisms in PC12 cells and rats (brain, heart and kidney)	[50, 51]
Prevents peroxynitrite-induced activation of caspase 3	[69]
Prevents peroxynitrite induced DNA laddering	[69, 58]
Prevents glutamate and NMDA induced neurotoxicity in hippocampal and cortical cell cultures [rasagiline only]	[67]
Prevent peroxynitrite and NM-(R)-sal induced fall in mitochondrial membrane potential and apoptosis	[51, 54, 69]
Protect against peroxynitrite (SIN-1) and 6-hydroxydopamine induced apoptosis	[53, 54]
Protect against cell death induced by ischemia and by glucose deprivation in PC12 cells	[67, 41]
Increased survival of dopaminergic neurons [rasagiline only]	[67]
Neuroprotection in closed head injury in mice	[41, 44, 47]
Prevents MPTP (mice) and 6-OHDA (PC12 cells and rats) neurotoxicity	[45]
Neuroprotection in models of motor and cognition disorders [rasagiline only]	[48]
APP preprocessing <i>in vitro</i> and <i>in vivo</i>	[65, 70]
Neuroprotection against myelinated fiber damage and microgliosis induced in rat brain by <i>icv</i> STZ [TV3326]	[41]
Prevent serum and NGF induced apoptosis in PC-12 cells	[51]
Neuro-rescue after MPTP treatment in mice (rasagiline)	[45]
PKC activation and dependent amyloid precursor protein processing in PC-12 cells and <i>in vivo</i> (mice and rats)	[65, 70]

profile of rasagiline. Both rasagiline and TVP1022 prevent neuronal damage caused by closed head injury in mice [47], focal ischemia [48], and cytotoxicity in cultured PC12 cells induced by growth factor withdrawal [39] or glucose-oxygen deprivation [49]. They also prevent apoptosis induced by the neurotoxins N-methyl-R-salsolinol, 6-hydroxydopamine, the peroxynitrite donor, SIN-1, and by aggregated A β amyloid peptide (A β) in SHSY-5Y neuroblastoma cells. Rasagiline and TVP1022 (0.10 nM–1 mM) prevent the loss of intact nuclei normally observed in partially-differentiated PC12 cells after serum and NGF withdrawal. This cytoprotective activity is related to their anti-apoptotic action, since the drugs significantly diminished the percentage of cell nuclei with chromatin condensation (an index of apoptosis) over the same concentration range. The anti-apoptotic action of these compounds is dependent on the synthesis of new Bcl-2 and SOD proteins, and the response is prevented by transcriptional (actinomycin) and translational (cycloheximide) inhibitors as measured in partially neuronally-differentiated PC12 cells. A similar effect is obtained with the racemic form of TV3326, TV3219. *In vivo*, chronic treatment rasagiline increase SOD and catalyse activities in the striatum hippocampus and cortex of rats [50]. Thus it can be inferred that TV3326 would also behave in a similar manner to rasagiline and TVP1022 [39, 51]. The mechanism of the anti-apoptotic effect and the identity of the proteins synthesized have not been fully determined, but they have been shown to be related to the ability of both drugs to prevent the decrease in Bcl-2 and Cu-Zn-SOD1 in response to growth factor withdrawal. In SHSY-5Y cells, rasagiline induces the expression of anti-apoptotic proteins as well as the mRNAs coding for Bcl-2 and Bcl-XI, while simultaneously decreasing the pro-apoptotic Bax and Bad proteins [52]. In fact, significant evidence exists to suggest that the anti-apoptotic activity exhibited by rasagiline and its derivatives is associated with their ability (1) to prevent the collapse of the mitochondrial membrane potential by opening mitochondrial permeability transition pores that are part of the voltage-dependent anion channel, (Fig. 5); (2) to inhibit cytochrome c; and (3) to activate the caspase cascade that involves caspase 3 [52–55] (Fig. 6). The MAO-inhibitory action of rasagiline is not a prerequisite for its neuroprotective action since its optical isomer TVP1022 [40], which exhibits poor MAO inhibitory activity, is an equipotent neuroprotective agent [56, 57]. The neuroprotective activity of rasagiline, TVP1022 and selegiline resides in the propargylamine moiety, since propargylamine itself exerts similar neuroprotective/anti-apoptotic activities [40, 53–55, 58]. TV3326 and TV3279 retain the neuroprotective properties of rasagiline and TVP1022 (Tab. 1).

Antidepressant and anti-Parkinson's activities of TV3326 and TV3279

TV3326 (but not TV3279) as a brain selective inhibitor of MAO-A and B induces significant increases in striatal, hippocampal, brainstem and hypothalamus dopamine, serotonin and noradrenaline in rats and mice [45], a neuro-

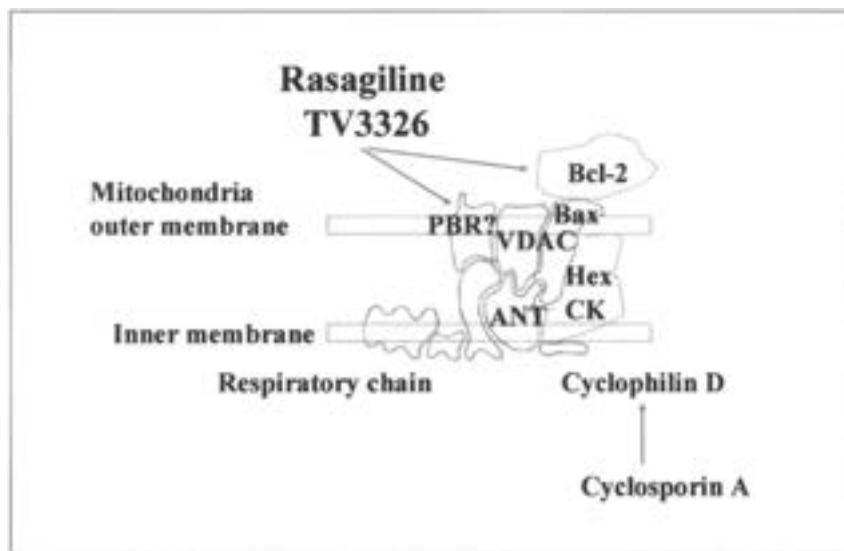


Figure 5. Possible site of action of rasagiline and its derivatives at the mitochondrial voltage dependent-anion channel (VDAC), which is part of MPT. The exact protein constituents of MPT is not known but several of the proteins, such as anti and proapoptotic proteins Bcl-2 and Bax respectively; porin; PBR, peripheral benzodiazepine receptor; ANT, adenosine nucleotide translocator; HEX, hexokinase and CK, creatine kinase have been identified. In a number of respects mechanism of neuroprotective action of rasagiline and its interaction with MTP resembles that of cyclosporin A, a drug with neuroprotective activity [52].

chemical profile is suggestive of potential antidepressant activity (Tab. 2). Classical antidepressant drugs such as amitriptyline and moclobemide (selective reversible MAO-A inhibitor) reduce the duration of immobility behavior in the forced swim test in rats for potential antidepressant activity [59, 60]. Administration of TV-3326 (26 mg/kg/day for two weeks, or 52 mg/kg for one week), inhibited brain MAO-A and B by more than 65%, and the drug significantly reduced the immobility duration to the same extent obtained after chronic treatment with amitriptyline (10 mg/kg/day) or moclobemide (20 mg/kg/day) [61].

The MPTP (N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) neurotoxin model of PD is used routinely for the screening of potential anti-Parkinson's drugs. MPTP is inert but it is converted by MAO-B within microglia to the active neurotoxin MPP⁺, which is then transported into striatal dopamine neurons where it induces neurotoxicity and depletion of dopamine. MAO inhibitors prevent MPTP neurotoxicity in monkeys and mice. As a non-selective MAO inhibitor TV3326 (but not TV3279) prevents the MPTP-induced degeneration of nigro-striatal dopaminergic neurons and the depletion of dopamine in mice [45]. In this regard the effects of TV3326 are similar to other MAO-B inhibitors.

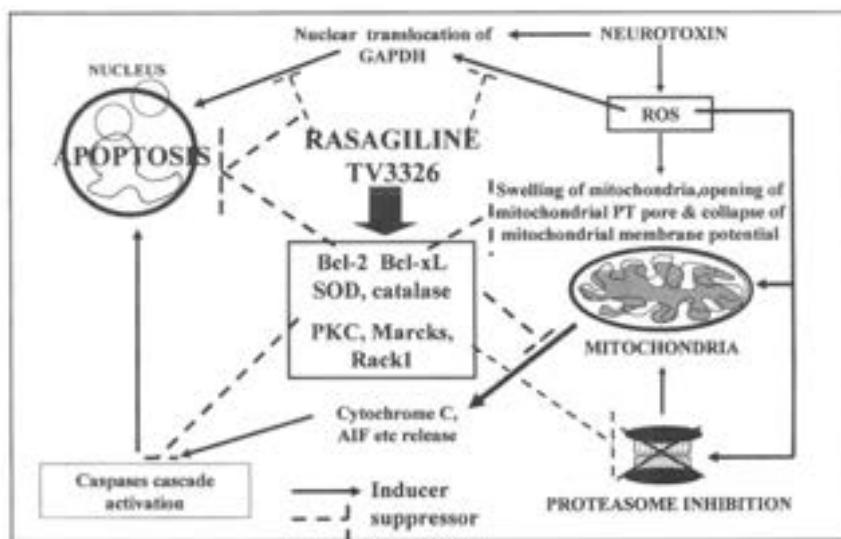


Figure 6. Mechanism of neuroprotective-antiapoptotic action of rasagiline and its anti-Alzheimer derivative cholinesterase-monoamine oxidase inhibitor, TV3326. Both drugs are N-propargyl-(1R)-aminoindan derivatives with TV3326 possessing a carbamate cholinesterase inhibitor moiety. It is the propargyl moiety in these drugs that confers the neuroprotective-antiapoptotic, Bcl-2 inducing activities and PKC activating properties. Rasagiline inhibits neurotoxin (SIN-1, NM-R-Sal) initiated apoptosis in SH-SY5Y and PC-12 cells by preventing the collapse of mitochondrial membrane potential, opening of the MPT, release of ubiquitin-proteasome dependent cytochrome C and caspase 3 activation resulting in its antiapoptotic activity. It also prevents the translocation of pro-apoptotic GAPDH in these cells. Its neuroprotective activity may also depend on its activation of SOD and catalase as has been observed *in vivo* in various tissues including brain and heart [52].

One major side-effect and limitation associated with the use of irreversible MAO-A inhibitors as antidepressant drugs is their ability to potentiate the sympathomimetic action of tyramine present in certain types of food. TV3326 exhibits limited tyramine potentiation and in this regard the drug appears to be equivalent (or superior) to other reversible MAO-A inhibitor antidepressant drugs [62]. This unique property of TV3326 is attributed to the fact that the drug appears to act selectively within the CNS, and as such it has limited effects on MAO-A and B activities in the periphery.

Cognitive enhancing property of TV3326

Recently we administered TV3326 to 7 year old Rhesus monkeys well trained to perform versions of a DMTS task [63]. An increasing dose regimen of TV3326 was administered orally according to a schedule that allowed the animals to perform the standard DMTS task and a self-titrating version of the DMTS task each week during the study. A distractor version of the task was

Table 2. Pharmacological properties of TV3326 and comparison with other cholinesterase and monoamine oxidase inhibitors

	TV3326	Riva-stigmine	Rasagiline	Clorgyline	Tranylcypromine
AChE inhibition	+	+	-	-	-
BuChE inhibition	+	+	-	-	-
MAO-A inhibition	+	-	-	-	-
MAO B inhibition	+	-	+	-	-
Increase brain acetylcholine	+	-	-	-	-
Increase brain dopamine	+	-	+	-	+
Increase brain norepinephrine	+	-	-	+	+
Increase brain serotonin	+	-	-	+	+
Tyramine potentiation	-	-	-	+	+
Antidepressant action	+	-	-	+	+
Hypothermic action	-	-	NC	NC	NC
Anti-Parkinson activity	+	-	+	-	+
Neuroprotection	+	-	+	-	-

NC, no change

administered during two of the doses of TV3326. Under the conditions of this experiment TV3326 failed to significantly affect accuracy on the standard DMTS task, however, the drug was very effective in improving the ability of subjects to titrate to longer duration delay intervals in the titrating version of the task. This version of the task is more sensitive to age-dependent cognitive impairment than is the standard DMTS task [64]. The maximal drug-induced extension of the self-titrated delay interval amounted to a 36.7% increase above baseline. TV3326 also significantly improved task accuracy during distractor (interference) sessions, a measure of attention deficit. The compound was effective enough to return group performance efficiency to standard DMTS vehicle levels of accuracy. Thus, TV3326 represents a new class of drug which is potentially suitable for the treatment of AD patients who require therapies that will delay the progression of the disease, and who suffer from impaired attention, impaired memory, extrapyramidal disorder and depression. The combination of the properties attributed to an MAO inhibitor and to a cholinesterase inhibitor may derive benefit from their combined cognitive enhancing properties, as well as from the ability of adrenergic/dopaminergic receptor activations to limit the side-effects of cholinesterase inhibition as discussed above for clonidine.

Amyloid precursor protein (APP) processing by TV3326

One of the debated current concepts regarding the neurotoxicity associated with AD is the processing of amyloid precursor protein (APP) by the three sec-

retases, α , β and γ , and the formation of aggregated A β . The potential reduction of A β through the administration β and γ secretase inhibitors is one approach being addressed. However, certain cholinesterase inhibitors have been shown to induce the release of neuroprotective-neurotrophic soluble amyloid precursor protein alpha (sAPP α) by selectively enhancing the action of the zinc-metaloprotease, α -secretase. TV3326, TV3279, rasagiline and TVP1022 induce the release of sAPP α in PC-12 and SHSY-5Y cells through activation of α -secretase. The mechanism of sAPP release has been shown to be directly linked to the propargyl moiety group on these drugs, since propargylamine itself is as effective as TV3326, rasagiline and their S-isomer derivatives. Employing several signal transduction pathway inhibitors, it has been established that this process is mediated via the PKC-MAPK dependent pathway, as a consequence of activation of PKC α and PKC ϵ and through ERK1/ERK2 phosphorylation [65] (Fig. 7). *In vivo*, chronic oral treatment with TV3326, TV3279 and rasagiline significantly reduced APP holoprotein in the hippocampus of rats and mice. The ability of these compounds to reduce A β in CHO cells and amyloid deposits in the CNS of transgenic mice that over-express this protein is being investigated.

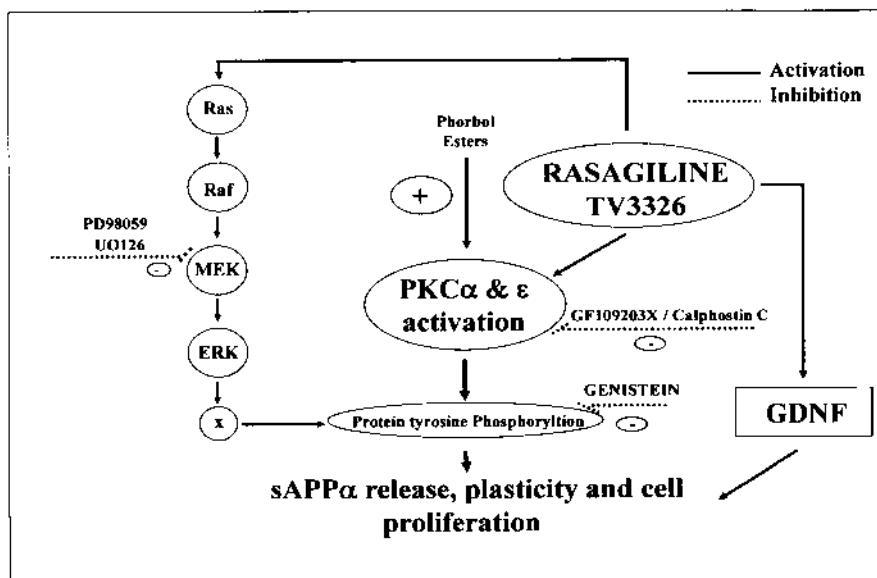


Figure 7. Signal transduction pathways mediating the activation of PKC dependent neuroprotection and plasticity by rasagiline and TV3326. Both drugs activate PKC and MAPK pathways in a time and concentration (0.1–10 μ M) dependent manner in PC-12 and SH-SY5Y cells in culture, resulting in activation of α -secretase dependent release of sAPP α . Phorbol esters have similar action and inhibitors of PKC and MAPK pathways, as indicated, prevent rasagiline and TV3326 induced release of sAPP α [Yogev-Falach et al. 2002]. *In vivo* both drugs activate mice and rat hippocampal PKC α and ϵ and promote their translocation from cytoplasm to the mitochondrial membrane [65].

The future

The potential for multi-functional drugs for the treatment of complex neurodegenerative diseases and perhaps even for the treatment of age-associated memory impairment has already been partially realized. The AChE inhibitor galantamine is in widespread use for the treatment of the symptoms of AD. Galantamine may offer additional potential for disease modification (neuroprotection) relative to its predecessors by virtue of its ability to allosterically activate nicotinic receptors [66], but has not been shown to possess the neuroprotective and APP processing properties of TV3326. Although, there continues to be debate as to the extent to which nicotinic receptor activation plays a role in the drug's profile of therapeutic benefit, the concept supports the continued development of bi-functional (AChE/nicotinic) drugs like MHP-133. There is no question that the development of multi-functional drugs presents additional problems for rationale drug design methodologies. Yet from our studies over the past decade in which we examined a wide variety of classes of pharmacological agents in basically the same non-human primate model it seems probable that drugs that target single functional components of cognition and memory will be limited in efficacy [26]. Moreover, it is likely that syndromes such as AD will require multiple drug therapy to address the varied pathological aspects of the disease. Even if the strategy of combining drugs with different therapeutic targets is workable, the development of multi-functional compounds will obviate the challenge of administering multiple single drug entities with potentially different degrees of bioavailability, pharmacokinetics, and metabolism. Also, the simplification of the therapeutic regimen for individuals with AD who have difficulty with compliance is important.

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