Multiple Central Nervous System Targets for Eliciting Beneficial Effects on Memory and Cognition

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ABSTRACT
The development of drugs for the treatment of disorders of cognition has benefited from a more precise knowledge of the loss of specific neural pathways associated with certain neurodegenerative diseases such as Alzheimer’s disease (AD). The loss of basal forebrain cholinergic neurons in AD has engendered the development of new compounds that target various aspects of the cholinergic system. However, limitations in the effectiveness of the most common of these, the anticholinesterases, have fueled the race to provide more efficacious compounds. In an attempt to avoid side effects and improve efficacy, other neuronal targets have been considered, including receptors for norepinephrine, dopamine, serotonin, excitatory amino acids, neural peptides, and others. Our laboratory has had the opportunity to study the memory-enhancing potential of many of the compounds developed expressly for these neuronal targets in macaques. Upon reviewing 21 such studies it was evident that: 1) To varying degrees, pharmacological manipulation of each target yielded improved task performance. 2) Combining pharmacological targets could lead to additive or synergistic effects on task performance. 3) Mature adult and aged monkeys provided equivalent estimates of drug effectiveness. 4) There appeared to be no limiting level of task improvement for compounds tested in aged and younger monkeys. 5) Certain of these agents also exhibited potential disease-modifying actions. Thus, certain memory-enhancing agents may prove more useful when implemented early in the course of a disease such as AD, and they also may enjoy a wide application for the treatment of the memory decline associated with normal aging.

Disorders of Cognition and Memory
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 those over 65 years of age, is now the third most expensive health care problem in the US 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 21st 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 also has 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, nondisease aging (Green, 1995). A measurable (albeit mild) decline in cognitive function can occur as a part of healthy aging in humans that begins at some point after the 5th decade of life. The changes observed in typical (nonpathological) aging are manifested primarily as mild deficits in working memory that are thought to result as a consequence of a reduction in the speed of central processing necessary for encoding and retrieval of information (Morris et al., 1991). Mild memory deficits that exceed those associated with normal aging, but that do not meet the (Diagnostic and Statistical Manual 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 (Green, 1995), a finding that has generated considerable concern and provided the impetus for rigorous investigation.

A wide variety of animal models and behavioral techniques has been applied to the study of drugs that affect memory. However, in recent years, efforts to mimic the symptom profile exhibited by AD patients in animals have been of paramount interest in this regard. Although there is no comparable animal model for the human syndrome, animals of advanced age, usually rodents and nonhuman primates, have provided a good level of predictability for the clinical efficacy of proposed therapeutics. In fact, many drug discovery programs continue to use rodents in general screening procedures for identifying potential cognitive-enhancing agents, electing to continue testing potential lead compounds in nonhuman primates. Our experience has been that evaluation of such compounds across different pharmacological classes and almost unique opportunity to compare the effectiveness of similar conditions in young and aged macaques, providing an almost unique opportunity to compare the effectiveness of such compounds across different pharmacological classes and subject age.

### Components of Cholinergic Neuronal Systems as Therapeutic Targets

Among the host of degenerative processes occurring in AD, reproducible cholinergic deficits are consistently reported, appear early in the disease process, and correlate well with the degree of dementia (for review, see Francis et al., 1999). These findings have contributed to the “cholinergic hypothesis” of AD. In addition, cholinergic neurons appear to be involved in β-amyloid precursor protein processing and consequently, abnormalities in these neurons may lead to β-amyloid deposition and formation of toxic neuritic plaques. Moreover, abnormalities in cholinergic function are frequently reported in other degenerative conditions such as...
Comparison of the effectiveness of potential cognitive-enhancing agents in macaques trained to perform a delayed matching-to-sample task

All data are presented as the calculated “Best Dose”, which was determined from dose-effect relationships of at least four doses. Each Best Dose was selected as that which improved average (of all four delay intervals) task performance to the greatest degree. All compounds were administered by i.m. injection between 10 and 60 min (as consistent with pharmacokinetic data) before initiating testing.

### TABLE 2

Comparison of the effectiveness of potential cognitive-enhancing agents in macaques trained to perform a delayed matching-to-sample task

<table>
<thead>
<tr>
<th>Drug</th>
<th>Class</th>
<th>Age</th>
<th>Species</th>
<th>Delay Interval</th>
<th>n</th>
<th>Increase in % Trials Correct</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physostigmine</td>
<td>ChE-inhibitor</td>
<td>Y</td>
<td>N</td>
<td>Long</td>
<td>6</td>
<td>9.5 ± 4.1</td>
<td>Terry et al., 1993</td>
</tr>
<tr>
<td>Velmecamine</td>
<td>ChE-inhibitor</td>
<td>A</td>
<td>R</td>
<td>Long</td>
<td>6</td>
<td>7.9 ± 2.2</td>
<td>Jackson et al., 1995</td>
</tr>
<tr>
<td>Tacrine</td>
<td>ChE-inhibitor</td>
<td>Y</td>
<td>N</td>
<td>Long</td>
<td>5</td>
<td>13.7 ± 3.0</td>
<td>Unpublished</td>
</tr>
<tr>
<td>Donepezil</td>
<td>ChE-inhibitor</td>
<td>A</td>
<td>R</td>
<td>Long</td>
<td>5</td>
<td>17.5 ± 4.3</td>
<td>Unpublished</td>
</tr>
<tr>
<td>Nicotine</td>
<td>nAChR agonist</td>
<td>Y</td>
<td>N</td>
<td>Long</td>
<td>10</td>
<td>15.1 ± 3.9</td>
<td>Buccafusco et al., 1995b</td>
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<tr>
<td>Nicotine</td>
<td>nAChR agonist</td>
<td>A</td>
<td>R</td>
<td>Short</td>
<td>13</td>
<td>10.6 ± 2.3</td>
<td>Buccafusco et al., 1999</td>
</tr>
<tr>
<td>ABT-418</td>
<td>nAChR agonist</td>
<td>Y</td>
<td>N</td>
<td>Long</td>
<td>5</td>
<td>17.3 ± 5.5</td>
<td>Buccafusco et al., 1995a</td>
</tr>
<tr>
<td>ABT-418</td>
<td>nAChR agonist</td>
<td>A</td>
<td>R, F</td>
<td>Long</td>
<td>6</td>
<td>15.7 ± 2.0</td>
<td>Prendergast et al., 1997</td>
</tr>
<tr>
<td>ABT-089</td>
<td>nAChR agonist</td>
<td>Y</td>
<td>N</td>
<td>Medium</td>
<td>6</td>
<td>8.9 ± 3.6</td>
<td>Decker et al., 1997</td>
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<tr>
<td>ABT-089</td>
<td>nAChR agonist</td>
<td>A</td>
<td>R, F</td>
<td>Long</td>
<td>6</td>
<td>12.7 ± 1.7</td>
<td>Decker et al., 1997</td>
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<tr>
<td>Isoarecolone</td>
<td>nAChR agonist</td>
<td>Y</td>
<td>N, F</td>
<td>Medium</td>
<td>5</td>
<td>11.0 ± 3.6</td>
<td>Buccafusco et al., 1995a</td>
</tr>
<tr>
<td>GTS-21</td>
<td>nAChR agonist</td>
<td>Y</td>
<td>N</td>
<td>Long</td>
<td>6</td>
<td>9.1 ± 5.5</td>
<td>Briggs et al., 1997</td>
</tr>
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<td>4OH-GTS-21</td>
<td>nAChR agonist</td>
<td>Y</td>
<td>N</td>
<td>Average</td>
<td>5</td>
<td>6.6 ± 1.3</td>
<td>Unpublished</td>
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<td>SIB-1553A</td>
<td>nAChR agonist</td>
<td>A</td>
<td>R</td>
<td>Short</td>
<td>5</td>
<td>18.5 ± 4.3</td>
<td>Unpublished</td>
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<td>Mecamylamine</td>
<td>nAChR antagonist</td>
<td>A</td>
<td>R</td>
<td>Short</td>
<td>6</td>
<td>14.4 ± 4.4</td>
<td>Terry et al., 1999a</td>
</tr>
<tr>
<td>WAY132983</td>
<td>Muscarinic (M1) agonist</td>
<td>A</td>
<td>R</td>
<td>Short</td>
<td>6</td>
<td>17.2 ± 4.7</td>
<td>Bartolomeo et al., 2000</td>
</tr>
<tr>
<td>Clonidine</td>
<td>Adrenergic (α2) agonist</td>
<td>Y</td>
<td>N</td>
<td>Medium</td>
<td>6</td>
<td>7.0 ± 1.9</td>
<td>Terry et al., 1993</td>
</tr>
<tr>
<td>RS56812</td>
<td>5HT1 antagonist</td>
<td>Y</td>
<td>N, F</td>
<td>Medium</td>
<td>5</td>
<td>9.2 ± 4.1</td>
<td>Terry et al., 1996</td>
</tr>
<tr>
<td>RS17017</td>
<td>5HT1 antagonist</td>
<td>Y</td>
<td>N, R</td>
<td>Long</td>
<td>6</td>
<td>17.3 ± 3.3</td>
<td>Terry et al., 1998</td>
</tr>
<tr>
<td>RS63331</td>
<td>5HT2 agonist/5HT3 antagonist</td>
<td>Y</td>
<td>N, F</td>
<td>Long</td>
<td>6</td>
<td>11.7 ± 11.2</td>
<td>Unpublished</td>
</tr>
<tr>
<td>RS62522</td>
<td>Angiotensin (AT1) agonist</td>
<td>Y</td>
<td>N, R</td>
<td>Long</td>
<td>6</td>
<td>13.2 ± 1.3</td>
<td>Unpublished</td>
</tr>
<tr>
<td>RS62522</td>
<td>Angiotensin (AT2) antagonist</td>
<td>A</td>
<td>R</td>
<td>Long</td>
<td>4</td>
<td>15.5 ± 2.8</td>
<td>Unpublished</td>
</tr>
</tbody>
</table>

A, aged (>19 years old); Y, young/mature adult (7–16 years old); N, Macaca nemestrina (pigtail macaque); F, Macaca fascicularis (long-tailed/eylonomolagus macaque); R, Macaca mulatta (cheus macaque); ChE, cholinesterase.

1 Delay intervals refer to the time interval between termination of the sample presentation and initiation of the presentation of the choice stimuli. Delay intervals were normalized for each subject to approximate the following levels of performance under baseline (vehicle) conditions: zero delay (85–99%), short delay (75–84%), medium delay (65–74%), long delay (55–64%) where % refers to % of trials answered correctly over the entire (24 trials/day × 4 delay) session. “Average” indicates that task performance was not delay-selective and that the data presented represent the average across all four delay intervals.

2 Each value (absolute increase from baseline levels of performance in units of % trials answered correctly) represents the mean ± S.E.M. for the indicated number of subjects who served as their own controls.

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4 Low (μg/kg) doses—note: higher doses are amnestic.

**Parkinson’s disease:** Diffuse Lewy body dementia, and Huntington’s disease. As in AD, such cholinergic deficits often correlate with memory decline and dementia.

Although a substantial degree of cholinergic neuronal degeneration is known to occur in AD, some function remains even in the very advanced stages of the disease (see Quirion et al., 1995). Post-mortem cortical and hippocampal tissues harvested from AD patients retain at least some ability to synthesize and release acetylcholine, and a portion of cholinergic receptor populations in these tissues do appear to be functional. Thus, the development of novel compounds designed to optimize synaptic acetylcholine levels remains an important approach for the amelioration of some of the symptoms of AD. However, the agents used in clinical practice to exploit the cholinergic hypothesis of AD, i.e., the acetylcholinesterase inhibitors (AChEIs) tacrine and donepezil, have proven to be generally disappointing from a therapeutic standpoint. In addition to the peripheral side effects and poor reliability of effect, these compounds mainly offer symptomatic therapy and they do not appear to reverse or retard the relentless neurodegeneration, nor do they significantly alter the eventual fatal outcome of the disease. However, AChEIs do provide modest improvements in cognitive function in some patients and thus offer some benefit in an otherwise untreatable condition. Use of AChEIs has been associated with a delay in nursing home placement and with improvement in a number of behavioral symptoms including depression, psychosis, and agitation, even in the absence of significant cognitive improvement (Kauffer et al., 1996). Therefore the development of new AChEIs, as well as other cholinergic compounds, seems warranted. Since AChEIs rely on intact cholinergic neurons, an advantage of direct-acting receptor agonists (such as the M1-selective agent talsaclidine), which act at postsynaptic cholinergic sites, is that they bypass the degenerated presynaptic terminals to enhance neuronal activity.

Nicotine and other centrally acting nicotinic acetylcholine receptor (nAChR) agonists have been demonstrated to improve the performance of a number of memory-related tasks in animal models and humans and to reduce distractibility (e.g., Buccafusco et al., 1995a,b; Prendergast et al., 1997; White and Levin, 1999). Accordingly, these agents may offer therapeutic options for several central nervous system (CNS) conditions including attention deficit-hyperactivity disorder, AD, Parkinson’s disease, and schizophrenia in which distractibility, inattention, and memory deficits are characteristic features. The discovery of potential neurotrophic and/or neuroprotective properties of nicotinic agents has further elevated the enthusiasm for their use, particularly for progressive neurodegenerative diseases.

It also is quite apparent that in AD, extensive neuropathology is common in areas normally rich in norepinephrine (locus ceruleus) and serotonin (dorsal raphe nucleus), particularly in the later stages of the disease. Therefore, agents designed as replacement therapies, both for the cholinergic deficits and for the deficits in other neurotransmitter systems, may be necessary for maximal symptomatic relief. Likewise, the manipulation of specific neurotransmitters (or their receptors) that participate in the regulation of cholinergic...
nergic activity may provide an additional approach to enhancing mnemonic function in AD patients.

**Components of Serotonin (5HT) Neuronal Systems as Therapeutic Targets**

A substantial accumulation of physiological and behavioral evidence supports the premise that 5-HT plays a significant role in the regulation or modulation of learning and memory processes. Serotonin receptor subtypes implicated to date include presynaptic 5HT1A, 5HT1B, 5HT2A/2C, and 5HT3 receptors, as well as postsynaptic 5HT2B/2C and 5HT4 receptors (Meneses, 1999). 5HT also appears to participate in modulating the activity of septal efferents to the hippocampal formation, a pathway that is of considerable importance to mnemonic processes and that is known to degenerate in AD. Such cholinergic-5HT interactions may offer a pharmacological substrate for memory-enhancing drugs (Cassel and Jeltsch, 1995). As mentioned above, the 5HT system can undergo significant degeneration in patients with AD. Accordingly, antagonists at the 5HT3 receptor or agonists at the 5HT4 receptor may provide additional approaches to enhancing synaptic acetylcholine levels in the CNS of patients with cognitive deficits. Ondansetron and several other 5HT3 receptor antagonists, e.g., granisetron, tropisetron, and itasetron, have been reported to improve performance efficiencies in several rodent memory tasks (see Terry et al., 1998). The newer agents RS 56812 and SEC-579 have been reported to improve memory-related task performance in monkeys (Terry et al., 1996; Arnsten et al., 1997). Alternatively, the 5HT4 agonist RS 17017 has been found to enhance working memory in both young and aged monkeys performing a delayed matching-to-sample task (Terry et al., 1998).

**Components of Noradrenergic, Dopaminergic and Histaminergic Neuronal Systems as Therapeutic Targets**

Consistent with the finding of diminished noradrenergic function associated with AD and AAMI, functional noradrenergic neurons are necessary for certain frontal lobe-mediated cognitive processes. These include attention and the prevention of distraction in the presence of irrelevant stimuli (Robbins and Everitt, 1995). Normal levels of this neurotransmitter appear necessary for optimal function of the prefrontal cortex (PFC), presumably because of its actions at postsynaptic α2A receptors. Accordingly, agonists at α2A receptors (e.g., clonidine, guanfacine) have been shown to improve PFC function in nonhuman primates, whereas antagonists at α2 receptors (e.g., yohimbine) have been shown to impair PFC function or antagonize the beneficial actions of agonists (see Mao et al., 1999). These findings have relevance to the present discussion since inattention and the susceptibility to distracting stimuli are common symptoms in AD that may be improved by compounds designed to optimize noradrenergic activity in PFC.

Human data collected from tissue samples, as well as data derived from imaging studies, indicate that dopaminergic cells, reuptake sites, and receptors also decline with age (Barili et al., 1998). Furthermore, a number of studies have indicated that normal dopaminergic function is necessary for the successful performance of a variety of memory tasks, particularly those that rely on the function of PFC (see Cai and Arnsten, 1997). Taken together, these findings suggest that agonists at dopamine receptors may have a role in the therapy of age-related disorders in which memory decline is a prominent feature. To date, however, few studies with potentially memory-enhancing dopaminergic drugs have been conducted with this hypothesis in mind. Cai and Arnsten (1997) did find that a narrow range of very low doses of D1-selective agonists (A77636 and SKF81297) improved performance of a spatial working memory task in aged monkeys.

In the CNS, histamine is primarily found in neurons with cell bodies located in the tuberomammillary nuclei of the hypothalamus. These cells, however, project to a number of areas of the brain including those important to mnemonic function: the basal forebrain, cortex, thalamus, and pontomesencephalotegmentum. The role of histamine as a significant neurotransmitter in the mammalian CNS, as well as an important modulator of cognitive processes, recently has gained attention. This amine not only binds to its own specific receptor subtypes in the CNS (designated as H1, H2, and H3), but it also appears to interact with the polyamine site on the N-methyl-D-aspartate (NMDA) receptor complex (Passani et al., 2000). Interactions at these receptors may influence a number of physiological processes in the brain including learning and memory. Studies designed to elucidate the cognitive effects of histamine (presumably via H1 and H2 receptors) have often provided equivocal results, with some studies demonstrating memory improvements with histamine (and histamine analogs) and some demonstrating impairment (Passani et al., 2000). Most recently, attention has focused on the H3 receptor subtype for its role in memory and attention and as a target for drug development (see Leurs et al., 1998). This subtype may serve not only as an autoreceptor (i.e., mediating negative feedback of histamine release) but also as a heteroreceptor, mediating the inhibition of serotonin, norepinephrine, dopamine, and acetylcholine release. Studies of antagonists at H3 receptors (e.g., thioperamide) as potential pro-cognitive agents have begun and some of these have generated encouraging results (Prast et al., 1996).

**Components of Amino Acid Neuronal Systems as Therapeutic Targets**

The NMDA receptor complex, which consists of a glutamate binding site, a strychnine-insensitive glycine co-agonist site, a polyamine allosteric site, and two channel sites, has been demonstrated to play an integral role in long-term potentiation (LTP) and learning and memory (Collingridge and Bliss, 1987). Glutamate-mediated neurotransmission is significantly compromised in the neocortex and hippocampus of AD patients, a factor that may contribute to the cognitive deficits associated with the disease (Palmer and Gershon, 1990). Conversely, in certain conditions (e.g., reduced cerebral blood flow) glutamate and aspartate may accumulate at NMDA receptors, causing prolonged depolarization and eventually cell death. Early studies with kainate and other glutamate analogs (now referred to as excitotoxins) further indicated the potential for toxicity associated with glutamate receptor stimulation (Doble, 1999). More recent studies indicate that compounds that act at the glycine site on the NMDA receptor complex may be more useful as pro-cognitive agents.
based on their ability to enhance LTP without producing neurotoxicity. The glycine prodrug milacemide and the partial agonist/antagonist, t-cycloserine, enhance the performance of memory-related tasks in animal models (Handelmann et al., 1989; Baxter et al., 1994), as well as in AD patients (Schwartz et al., 1996).

Components of Angiotensin and Other Peptidergic Neuronal Systems as Therapeutic Targets

Certain neuropeptides, including substance P, arginine-vasopressin, and thyrotropin-releasing hormone, that function in the CNS as neurotransmitters or neuromodulators are now known to be important for learning and memory. Also, the levels of these peptides have been reported to be deficient in the cerebral cortex and hippocampus of AD brains (for review, see Pan et al., 1999). Compounds designed to restore or mimic the effects of these peptides may provide novel approaches to the treatment of AD. The renin-angiotensin system, and particularly angiotensin II (ANG II), have been implicated in learning and memory processing, and in the cognitive decline in AD. Contrary to the disposition of other peptides and the classical neurotransmitters mentioned above, angiotensin-converting enzyme (ACE) activity has been found to be elevated in cerebrospinal fluid (Zubenko et al., 1985) as well as in several brain regions in AD (Barnes et al., 1991). Likewise, AT\(_2\) receptor expression was reported to be increased by over 200% in the temporal cortex of AD brains (Ge and Barnes, 1996). In behavioral studies, inhibition of ANG II formation by a number of ACE inhibitors was shown to improve cognitive function in both animals and humans (Domeney, 1994). Conversely, direct central administration of renin was reported to disrupt the retention of passive avoidance learning in rats, an effect that was attenuated by ACE inhibitors (DeNoble et al., 1991). ANG II plays a role in the inhibition of LTP induced by ethanol and diazepam, an effect that is mediated by the AT\(_1\) receptor subtype (Wright and Harding, 1997). In addition, ANG II inhibits the release of acetylcholine in slices prepared from rat and human cortex (Barnes et al., 1989), and the peptide blocks hippocampal LTP (Denny et al., 1991). Taken together, these findings appear to suggest a possible reciprocal relationship in terms of memory function between ANG II and cholinergic activity in specific regions of the mammalian brain. Such a relationship could be of importance in AD and in consideration of the design of novel AD drugs. Based on this hypothesis, we studied a novel AT\(_1\), receptor antagonist, RS 66252, in both young adult and aged macaques. The drug produced robust improvements in the accuracy of these subjects in performance of a delayed matching-to-sample task (Table 2).

Potential Multiple Synergistic Targets for Memory Enhancement

Over the past several 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 often is 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 Parkinson’s disease 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 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. Indeed, several neurotransmitter systems likely subserve the various components of memory and cognitive ability. 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 AChEIs to fully reverse the cognitive defects resulting from combined lesions of adrenergic and cholinergic neuronal pathways (Haroutunian et al., 1990). From a different perspective, there may be another rationale for considering that combined therapy may be superior for treating AD. We have reported that clonidine is a potent inhibitor of the biosynthesis and the release of acetylcholine within specific brain regions in the rat and the drug can inhibit the expression of cholinergic signs of toxicity to phsysostigmine and other cholinesterase inhibitors (see Buccafusco, 1992). As indicated in Fig. 1A, clonidine is without effect on cholinergic function in the hippocampus, a potentially important site of action for the memory-augmenting actions of potential AD drugs. Thus, we reported that combined treatment with clonidine and phystostigmine resulted in at least additive, possibly synergistic, effects on delayed matching-to-sample performance by mature adult and aged macaques (Terry et al., 1993). In the presence of clonidine, the therapeutic window for phystostigmine appeared to widen such that addition of clonidine to the phystostigmine regimen allowed the animals to tolerate higher doses of phystostigmine. Whereas suppression of potential phystostigmine-induced adverse reactions was one consequence of clonidine’s effects, the \(\alpha_2\)-receptor agonist also was shown to exert a small, but significant level of task improvement of its own in these same animals (Tables 1 and 2).

A few other examples of additive or synergistic actions of different drug classes on memory-related task performance exist in the literature. In one such study, the partially selective muscarinic M1 receptor agonist, milacemide, was reported to augment the ability of the AChEI tacrine to reverse a scopolamine-induced decrement in efficiency of maintaining a continuous performance task by rhesus monkeys (Calabhan, 1999). As with the clonidine-phystostigmine situation, there was a widening of the effective dose-range in reversing the scopolamine-induced task deficit. One disadvantage of combining drug classes is that compounds with differing pharmacodynamic and pharmacokinetic properties may be difficult to manage clinically. This disadvantage would be
largely eliminated through the development of compounds that have the potential to act at multiple therapeutic targets. To suggest this possibility is our recent study of the effectiveness of the ranitidine analog JWS-USC-75IX in several memory-related tasks in rats (Terry et al., 1999b). JWS-USC-75IX is a relatively potent AChEI, but it also exhibits high-affinity antagonism for the muscarinic M2 receptor. As AChEIs 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 AChEI-induced feedback inhibition (Fig. 1B). 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 drug also exhibited a marked safety profile relative to pure AChEIs. Of course, this approach may not always provide pharmacological dividends. An example is the compound RS66331, which (neurochemically) exhibits the properties of a 5HT4 agonist and a 5HT3 antagonist (Table 2). Rather than this combination of properties imbuing the drug with augmented memory-enhancing action, the effectiveness of RS66331 was similar to the 5HT3 antagonist RS56812, but it was considerably reduced in effectiveness compared with the 5HT4 agonist RS17017 (Table 2).

In considering some of the compounds discussed in the paragraphs above and those listed in Table 2, it may be determined that many of these already affect more than one CNS target. For example, AChEIs such as tacrine and donepezil (notwithstanding the potential for negative feedback on acetylcholine release discussed above) have the potential for activating various subtypes of muscarinic and perhaps nicotinic cholinergic receptors. That donepezil remains one of the most effective (on an acute basis) compounds we have studied in aged monkeys for memory-related task improvement (Table 2) may reflect the multiplicity of its neural targets. Likewise, nicotinic receptor agonists, which have the potential for enhancing transmitter release from cholinergic and biogenic amine nerve terminals, can elicit very efficacious responses in primates. The inclination to develop more selective agents such as the subtype (α7)-selective nicotinic receptor agonist GTS-21 has not resulted in a greater level of effectiveness compared with the other less selective compounds (Table 2, Fig. 2). Rather, receptor specificity/selectivity and high potency could engender the expression of severe side effects (e.g., Bartolomeo et al., 2000).

**Future Trends**

Over the past 10 years we have had the opportunity, partly through our collaborative efforts with several pharmaceutical companies, to study many pharmacological classes of...
potential memory-enhancing agents. Some of these have already been discussed. To varying degrees many of these test compounds were effective cognitive-enhancing agents in adult mature and aged rhesus monkeys. Table 2 was prepared from this database of nonproprietary compounds obtained from sessions with mature adult and aged macaque subjects who were well trained in the performance of our computer-assisted delayed matching-to-sample task (see Paule et al., 1998). In addition to exhibiting various levels of effectiveness, the drugs often exhibited specificity for a particular recall duration (time interval between extinguishing the sample stimulus and presentation of the choice stimuli). Also, because of marked subject sensitivity to a particular dose or doses within a dose-response series, it was felt that a rational approach to comparing relative effectiveness was to select a “Best Dose” (the most effective dose, independent of delay interval) for each subject from the dose-response data and to provide an averaged Best Dose for each compound. Therefore, in Table 2, comparison is made among compounds in terms of the Best Dose and most improved recall delay interval for each drug series. Despite the inherent limitations in this type of approach, it provides a tentative means for comparing the relative effectiveness for each compound under conditions that would mimic their potential clinical application (i.e., dose titration for best effect). Perusal of Table 2 reveals that, where comparable data exist, there is not a great difference between data obtained for the two different age groups or among the different macaque species that served as subjects. It was perhaps somewhat surprising that non-aged subjects generated data that were quite similar to those derived from aged subjects in terms of drug effectiveness (even though the older group, on average, exhibited reduced levels of baseline performance compared with their younger counterparts). This finding may be due to the nature of the paradigm, which was designed to assess the animals, young or aged, to their mnemonic limit (e.g., long delay intervals were adjusted to produce performance efficiencies that were just above chance). Subtle differences in perfor-
mance strategies used by the two age groups before and after receiving drugs are no doubt present (as we have described in our earlier studies, e.g., Terry et al., 1996), but discussion of these differences is not within the scope of this review.

Figure 2 illustrates the data from Table 2 plotted in terms of order of relative effectiveness, as determined by the ability of each compound to increase delayed matching-to-sample performance efficiency after administration of a Best Dose. For those drug classes represented by multiple compounds, such as the nicotinic and 5HT receptor ligands and the AChEIs, there is a broad range of efficacy. Thus, at least for acute administration, the effectiveness of these compounds may not be solely predicted from receptor binding or enzyme affinities. In this regard, we have noted a very good level of clinical predictability for these compounds using our testing paradigm in macaques. Panel A of Fig. 2, which provides data from both age groups, describes a plot that is fit very well by a linear expression. In terms of future trends, then, it would appear that we have yet to reach an upper limit in terms of agent effectiveness, and that there is every reason to expect that new, even more promising drugs are possible. Examination of panels B and C of Fig. 2, where the data are segregated into young (mature adult) and aged subjects, respectively, would support this conclusion.

Some of the compounds listed in Table 2 also may possess disease-modifying potential. For example, nicotinic compounds appear to offer neuroprotection (see Fig. 1C) under many different experimental conditions possibly through different biological mechanisms (e.g., Li et al., 1999). Also, muscarinic M1 receptor agonists may alter the processing of amyloid precursor molecules such that less toxic fragments are produced (Müller et al., 1997). Therefore, the possibility exists for using a single molecular entity to offer both cognitively and disease-modifying effects for the treatment of AD and related disorders. Notwithstanding this interesting possibility, it is likely that drugs designed solely to improve learning, memory, and general cognitive performance will have a place in the future therapy of such diseases. There is also a strong possibility that “cognitive pharmacology” will have a place in the treatment of AAMI. Aged monkeys are perhaps a more relevant model for this condition than they are for AD. Indeed, it is somewhat surprising that memory loss associated with “normal” aging has not been targeted more by the pharmaceutical industry. Based on our experience with aged monkeys, it is more likely that pharmacological success will be achieved in this population than in moderately or severely demented individuals. If mild cognitive impairment is a risk factor for AD, there is reason to anticipate that successful treatment with cognitive and disease-modifying agents may result in a longer duration of cognitive health and a reduced incidence of AD.

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