Anticholinergic Medications and Cognition in Older Adults

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A significant portion of the cognitive decline seen in older adults may be due to anticholinergic medications (i.e., muscarinic receptor antagonists) which are known to cause memory loss, confusion, and delirium. A competitive radioligand binding assay has been used in the research setting to measure the cumulative level of muscarinic receptor binding present in an individual's serum, referred to as serum anticholinergic activity (AA). Serum AA is the measure of binding of all compounds present in a person's serum (e.g., medications, metabolites, and possibly endogenous substances) to muscarinic receptors. Multiples studies have shown that even low serum AA levels are associated with impaired cognitive performance, impaired self-care capacity, and the presence of delirium in nondemented or mildly demented elderly. Serum AA has the potential to be a useful tool for clinicians. However, there are multiple items which first need to be addressed to enhance the reliability and clinical applicability of this assay.

One concern is that the muscarinic receptor binding profiles of most medications and their metabolites have never been examined. Thus, even if a clinician decides that a patient is suffering from anticholinergic-induced toxicity, he/she has little guidance on which medication(s) to adjust. To address this issue, we investigated the *in vitro* AA of 106 commonly used medications and estimated the relationship between dose and AA in older adults.

The change in serum AA over time in the absence of medication adjustments is not known. Another limitation is that serum AA is a peripheral measure, while the central anticholinergic effects of a medication are dependent on its distribution into the CNS. An optimal tool to predict medication-induced cognitive impairment would be one which better estimates drug distribution into the CNS. To address these issues, we conducted a pilot study investigating the utility of using centrally mediated pupillary oscillationsin conjunction with serum AA as a possible predictor of cognitive performance. Serum AA levels and ocular response were measured in a double-blind, cross-over study across an 8 hour time period following administration of placebo or the anticholinergic medication, oxybutynin.

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LIST OF ABBREVIATIONS

AA----------Anticholinergic Activity

AD----------Alzheimer's Disease

BBB----------Blood-Brain Barrier

BOB----------Blood-Ocular Barrier

CNS----------Central Nervous System

DSST----------Digit Symbol Substitution Test

IR----------Immediate Release

IV----------Intravenous

MS/DB----------Medium septum and diagonal band of Broca

NBM----------Nucleus basalis of Meynert (MS/DB)

NMS----------N-methylscopolamine

OTC----------Over-the-counter

QNB---------- L-quinuclidinyl benzilate

RRA----------Radiorecptor assay

SERUM AA----------Serum Anticholinergic Activity

SD----------Standard Deviation

T----------Time

PREFACE

To my family and friends, thank you for all of your support. To SKOB, I have not forgotten you.

1.0 ANTICHOLINERGIC MEDICATIONS AND COGNITIVE IMPAIRMENT

INTRODUCTION

Cognitive performance (e.g., verbal learning and processing speed) tends to decline with age in nondemented older adults.[1] This phenomenon is usually attributed to age-related changes in the central nervous system (CNS). However, a significant portion of this decline may actually be due to anticholinergic medications (i.e., muscarinic receptor antagonists). Studies conducted in elderly have shown that drugs, which block muscarinic receptors, cause impairment in a plethora of cognitive aspects including working memory, episodic and semantic memory, language, processing speed, and praxis.[2, 3] Elderly individuals are particularly at risk for anticholinergic adverse events due to age-related decreases in cholinergic neurons and receptors,[4, 5] as well as an increase in medication use.[6] Moreover, patients with dementia suffer from greater impairment on specific cognitive performance tasks and at lower doses of anticholinergic drugs than aged-matched elderly.[2]

Up to 50% of community-dwelling and 40% of institutionalized elders are taking a medication known to be anticholinergic.[7-9] Anticholinergic medications are often prescribed for specific indications; for example, meclizine for dizziness, oxybutynin for spastic bladder, and hyoscyamine for irritable bowel syndrome. In addition, there are many medications with nonanticholinergic indications (e.g., diphenhydramine, olanzapine, and paroxetine) that have potential mild to moderate anticholinergic side effects which alone, or in combination, can have significant effects on the overall cognition of the patient.

1.1 CHOLINERGIC SYSTEM

Cholinergic neurons and receptors are found throughout the peripheral and central nervous systems. Acetylcholine, the neurotransmitter for this system, binds to two classes of receptors, the ionotropic family of nicotinic receptors and the metabotropic family of muscarinic receptors. The nicotinic receptors are ligand-gated ion channels and have sparse, but widespread distribution throughout the brain.[10-12] Muscarinic receptors are G protein-coupled receptors and are also widely distributed throughout the CNS.[4, 11-13] Both receptor classes are thought to have a distinct, as well as an overlapping role in learning and memory.[14-19] This doctoral dissertation focuses exclusively on the effects of antimuscarinic medications on cognitive performance.

The majority of central cholinergic neurons are located in the basal forebrain and midbrain and send a comprehensive projection throughout the CNS. The nucleus basalis of Meynert (NBM) and the medium septum and diagonal band of Broca (MS/DB) are cell bodies located in the basal forebrain. The NBM is the principal contributor of cholinergic input to all regions of the cerebral cortex, whereas the MS/DB project to all cell types in all areas of the hippocampus.[20] Additional cholinergic projections from the forebrain include the amygdala, specific thalamic nuclei (e.g., intralaminar nuclei), the medial dorsal nucleus, the reticular nucleus, and olfactory structures.[21, 22] Midbrain cholinergic nuclei, the pedunculopontine nucleus and the lateral dorsal tegmental nucleus, project to the NBM, all thalamic nuclei, the amygdala, and the primary visual and olfactory cortices.

For over 100 years, it has been recognized that naturally occurring antimuscarinic alkaloids may cause memory impairment in some individuals. Formal pharmacological studies conducted in the 1960s and 70s substantiated initial case reports.[23-25] Since then, an abundance of studies, using a variety of agents, have been performed. The following sections review in greater detail the cognitive domains impaired by anticholinergic agents, the specific roles of individual muscarinic receptor subtypes, clinical implications, and unanswered questions.

1.2 MUSCARINIC RECEPTOR SUBTYPES

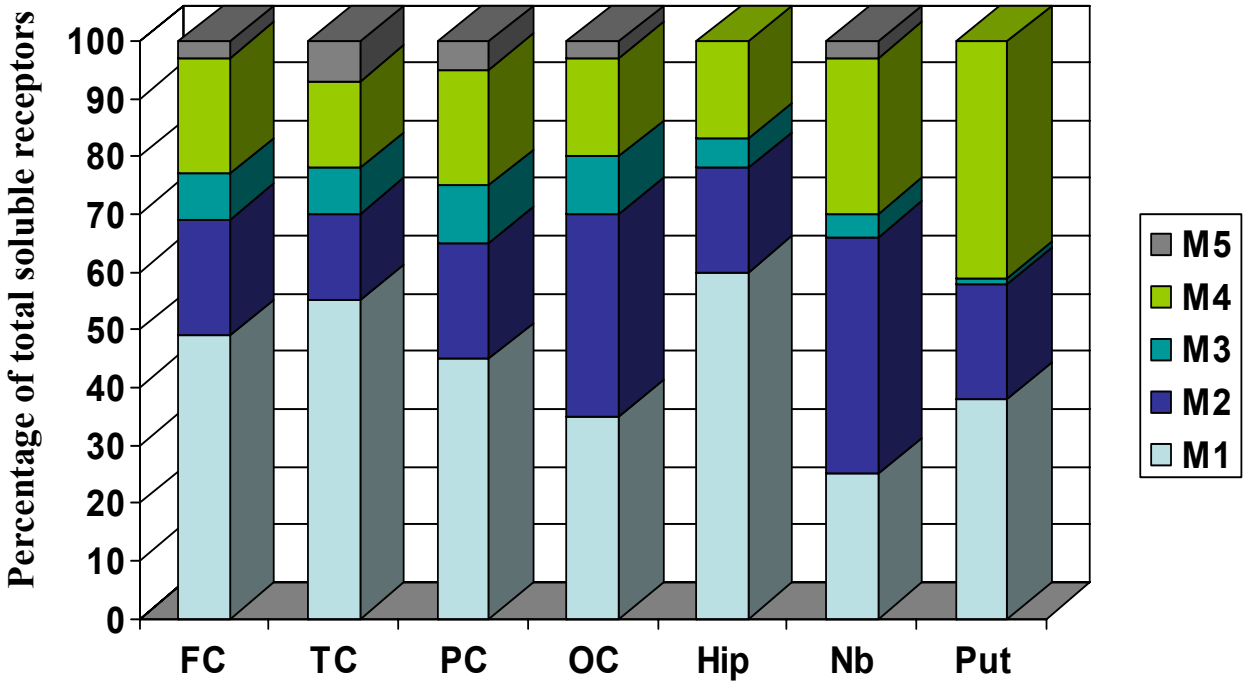
To date, five specific muscarinic receptor subtypes have been identified.[13, 26, 27] All five subtypes are G protein-coupled receptors and are made up of seven highly conserved transmembrane regions. The large cytoplasmic third intracellular loop between transmembrane domains 5 and 6 interacts with the G protein (e.g., heterotrimeric guanine nucleotide binding protein) and is the most highly divergent domain among the subtypes. All subtypes are activated by the binding of a ligand which causes a conformational change in the receptor. The ligand-bound receptor then activates the coupled G-protein. Subsequent downstream effects are dependent upon the type of G protein. It is thought that the M1, M3, and M5 subtypes preferentially couple to $G_{\alpha q}$ and M2 and M4 to $G_{\alpha i}$.[13] Through the activation of these G subunits, M1, M3, and M5 signaling mechanism is via mobilization of inositol phosphoinositides; whereas M2 and M4 inhibits adenylyl cyclase activity (e.g., inhibits the messenger cyclic adenosine monophosphate or cAMP from being produced).[13]

Areas of the brain involved in cognition (e.g., cortex and hippocampus) are rich in all five muscarinic receptor subtypes, with a predominance of subtype M1 (Figure 1.1).[4, 28] It is thought that in the central nervous system M1, M3, and M5 receptors function primarily as postsynaptic receptors. M2 receptors are thought to act as autoreceptors (i.e., inhibiting release of acetylcholine from cholinergic terminals), and there is evidence that M4 functions as both a postsynaptic receptor and an autoreceptor in a region specific manner.[29] Thus, antagonism at different receptors, in different regions, may have differing effects.

The subtype specific binding profile of most drugs and their metabolites has not yet been investigated. Among those examined, the majority have been shown to have relatively similar binding affinities at all five subtypes. It has only been in the last several years that specific muscarinic receptor antagonists and knock-out mice have become available to study the role of individual muscarinic receptor subtypes. Studies conducted in humans and rodents have suggested that M1, M2, M4, and M5 are all critical in certain learning and memory processes.[30-34] M1 knockout mice display a severe deficit in working memory,[30] but maintain spatial memory and have an enhanced response to emotional learning tasks. M5 knockout mice display deficits in recognition tasks and spatial memory.[35] Administration of an M2 specific antagonist (M2 is thought to be an autoreceptor) to rodents produces an improvement in emotional learning.[36] The opposite outcome occurs with an M4 specific antagonist.[37] In contrast, M3 knockout mice perform normally across a wide range of cognitive testing.[38] Moreover, preliminary evidence indicates that cognitive function is spared with chronic administration of the M3 selective incontinence agent, darifenacin.[39] However, further testing still needs to be conducted before it can be unequivocally stated that M3 is not involved in cognition. Moreover, the same cognitive

tests need to be employed across all 5 subtype knockouts before specific roles can better be elucidated. Despite the limitations, these results raise the possibility that antimuscarinic activity at each receptor subtype may have different (and sometimes opposite) effects.

Figure 1.1. Localization of muscarinic receptor subtypes in various human brain regions



Relative proportions of M1-M5 muscarinic receptor subtypes in post-mortem brain regions from nondemented older adults. The level of each receptor subtype is expressed as the percentage of the total soluble receptors determined via the radioligand N-methylscopolamine. FC=frontal cortex, TC=temporal cortex, PC=parietal cortex, OC=occipital cortex, Hip=hippocampus, Nb=nucleus basalis, and Put=putamen. Modified from Flynn and colleagues[4]

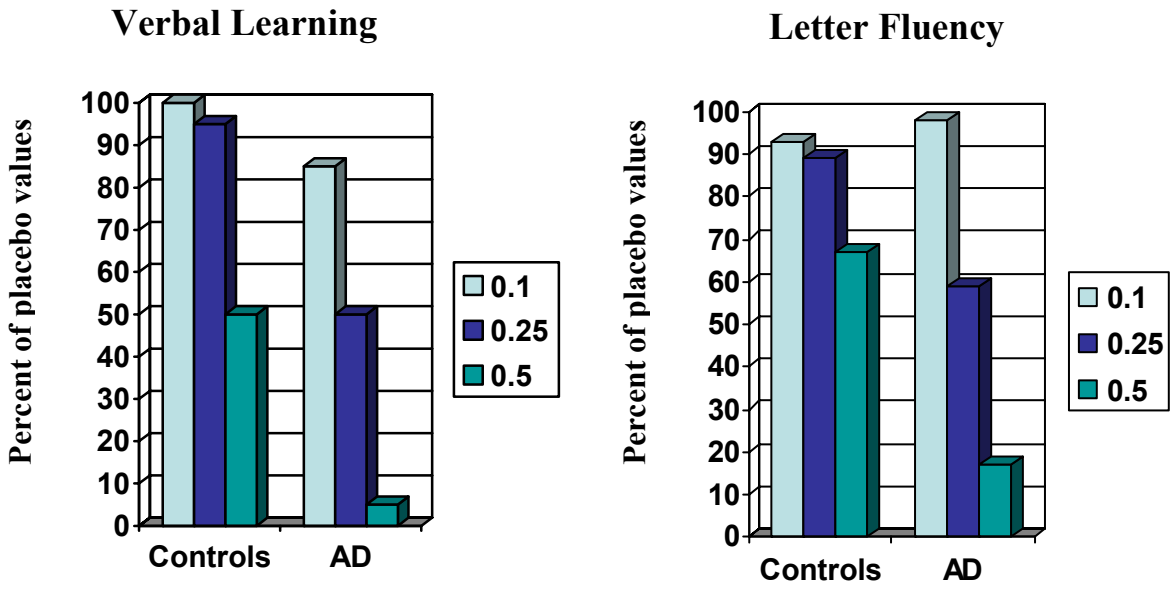
1.3 ACUTE ADMINISTRATION OF ANTICHOLINERGIC AGENTS

The majority of older adults suffer from a decline in multiple areas of cognition following a single-dose administration of an anticholinergic agent (see Appendix A for brief descriptions of various memories). Scopolamine, a potent muscarinic receptor antagonist at all five muscarinic receptor subtypes, is the most common agent used to assess these effects. In nondemented older adults, small doses of scopolamine produce a deficit in complex attention, facial recognition, praxis, reaction time, and verbal learning.[2, 3, 16, 40-45] Some[2, 3, 40, 44], but not all[16, 42, 45] studies have reported an impairment in semantic and working memory as well.

Older subjects suffer from a greater impairment than younger, while patients with dementia are at the greatest risk.[2, 3, 40, 42, 43, 45] Zemishlany and colleagues reported that a low dose of scopolamine (0.2 mg subcutaneous) produced no apparent deficits in younger adults, whereas older adults suffered from a significant impairment in verbal learning, as well as the ability to carry out complex motor functions.[45] This disparity remains at higher doses as well. Following a 0.5 mg intravenous (IV) dose of scopolamine, elderly subjects are notably more impaired on measures of episodic and semantic memory.[3] Moreover, older subjects continue to be impaired even after young subjects return to baseline functioning.[43] Naranjo and colleagues administered a single dose of 0.5 mg of IV scopolamine to both younger and older adults and then assessed verbal learning every 30 minutes for 120 minutes following medication administration. Younger subjects returned to baseline functioning by the final 2 hour assessment, whereas older adults were still significantly impaired, scoring on average 50% lower than their baseline performance.[43]

Patients with dementia suffer from the greatest decline and at lower doses than any other population examined. Sunderland and colleagues investigated the effects of multiple low doses of IV scopolamine on cognition in patients with mild-to-moderate Alzheimer's Disease (AD) and aged matched controls. For AD patients, the highest dose examined (0.5 mg) resulted in a marked decline of 75% or more in the majority of the cognitive areas assessed including complex attention, semantic memory, verbal learning, and working memory (Figure 1.2).

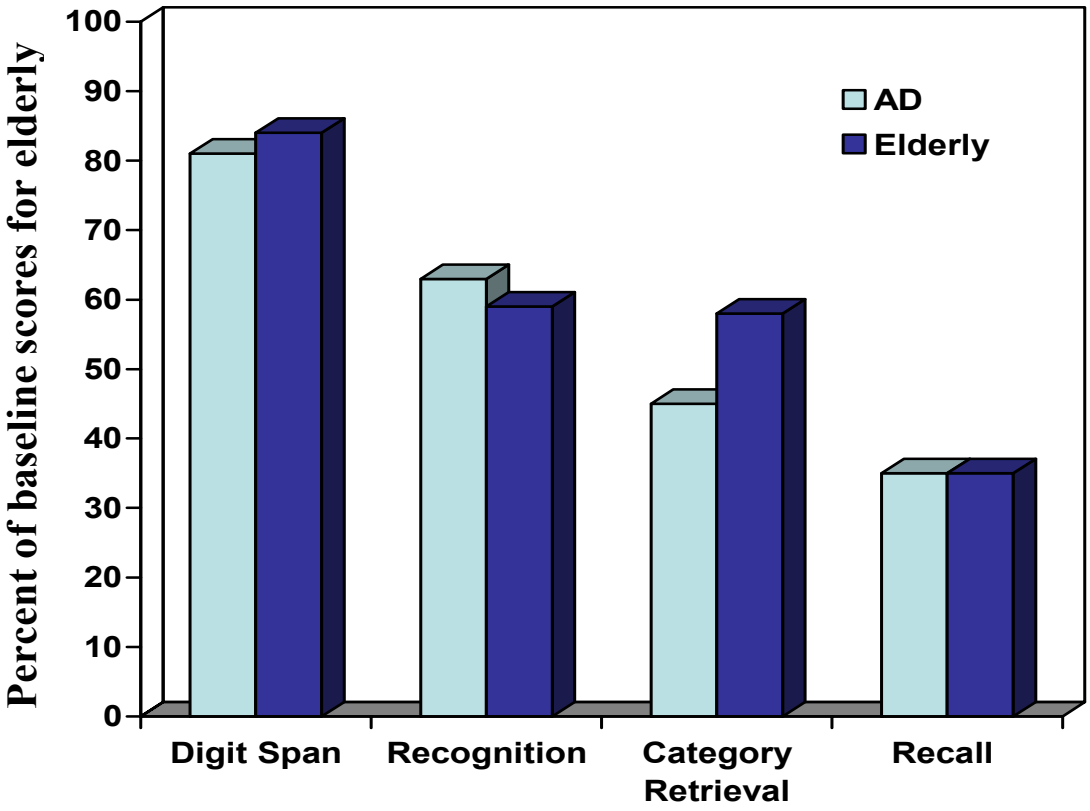
Figure 1.2. Cognitive performance following IV scopolamine



Cognitive performance was investigated in a double-blind, cross-over study approximately 90 minutes following IV placebo saline and IV scopolamine administration of 0.1, 0.25, and 0.5 mg doses. Modified from Sunderland et al.[40]

Interestingly, cholinergic blockade mimics some of the deficits present in AD, especially those prevalent in the mild-to-moderate stages.[46-48] In a small cohort of older adults (n=10, mean age 61 years), a single dose of 0.5 mg IV scopolamine resulted in cognitive test scores similar to baseline scores of AD patients (Figure 1.3). Moreover, the impairment to scopolamine was such that experienced clinical staff noted the resemblance to AD even prior to comparing test scores.[49] The scopolamine model of dementia is incomplete though, as anticholinergic agents do not appear to impair autobiographical memory, previously learned material, or procedural memory (e.g., areas impacted with the progression of AD)[46, 50, 51] Moreover, the degree of impairment following scopolamine administration to nondemented older adults is considerably less than the deficits typically seen with moderate-to-severe AD. Nevertheless, from these findings arise a serious concern of possible misdiagnosis and mistreatment of anticholinergic-induced cognitive impairment in a clinical setting.

Figure 1.3. Comparison of cognitive performance



Comparison of mild-to-moderate Alzheimer's Disease subjects at baseline (e.g., no anticholinergic medication) and normal elderly controls following administration of a single dose of scopolamine (0.5 mg IV). Bars represent test results as a function of the elderly normal baseline scores for each task (e.g., prior to scopolamine administration). The digit span task assesses working memory, category retrieval assesses semantic memory, recognition/recall assesses verbal learning. Modified from Sunderland et al.[52]

Unfortunately, very few studies have reported possible covariates associated with the presence or extent of anticholinergic-induced impairment. In one small study, conducted in younger adults, baseline verbal performance was inversely correlated to the degree of impairment produced by scopolamine ($p=<0.05$, $r=-0.50$, $n=14$).[25] Also in a younger adult population, a significant increase in the detrimental effects of scopolamine was seen with sleep deprivation,[53] while an attenuation occurred with concurrent caffeine or nicotine use.[54] In an older adult population, Nebes and associates reported that cognitive impairment associated with cerebrovascular disease (in the form of white matter hyperintensities) was exacerbated in the presence of anticholinergic medications.[55] In post-menopausal women, Dumas and colleagues found that 3 month estrogen treatment attenuated the effects of scopolamine on tests of attention and tasks with a speed component. However, pre-treatment with estrogen had minimal or no effects on scopolamine-induced impairments in verbal learning.[41] Larger studies need to be conducted in order to adequately characterize the level of risk associated with anticholinergic medication

1.4 CHRONIC USE OF ANTICHOLINERGIC AGENTS

Although not as well researched as acute effects, chronic exposure to anticholinergic agents has been found to have a significant impact on cognitive performance and may cause a more rapid decline associated with neurological disease processes (e.g., Alzheimer's or Parkinson's Disease). Long-term anticholinergic use is associated with declines in attention, simple reaction time, psychomotor speed, executive functioning, visual memory and overall global cognition.[56-58] In one study (n=372), 80% of nondemented older adults consistently taking an anticholinergic medication met the criteria for mild cognitive impairment (MCI).[58] In other words, 80% of a

nondemented population suffered from a noticeable functional impairment such as a decrease in normal every day functioning; a significant decline in at least one area of cognition; and difficulty completing daily activities of living.[59] Only 35% of "non-users" (e.g., not known to be taking an anticholinergic medication) met the criteria for MCI. Recent evidence has also suggested that chronic exposure to anticholinergic agents may adversely affect the course of both AD and Parkinson's Disease. In Parkinson's patients, individuals with a history of chronic anticholinergic use had twice as many tangles and plaques post-mortem as did non-users.[60] Over the course of 2 years, chronically exposed AD patients suffered from a more rapid decline in global cognition.[61]

Limited studies have been conducted assessing the specific anticholinergic effects of clinically relevant medications in older adults. Single dose and 4 day administration of the atypical antipsychotic olanzapine to elderly volunteers resulted in impairment in attention, reaction time, motor control tasks, and verbal learning. Interestingly, on day 4, deficits were only present for approximately six hours following oral administration.[62] The incontinence agent oxybutynin has been shown to have effects on verbal learning and working memory.[63] Acute administration of a low dose of diphenhydramine produces impairment in working memory and reaction time.[63, 64] There are some additional examples available. However, the majority of medications have never been examined for possible muscarinic receptor binding or cognitive effects.

1.5 CLINICAL IMPLICATIONS

It is well known that acute and chronic administration of anticholinergic medications causes impairment in multiple cognitive domains. Unfortunately, given the large heterogeneity in cognitive performance in the elderly and the similarities between anticholinergic-induced cognitive deficits and the early stages of Alzheimer's disease, symptoms of central anticholinergic toxicity are often ignored or viewed as normal aging processes. Moreover, it is difficult to predict who is at risk for anticholinergic toxicity based on medication consumption alone. Community-dwelling and institutionalized elderly take an average of up to eight medications daily.[5,12,13] The cholinergic properties of most prescribed and over-the-counter (OTC) drugs and their metabolites are unknown. Furthermore, the cumulative anticholinergic effect of exposure to multiple drugs is not known and is difficult to predict given the large interindividual variability found in metabolism and excretion for the elderly. A tool is needed which would better allow clinicians to determine which individuals are at risk for anticholinergic-induced cognitive impairment. The serum AA assay outlined below holds promise as a useful clinical tool to determine which individuals may be at risk for anticholinergic toxicity.

1.6 SERUM ANTICHOLINERGIC ACTIVITY

A radioreceptor assay has been used in research to quantify a person's overall anticholinergic burden, referred to as serum anticholinergic activity (AA).[7, 65, 66] Serum AA is the measure of binding of all of the compounds present in a person's serum (e.g., medications, metabolites, and

possibly endogenous substances) to muscarinic receptors. Up to 90% of older adults living in the community have detectable serum AA.[7, 67] Thus up to 90% have compounds (e.g., medications, metabolites, and possibly endogenous substances) present in their sera that block muscarinic receptors. Several studies have shown that serum AA is associated with global cognitive performance, verbal memory, self-care capacity, and presence of delirium in older nondemented or mildly demented elderly.[7, 67-70] Moreover, serum AA was found to be a stronger predictor of cognitive impairment than age or total number of prescribed and over-the-counter medications.[7]

1.6.1 The relationship between serum AA and serum drug levels

Serum anticholinergic activity has been correlated with serum drug levels of specific anticholinergic agents. In a small psychiatric population, the relationship between serum AA and drug concentrations was examined in individuals receiving maintenance amitriptyline or doxepin.[71, 72] Serum levels of both the parent as well as the active metabolite (e.g., nortriptyline or desmethyldoxepin) were measured. There was a significant correlation between amitriptyline ($p<0.001$, $r=0.92$) and nortriptyline ($p<0.001$, $r=0.79$) concentrations and serum AA. The correlation was less strong, although still significant with doxepin ($p<0.05$, $r=0.42$) and its active metabolite ($p<0.1$, $r=0.58$). The sum of amitriptyline and nortriptyline or doxepin and desmethyldoxepin serum levels was correlated to serum AA as well ($p<0.001$, $r=0.86$ and $P<0.01$, $r=0.60$, respectively). In a separate study, a similar relationship existed with the anticholinergic medication disopyramide ($p<0.001$, $r=0.66$).[72]

Preliminary evidence has suggested the possibility of endogenous substances which affect muscarinic receptor binding.[73, 74] Clinically relevant serum AA (mean 3.45; standard deviation 4.25 pmol/mL) has been detected in patients with delirium not taking any known anticholinergic medications.[73] For this study (n=10), the authors recruited individuals for participation who had no history of known anticholinergic medication for one week prior to the study. Moreover, all of the medications used by the subjects at the time of the serum AA blood draw, were assessed and found to have no *in vitro* AA at "therapeutic concentrations". Unfortunately, the authors did not provide the actual *in vitro* concentrations assessed and did not appear to list medications which were administered prior to hospital admission. Nevertheless, this article did provide a convincing argument that this topic needs further discussion. Multiple naturally occurring substances have been shown to have antimuscarinic activity *in vitro* (e.g., progesterone, oubain, eosinophil major basic protein, myelin basic protein, arachidonic acid, dynorphin A, and protamine) in specific assay environments.[75, 76] However, some of these agents such as dynorphin are rapidly degraded under the conditions in which serum is processed for AA. More research is needed to be conducted in order to clarify if indeed endogenous substances impact the serum AA assay and to characterize these compounds.

1.6.2 The relationship between peripheral and central anticholinergic activity

Many investigators have used serum (peripheral) AA as a possible measure of central anticholinergic burden. However, only two small studies have directly examined the relationship between peripheral and central AA. Miller and colleagues measured serum and cerebral spinal fluid (CSF) AA in older adults approximately 2 hours following intramuscular scopolamine (n=5) or saline (n=4) administration.[77, 78] Mean CSF AA levels were 74 and 0 pmol/mL of

atropine equivalents between scopolamine and saline treatment groups, respectively. In these nine patients, CSF AA correlated significantly with serum AA levels ($p<0.05$, $r=0.69$). Plaschke et al measured the AA in older adults receiving a range of anticholinergic agents of mild to moderate potency.[79] A strong relationship was found between peripheral and CSF AA ($p<0.001$, $r=0.86$). Interestingly, in all subjects, CSF AA was approximately 2.5 times higher than that found in serum. A likely explanation for this is a discrepancy between plasma and CNS pharmacokinetics.

1.6.3 The relationship between serum AA and cognitive performance

With few exceptions, serum AA has consistently been associated with cognitive performance or the presence of delirium in older adults (Table 2.1). In the largest study conducted to date, Mulsant and colleagues reported that serum AA in 201 community-dwelling elderly was associated with overall cognition as measured by the Mini-Mental State Exam (MMSE).[7] Specifically, individuals with moderate levels of serum AA (e.g., greater than 2.8 pmol/mL atropine equivalents) were 13 times more likely to perform poorly on the MMSE. Nebes and associates examined serum AA in a group of depressed geriatric patients. Individuals with detectable levels of serum AA had lower scores on measures of verbal learning.[67] Of note was that in this small cohort, there was no difference in medical burden between individuals with AA (n=19) and without detectable AA (n=17). Tollefson et al conducted an intervention study in an institutionalized, psychiatric older adult population that was designed to reduce the total amount of anticholinergic medications taken. An improvement in multiple cognitive domains and a decrease in serum AA levels were seen at postintervention. Moreover, baseline and

postintervention serum AA levels correlated to both pre and post-cognitive performance, respectively.[68]

1.7 METHODS: SERUM ANTICHOLINERGIC ASSAY

Serum samples are collected in a 5 or 10 cc red top (e.g., lacking heparin or EDTA) vacutainer and allowed to sit for 30 minutes at room temperature. Samples are then centrifuged for 10 minutes at 1300 g. Serum is then aliquoted into polypropylene tubes and stored at -20˚ Celsius (C) for up to 7 days and at -80˚ C for longer periods.

Serum AA levels are analyzed as previously described.[7, 66] Tritiated L-quinuclidinyl benzilate (^{3}H-QNB), an antagonist that has high and specific affinity for muscarinic receptors, is used as the radioligand. Atropine, also a specific muscarinic receptor antagonist, is used for the standard curve. The assay is conducted on ice and all solutions used are at 4° C. For the standard curve samples, 0.1 mg of a homogenized mixture of rat (Sprague-Dawley males, approximately 200-225 grams) forebrain and striatum are added to a mixture of varying concentrations of atropine (0.1 nM – 10 nM), 200 µl drug-free human off-the clot serum (Scantibodies), and 50 mM sodium phosphate buffer, pH 7.7. For subject samples, no atropine is added and blank serum is replaced with 200 µl of subject's serum. Following the addition of muscarinic receptors, samples are vortexed and incubated on ice for ten minutes. A fixed amount of ^{3}H-QNB (3 nM) is then added giving a total sample volume of 2 mL. Samples are vortexed and incubated in a shaking water bath at 22°C for 60 minutes. Post-incubation, 2 mL of cold buffer is added to each sample and the reaction is

stopped by filtering samples under reduced pressure through Whatman GF/B filters using a Brandel Cell Harvestor (Brandel Scientific).

All subject samples are compared to the atropine standard curve which is run with each assay. In other words, the amount of ^{3}H-QNB that is displaced from the muscarinic receptors in response to subject's serum is compared to the amount of atropine which is required to displace the same amount of ^{3}H-QNB. Serum AA results are then reported as picomoles of atropine equivalents per milliliter (pmol/mL). The limit of reliable detection of atropine is 0.5 pmol/mL. Serum AA values less than 0.5 pmol/mL are reported as 0. All standard curve and drug samples are run in triplicates. Previously made controls of low and medium atropine concentrations are run with each assay. Oxotremorine sesquifumarate (100 μM, Sigma-Aldrich) is used to assess for nonspecific binding. Atropine standard curve and subject serum AA values are calculated using logit-log regression.

1.8 UTILITY OF SERUM ANTICHOLINERGIC ACTIVITY

Serum AA has the potential to be a useful tool for clinicians. It allows a single measurement, standardized with atropine so values can easily be compared. Serum AA takes into account the affinity of an agent at muscarinic receptors and individual differences in pharmacokinetics.[66] However, there are multiple items which need to be examined to enhance the reliability and clinical applicability of the assay.

1.8.1 A peripheral measure to predict central effects

A limitation of the assay is that serum AA is a peripheral measure. The central anticholinergic effects of a medication are thought to be primarily dependent on its distribution into the CNS. As discussed above, a correlation between serum and central AA has been found in two small groups taking a specific subset of anticholinergic medications. However, there are multiple muscarinic receptor antagonists that are not readily distributed into the brain (e.g. the metabolite desmethylloratadine, trospium, and glycopyrrolate), yet would still cause an increase in serum or peripheral AA. Ray and colleagues examined the cognitive effects of glycopyrolate, an agent which has minimal distribution into the CNS and scopolamine.[80] Using a radioligand binding assay similar to that outlined in section 1.7, mean AA levels in subjects receiving glycopyrrolate were similar to AA in the scopolamine group (e.g., 1.24 nmol/L and 1.27 nmol/L scopolamine equivalents, respectively). However, cognitive performance was only impaired in the group receiving scopolamine. A superior biomarker for central anticholinergic burden will be a measure which takes into account drug distribution into the CNS.

1.8.2 Multiple serum AA unknowns

The change of serum AA across a 24 hour period in the absence and presence of medication changes is not known. It is assumed that serum AA levels change across the day as a subject's medication levels decline. However, this has never been specifically examined.

The stability of serum AA and the relationship between serum AA and cognition across time is also unknown. There is some evidence to suggest that with multiple doses of an anticholinergic drug, muscarinic receptors may be modestly upregulated centrally in nondemented older adults. Thus, it is possible that some individuals experience a reduction in cognitive impairment over time with chronic use of an anticholinergic medication.

The exact relationship between serum AA and cognitive performance is still not known. Mulsant and colleagues reported a nonlinear relationship between serum AA and MMSE (e.g., a measure of global cognition) performance in older community-dwelling persons.[7] In contrast, in two separate studies, a linear relationship ($r = -0.5$) was found between serum AA levels and verbal learning in persons with schizophrenia under 60 years of age.[81, 82] However, all of the studies published to date have used 1) a relatively small number of subjects, 2) a specific patient population, 3) and limited cognitive assessments. The association between AA and performance most likely varies depending on the population examined, which cognitive domain is being assessed (e.g., verbal learning, working memory, global cognition),[67 7] and possible covariates such as age, sex, level of education, and disease state.

It is not known why some persons with higher serum AA do not demonstrate clinically apparent anticholinergic effects, while other patients become confused or even delirious with more modest AA levels. Part of this discrepancy may be due to agents contributing to serum AA, but not crossing the blood-brain barrier. It is also possible that these individuals with higher AA may actually be suffering from cognitive impairment, but given the large heterogeneity of performance in older adults, they still fall within "normal" ranges.

1.8.3 Lack of guidance

The muscarinic receptor binding profiles of most medications and their metabolites have never been examined. Thus, even if a clinician decides that a patient is suffering from anticholinergic-induced toxicity, he/she has little guidance on which medication(s) to adjust.

1.8.4 Sensitivity

The current assay lacks the sensitivity for detection of all possible clinically relevant agents. Specifically, lower concentrations of some medications or drugs with only modest affinity at muscarinic receptors may not show detectable AA. In depressed, institutionalized older adults, no difference was evident between trough serum AA levels in patients receiving the anticholinergic antidepressant paroxetine, as compared to those receiving placebo.[83] However, two out of the twelve subjects receiving paroxetine had to discontinue treatment, due to the development of delirium. Moreover, subjects receiving paroxetine were more likely to have a decrease in global cognitive impairment as measured by the MMSE ($p=0.03$). In fact, all but one of the eight subjects who completed at least four weeks of paroxetine treatment had decreases in MMSE scores as opposed to four out of eleven receiving placebo.

A similar finding was reported in subjects receiving a single oral dose, 20 μg/kg, of the potent anticholinergic agent, scopolamine.[84] Using a slightly modified version of the method reported in section 1.7, serum AA was very low and in most cases below the limit of detection. However, clinically relevant antisialogogue and sedative effects (e.g., anticholinergic adverse effects) were evident within 40 to 60 minutes of scopolamine administration.

1.8.5 A lack of uniformity

Methods for serum AA measurement vary from investigator to investigator and are poorly defined in most publications. This makes it difficult to compare findings across studies and to evaluate cognitive effects at varying levels of serum AA.

1.8.6 Use of rat brain homogenate

Rat forebrain and striatum homogenate is typically used for the source of muscarinic receptors for the serum AA assay. This practice has both advantages and disadvantages as outlined below:

Advantages

1) Serum AA is derived from a single assay which takes minimal time to complete and requires less than 5 mL of blood per subject.

2) Another advantage is the use of native tissue. The current alternative is the utilization of stably transfected cell lines with individual muscarinic receptor subtypes. The use of transfected cell lines is informative, however, is accompanied by a multitude of shortcomings. Foremost, the binding properties and downstream signaling pathways may differ between native and cell line muscarinic receptors (e.g., due to different post-translational modifications, number or type of G proteins present, and muscarinic receptor densities).

Disadvantages

1) Serum AA only informs us that an individual has compound(s) present in his/her serum that affects binding at one or more muscarinic receptors. All five muscarinic receptor subtypes are represented in rat forebrain and striatum homogenate. However, M1 and M4, and to a lesser extent M2 are predominantly expressed.[4] Although many medications have a similar binding profile at each of the muscarinic receptor subtypes, there are multiple agents that exhibit a considerable variation in binding (Table 1.1). The assay may be less sensitive to medications which preferentially bind at M3 and/or M5. Moreover, as reviewed in section 1.2, binding at different muscarinic receptor subtypes may have different effects on memory. Thus an agent contributing to serum AA via M1, M3 and M4 subtypes may have a different correlation with a specific cognitive assessment than an agent that primarily contributes to serum AA via the M4 receptor subtype. In addition, although there are only a few examples of this in the literature, an antagonist that has significantly greater affinity at the M2 receptor (e.g., an autoreceptor), as compared to the other subtypes, may actually demonstrate an improvement in cognitive performance.

2) Although there is considerable homology between human and rodent muscarinic receptors, there may be a discrepancy in ligand binding between species.

Table 1.1. Binding of various agents at muscarinic receptors[a]

Compound	Indication	M1	M2	M3	M4	M5
Acetylcholine[85] IC_{50} for NMS (μmol)	Neurotransmitter for muscarinic and nicotinic receptors	40.1	1.3	49.7	28.4	1.8
Darfenacin[86]	Incontinence	31.1	56.8	2.5	16.1	9.6
Olanzapine[87]	Atypical antipsychotic used for the treatment of schizophrenia and other psychotic disorders	2.5	18	13	10	6
Pirenzepine[88] IC_{50} for NMS	Older agent used for the management of peptic ulcer disease; antagonist used to study binding characteristics	9	540	170	61	28
Quetiapine[87]	Atypical antipsychotic used for the treatment of schizophrenia and other psychotic disorders	120	630	1320	660	2990

[a]Unless otherwise noted, values reported are either equilibrium dissociation (K_d) or inhibitory (K_i) constants in nanomolar (nM) units. Both constants reflect affinity; smaller values indicate greater receptor affinity.
NMS = N-methylscopolamine; IC50 = the concentration of the substrate (e.g., acetylcholine) needed to inhibit 50% of the radioligand (e.g., NMS) from binding to muscarinic receptors; μmol = micromolar

1.8.7 Possible agonist interference with assay

The serum AA assay is designed to limit binding of agonists to muscarinic receptors (e.g., utilization of a buffer with a high molarity salt concentration and use of a radioligand which is a potent antagonist). However, agonist binding affinity has never been formally investigated in the serum AA assay. Low and higher potency agonists need to be examined to determine the extent to which medications with agonist properties could be interfering with the results.

1.9 ADDRESSING SOME OF THE LIMITATIONS OF SERUM AA

This doctoral dissertation addresses in part the first three items outlined in section 1.8. Specifically, we have 1) examined the association between serum AA and cognitive performance in patients with moderate-to-severe dementia [Chapter 2]; 2) investigated the *in vitro* AA of 106 commonly used medications [Chapter 3]; 3) assessed serum AA following administration of the anticholinergic incontinence agent oxybutynin and compared these "*in vivo*" values with the *in vitro* AA of oxybutynin [Chapter 6]; 4) assessed serum AA across an 8 hour time period in older adults following the administration of subjects' regular medication regimen [Chapter 6]; and 5) explored the feasibility of using centrally mediated pupillary oscillations as a marker for central anticholinergic toxicity [Chapter 6].

2.0 SERUM ANTICHOLINERGIC ACTIVITY AND COGNITION IN PATIENTS WITH MODERATE-TO-SEVERE DEMENTIA

[Chew ML, Mulsant BH, Pollock BG. Serum Anticholinergic Activity and Cognition in Patients with Moderate-to-Severe Dementia. *Am J Geriatr Psychiatry* 2005; 13:535-538.]

2.1 INTRODUCTION

Chapter 1 reviewed the possible detrimental effects of anticholinergic medications (e.g., muscarinic receptor antagonists) and the possible utility of serum AA. Medications with anticholinergic properties are associated with a variety of central adverse events including sedation, agitation, cognitive impairment, confusion, and delirium. However, it is difficult to predict who may be at risk for these anticholinergic effects based on medication alone, as the muscarinic binding profile of most drugs and their metabolites is not known. Serum anticholinergic activity (AA), as measured by radioreceptor assay, reflects the cumulative binding of all drugs and their metabolites to muscarinic receptors. Serum AA has been correlated to both serum levels of anticholinergic medications[89] and anticholinergic activity in cerebral spinal fluid following specific agents.[78] In older adults, low serum AA has consistently been associated with cognitive impairment on various specific tasks, while higher serum AA has been associated with frank delirium (see Table 2.1). Unfortunately, the impact of various possible covariates (e.g., Alzheimer's Disease, age, education, intelligence, disease state, caffeine) on the relationship between serum AA and cognition is still not clear. Chapter 2 focuses on the initial exploration of the relationship between serum AA and cognitive performance in patients with moderate to severe dementia.[65]

Patients with dementia are at high risk for cognitive impairment when they receive medications with anticholinergic properties. One study examined changes in serum AA levels in 10 patients with mild to moderate Alzheimer's Disease and 18 non-demented depressed elderly before and after psychotropic adjustment. Increase in serum AA was associated with impairment on measures of recognition and concentration in the Alzheimer's group, but not in the non-

demented group.[90] In another study that measured serum AA as percent ^{3}H-QNB binding (i.e., the assay was not standardized with atropine) a significant correlation was found between muscarinic receptor binding and MMSE scores in 48 subjects with Alzheimer's dementia and 17 with vascular dementia.[91] Finally, patients with mild to moderate Alzheimer's disease were found to suffer from greater cognitive impairment than age-matched control subjects following a single administration of the anticholinergic drug scopolamine.[2]

Since cholinergic neurons and receptors decrease as dementia progresses,[4, 5] patients with more advanced dementia should be at even higher risk for anticholinergic toxicity. However, to our knowledge, only one small study has examined the relationship between serum AA and cognitive performance in patients with moderate to severe dementia: in 22 demented nursing home residents, higher serum AA levels was associated with greater impairment in self-care capacity, but not lower MMSE scores.[69]

We examined the association between serum AA levels and cognitive performance in patients with moderate to severe dementia. We hypothesized that serum AA was associated with cognitive performance as measured by the MMSE and the Severe Impairment Battery (SIB).

Table 2.1. Relationship between serum anticholinergic activity and cognition in older persons: Published studies[65]

Reference	Setting; N	Mean (SD) Age Range	Design	Serum AA (atropine equivalents in pmol/ml)*	Relationship between serum AA and Cognition**
Flacker JM et al: The American Journal of Geriatric Psychiatry, 1998; 6:31-41.	Medical ward; 67	85 (6) ≥ 75	20 delirious pts vs. 47 non-delirious pts	9 (8) 3.5 (4)	Serum AA associated with delirium in multivariate analysis
Flacker JM et al: J of Gerontology: Medical Sciences, 1999; 54A:M12-M16.	Nursing home; 22	88 (5)	8 delirious pts vs. 14 non-delirious pts; all 22 pts were febrile	3.45 (4.25) 3.25 (2.55)	Serum AA not associated with delirium. Serum AA decreased in both groups after recovery ***
Golinger RC et al: Am J Psychiatry, 1987; 144:1218-1220.	Surgical ICU; 25	58 29-76	9 delirious pts vs. 6 non-delirious pts	16.13 (11.4) 2.8 (3.46)	Serum AA higher in patients with delirium
Mach JR, Jr et al: J Am Geriatr Soc, 1995; 43:491-495.	Medical ward; 22	71 (7) ≥60	11 delirious pts vs. 11 control pts	6.05 (3.0) 3.38 (2.5)	Resolution of delirium assoc. with decrease in serum AA
Miller PS et al: Am J Psychiatry, 1988; 145:342-345.	Surgery pts; 30	67 (6) 59-81	Scopolamine (N=14) vs. placebo (N=16)	121.1 (85.5) 11.6 (18.2)	Higher serum AA assoc. with lower cognitive performance
Mondimore FM et al: Am J Psychiatry, 1983; 140:930-931.	Post-ECT pts treated with atropine; 20	49 (17) 17-76	8 pts with lower serum AA 12 pts with higher serum AA	≤ 51.8 > 51.8	Higher serum AA assoc. with decrease in MMSE
Mulsant BH et al : Arch Gen Psychiatry, 2003 ; 60 :198-203	Community; 201	78 (5) 71-95	21 pts with undetectable serum AA 159 pts with low serum AA 21 pts with high serum AA	< 0. 25 0.25-2.79 ≥ 2.8	Higher serum AA assoc. with lower MMSE
Mussi C et al: J Geriatr Psychiatry Neurol, 1999; 12:82-86.	Geriatric ward; 61	79 (12) 66-95	12 pts with delirium 49 pts without delirium	23.0 (15.5) 3.9 (8.4)	Serum AA higher in pts with delirium
Nebes RD et al: Psychopharmacol Bull, 1997; 33:715-720.	Geropsychiatric ward; 36	69 (6)	17 with undetectable serum AA 19 with detectable serum AA	< 0. 25 1.4 (1.3)	Detectable serum AA assoc. with lower cognitive performance
Rovner BW et al: Am J Psychiatry, 1988; 145:107-109.	Nursing home; 22	81 (10)	All residents with cognitive impairment	0.0-9.95 Median: 0.83	Higher serum AA assoc. with greater impairment in self-care
Thienhaus OJ et al: Eur Arch Psychiatry Clin Neurosci, 1990; 240:28-33.	Geropsychiatric ward; 28	65 (9)	10 pts with AD 18 pts without AD	6.17 (4.47) 6.66 (6.23) ****	Higher serum AA assoc. with increase in AC drugs and with lower cognitive performance in AD pts, but not in non-AD pts
Tollefson G et al: The Journal of Neuropsychiatry and Clinical Neurosciences, 1991; 3:314-319.	Nursing home; 34	79 (10) >65	15 "intervention" pts 19 control pts	6.53 (11.75) 11.16 (12.78)	Decrease serum AA associated with decrease in AC drugs and improved cognitive performance
Tune LE et al: Lancet, 1981; 2:651-653.	Postcardiotomy pts; 29	55 29-75	10 delirious pts 19 control pts	SAA > 7.5 in 7/8 SAA > 7.5 in 4/17	Higher serum AA assoc. with lower MMSE
Chew ML et al: Am J Geriatr Psychiatry, 2005, 13:535-538	Geropsychiatric ward; 26	84 (6) 68-95	26 pts with dementia	1.06 (1.20)	Higher serum AA assoc. with worse cognitive performance

Note: AD: Alzheimer's Disease; ICU: intensive care unit; ECT: electroconvulsive therapy

*1 nM of atropine equivalent=1 pmol/mL; 1 pmol/0.200mL=5 pmol/mL; 1 ng/mL=3.455 pmol/mL; **Cognitive performance was assessed on various specific cognitive tests; ***After resolution of fever, mean (SD) of serum AA: 0.5 (0.8) pmol/mL (delirium) and 0.4 (0.6) pmol/mL (no delirium), respectively; ****Baseline mean(SD) serum AA: 3.50 (2.39) and 4.09 (4.83), respectively.

2.2 METHODS

All subjects in this report participated in an ongoing federally-funded clinical trial ("Continuation Pharmacotherapy for Agitation of Dementia", MH59666) for the treatment of behavioral disturbances or psychosis associated with dementia. Between February 2000 and April 2002, all patients admitted to Western Psychiatric Institute and Clinic's geriatric inpatient unit were considered for inclusion if they presented with: behavioral disturbances or psychosis associated with dementia of the Alzheimer type, vascular dementia, dementia with Lewy bodies, mixed dementia, or dementia not otherwise specified. The rating of at least a 3 (i.e., moderate severity) on at least one of the Neurobehavioral Rating Scale (NBRS) agitation items (aggression, agitation, hostility) or psychosis items (delusions, hallucinations, suspiciousness) was also required. Exclusion criteria included current or past diagnosis of schizophrenia, schizoaffective disorder, delusional disorder, Psychotic Disorders Not Otherwise Specified (NOS), or bipolar disorder. Patients with a current diagnosis of delirium, substance-induced persisting dementia, Parkinson's disease, drug/alcohol abuse or dependence, or depression were also excluded. As required by the Institutional Review Board at the University of Pittsburgh, after study procedures were fully explained, written informed consent was obtained from patients' authorized legal representatives, with patients' verbal assent given. Participants were permitted to continue receiving a cholinesterase inhibitor if they had been taking it for at least 12 weeks prior to the study and had been maintained on the same dose for at least 4 weeks. Use of lorazepam was permitted for immediate control of severe agitation and aggression. All other psychotropics were discontinued.

Only baseline data was used for this analysis. In other words, all subjects met the criteria for behavioral disturbances or psychosis at the time of cognitive testing and blood sampling. During the recruitment period, 50 individuals met the eligibility criteria and consented to participate. Baseline serum AA was available for 35 of them. Reasons for missing serum AA included: excessive agitation or aggression (6), refusal of blood draw (2), and other (7). Within three days of obtaining baseline serum AA, the MMSE and the SIB were administered. Of the 35 subjects with serum AA, 17 subjects were able to complete both instruments; 8 subjects completed only the MMSE; one subject completed only the SIB. The primary reasons for incomplete assessments were excessive agitation and/or subject refusal.

The assay used to measure serum AA is described in detail in section 1.7 above.

Descriptive statistics were calculated for age, sex, diagnoses, number of prescription medications, number of subjects receiving a cholinesterase inhibitor or lorazepam, serum AA, MMSE scores, and SIB scores. Nonparametric analyses were performed as serum AA levels were not normally distributed. The association between MMSE or SIB scores and serum AA was assessed with an Exact Spearman using a Monte Carlo method, with 100,000 tables sampled.

2.3 RESULTS

The subjects' mean (SD) age was 83.6 (5.8) years; 12 (46%) were women; 22 (85%) and 4 (15%) had an admission diagnosis of Dementia of the Alzheimer type and Dementia NOS, respectively. The mean (SD) number of prescription medications was 6.2 (3.5); 6 subjects (23%)

were taking a cholinesterase inhibitor; 15 subjects (58%) had received a PRN dose of lorazepam within 24 hours of serum AA blood draw or cognitive assessments.

Serum AA was detectable in 16 (62%) of the 26 subjects; the mean (SD) serum AA was 1.06 (1.20; range: 0-3.70) pmol/mL. The mean (SD) MMSE and SIB scores were 12.4 (8.5) and 76.3 (25.6), respectively. There was a significant correlation between serum AA and MMSE (Spearman r = -0.398, n=25, p = 0.049). Serum AA and SIB were similarly correlated, although this did not reach significance (r = -0.405, n=18, p = 0.095).

2.4 DISCUSSION

We found an association between serum anticholinergic activity (AA) and cognitive performance in a group of patients with moderate to severe dementia. Our results extend the findings from thirteen previous studies conducted in older adults (Table 2.1), mostly in nondemented persons or patients with mild dementia. Taken together, these data support that medications that have anticholinergic properties should be avoided in patients with dementia. This includes not only medications classified as "anticholinergic medications" (e.g., benztropine or oxybutynin) or medications with well recognized anticholinergic properties (e.g., amitriptyline or diphenhydramine), but also medications that have more modest binding to muscarinic receptors individually (e.g., digoxin, prednisone, or warfarin) that can result in elevated serum AA when taken together.[7]

Patients with dementia have been found to be more likely to receive medications with anticholinergic properties than age-matched controls.[8] However, discontinuing medications in older frail elderly is often perilous. In the future, measurement of serum AA could be used to identify at-risk patients who should be targeted for aggressive discontinuation of medications with anticholinergic properties.

This study was limited by a small sample size. In addition, some subjects were unable to complete one or both cognitive assessments, typically because of severe behavioral disturbances. This limits our conclusions regarding this subset of patients. Nevertheless, the results of our study support the need for future investigations of the association of serum AA and cognitive performance, including those with moderate to severe dementia in whom anticholinergic medications may be particularly deleterious.

3.0 *IN VITRO* ANTICHOLINERGIC ACTIVITY OF 106 COMMONLY USED MEDICATIONS

[Chew ML**,** Mulsant BH, Pollock BG, Lehman ME, Greenspan A, Kirshner MA, Bies RR, Gharabawi G. A model of Anticholinergic Activity of Atypical Antipsychotic Medications. *Schizophrenia Research.* 2006; 88(1-3): 63-72.]

[Chew ML, Mulsant, BH, Pollock BG, Lehman ME, Greenspan A, Mahmoud RA, Kirshner MA, Sorisio DA, Bies RR, Gharabawi G. Anticholinergic Activity of 106 Medications Commonly Used by Older Adults. *JAGS*, Under Review.]

As stated, serum AA holds promise for use in a clinical setting to determine possible anticholinergic-induced cognitive impairment. However, the muscarinic receptor binding profiles of most medications and their metabolites have never been examined. Moreover, the central effects of an anticholinergic medication depend on the dose administered and subsequent plasma concentrations. Therefore, even knowing whether or not a medication is anticholinergic may not be sufficient. Thus, if a clinician decides that a patient is suffering from anticholinergic-induced toxicity, he/she has little guidance on which medication(s) to adjust.

We examined the *in vitro* AA of 106 common medications. Our objective was to assess AA at various therapeutic concentrations and to then translate this data into a dose-AA relationship. The following articles summarize our findings, with a focus on atypical antipsychotics.

3.1 A MODEL OF ANTICHOLINERGIC ACTIVITY OF ATYPICAL ANTIPSYCHOTIC MEDICATIONS[92]

3.1.1 Introduction

Over the past decade, several atypical antipsychotics have become widely used.[93] These atypical antipsychotics are hypothesized to improve psychotic symptoms through similar mechanisms. However, each of these medications has a unique pharmacological profile and has been associated with differing frequencies of adverse effects.[93] From a pharmacological perspective, one important difference between the atypical antipsychotics is the variation in binding to muscarinic receptors.

Muscarinic receptors are thought to be involved in multiple central processes including body temperature, movement, analgesia, arousal, attention, and cognition. To date, five specific muscarinic receptor subtypes have been identified.[13, 27, 94, 95] All 5 subtypes are present in the central nervous system (CNS) with a predominance of M1, M2, and M4.[4]

Specific muscarinic receptor substrates and knock-out mice have become available to study the role of individual muscarinic receptor subtypes only during the past several years. Recent studies have suggested that M1, M2, and M4 are important for learning and memory.[36, 37, 94, 96-98] M1 and M4 are also thought to play a role in motor control[99, 100] and pharmacotherapy of psychoses (e.g., muscarinic receptor agonists have antipsychotic activity).[95, 100, 101]

Early pharmacological data have documented that clozapine, olanzapine, and quetiapine have significant affinity at muscarinic receptors, relative to their affinity at dopamine 2 (D2) receptors (Table 3.1). Conversely, *in vitro* binding at muscarinic receptors is negligible for aripiprazole, risperidone or ziprasidone. These *in vitro* differences may have clinical significance. Mulsant and colleagues reported elevated anticholinergic activity (AA) in patients with dementia following initiation of treatment with olanzapine.[102] Similarly, Tracy and colleagues found that schizophrenic patients taking clozapine or olanzapine had higher AA than those receiving risperidone.[103] Higher AA was associated with impairment in performance, verbal learning and executive control.

Table 3.1. Binding of atypical antipsychotics at dopamine 2 (D2) and muscarinic receptors (MR)[a]

Medication	D2[b]	MR[b]	D2/MR	MR1[d]	MR2[d]	MR3[d]	MR4[d]	MR5[d]
Aripiprazole	0.45[c]	>10,000[c]	<0.0001	6780[e]	3510[e]	4680[e]	1520[e]	2330[e]
Clozapine	210	9	23.3	1.4	10	7	6	5
Olanzapine	20	36	0.56	2.5	18	13	10	6
Quetiapine	770	1400	0.55	120	630	1320	660	2990
Risperidone	3.77	34,000	0.0001	>10,000	>10,000	>10,000	>10,000	>10,000
Ziprasidone	2.6	2440	0.001	5100[f]	>3000	>1300	>1600	>1600

[a]Values reported are either equilibrium dissociation (K_d) or inhibitory (K_i) constants in nanomolar (nM) units. Both constants reflect affinity; smaller values indicate greater receptor affinity.
[b]Used human brain (caudate nucleus) homogenate in buffer with [^{3}H]-spiperone for D2 and [^{3}H]-QNB for MRs.[104]
[c]Specific details regarding the source of receptors and the radioligands used were not provided (Bristol-Myers Squibb, data on file, [105]).
[d]Used clonal cell membranes in buffer with [^{3}H] N-methylscopolamine.[87, 94, 106]
[e]Used clonal cell membranes in buffer with [^{3}H]-QNB.[107]
[f]Used clonal cell membranes with [^{3}H] N-methylscopolamine.[108]
Please note, Bymaster and colleagues reported the binding affinity of ziprasidone at MR1 to be 300 nM.[94]

In addition, anticholinergic medications may reduce the effectiveness of antipsychotic medications.[109] In one small cross-over study, increases in muscarinic receptor binding in the striatum with a change in olanzapine dose from 5 to 20 mg was correlated to an increase in negative symptoms in persons with schizophrenia.[110] On the other hand, muscarinic receptor blockade may protect an individual from developing extrapyramidal symptoms associated with antipsychotic use.[66, 111, 112] Thus, patients receiving an antipsychotic medication with anticholinergic properties may have an improved clinical outcome by virtue of enhanced tolerability and compliance.[113]

Thus far, the comparisons of the anticholinergic potential of the atypical antipsychotics have been based mostly on their equilibrium dissociation (K_d) or inhibitory (K_i) constants for the muscarinic receptors. However, it is difficult to predict the *in vivo* anticholinergic effects of various doses of a given medication based on K_d or K_i alone. Data obtained using Single Photon Emission Tomography (SPECT) imaging has provided preliminary evidence of the relationship between dose and central muscarinic receptor binding.[110, 114, 115] Both clozapine and olanzapine showed dose-dependent increases in muscarinic receptor occupancy in multiple brain regions of schizophrenic patients including the thalamus and frontal and temporal cortices.[110, 115] Patients treated with risperidone showed a small increase in muscarinic receptor occupancy in the striatum, although these findings may have been an artifact of comparing occupancy with healthy controls.[114] Although informative, the above studies had small samples sizes with a limited number of doses examined.

A radioreceptor assay (RRA) is available to quantify a person's overall anticholinergic burden caused by the cumulative effects of all drugs, metabolites, and potentially endogenous substances — referred to as anticholinergic activity (AA).[7, 65, 66] This *in vitro* assay examines the amount of displacement of the muscarinic receptor antagonist tritiated quinuclidinyl benzilate (^{3}H-QNB) caused by compounds present in an individual's serum (or plasma). ^{3}H-QNB is a specific antagonist with high affinity for all muscarinic receptor subtypes.[116] Peripheral AA has been correlated with serum levels of anticholinergic medications[71, 72, 112], as well as AA in cerebral spinal fluid.[78]

We used such a RRA to develop an *in vitro* model to assess the potential *in vivo* AA of various dosages of six atypical antipsychotics. The assay we utilized employs similar methods used previously to determine the K_i or K_d of medications with the most relevant differences being the use of human serum in each sample, an atropine standard curve to allow comparisons between medications, and focusing on clinically relevant concentrations. The concentrations of atypical antipsychotics that we examined were based on published reports of typical serum or plasma drug levels (Table 3.2). Subsequently, to make these data more useful to clinicians, we used published pharmacokinetic data to translate the concentration-AA relationship into an estimated dose-AA relationship. We propose that clinicians can use this model to estimate the relative anticholinergic risks and benefits of a given dose of an atypical antipsychotic.

Table 3.2. Typical dose and concentration ranges of atypical antipyschotics[a]

Drug	Typical dose range in adults (mg/day)	Typical dose range in elderly (mg/day)	Typical concentration ranges (ng/mL)	Concentrations tested for *in vitro* AA (ng/mL)
Aripiprazole	10–30	10–30	0–1000	10, 50, 100, 250, 500, 1000
Clozapine	300–900	25–400	0–1500	10, 50, 100, 250, 500, 1000, 1500
Olanzapine	7.5–30.0	5–15	0–150	10, 25, 50, 100, 150, 250
Quetiapine	200–800	50–300	0–1500	50, 200, 500, 1000, 1500, 2500
Risperidone	1.5–6.0	0.5–3.5	0–50	10, 50, 100, 250, 500, 1000
Ziprasidone	40–160	40–160	0–750	10, 50, 100, 250, 500, 1000

[a]Aripiprazole, package insert; Clozapine, package insert; Olanzapine package insert; Quetiapine, package insert; Risperidone package insert; Ziprasidone, package insert; [117-119] [93, 120-123] [124, 125]

3.1.2 Methods

Materials

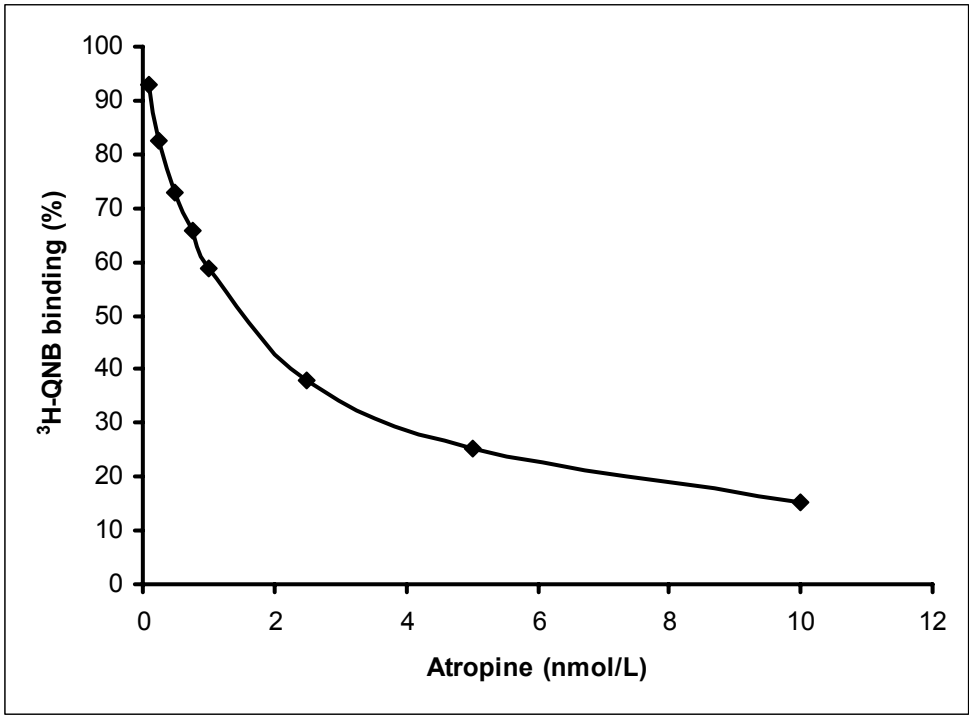
Aripiprazole, olanzapine and quetiapine fumarate were purchased from Sequoia Chemicals and clozapine from Sigma-Aldrich, risperidone and ziprasidone hydrochloride were purchased in pill form from our hospital pharmacy. Stock concentration of all medications was 1 mg/mL. Aripiprazole/clozapine and olanzapine/quetiapine were completely dissolved in methanol and 0.1 N HCl, respectively. Drugs purchased in pill form were placed in 0.1 N HCl, vortexed, and placed on an Eberbach shaker on high for 30 minutes and then centrifuged at 600 g for 5 minutes, at 4°C to remove excipients.

Procedure

In vitro AA of the antipsychotics was measured using a modified version of a competitive radioreceptor binding assay previously described (Section 1.7)[65, 66, 102] For the standard curve samples, a homogenized mixture of rat (Sprague-Dawley males, approximately 200-225 g) forebrain and striatum were added to a mixture of varying concentrations of atropine (0.1 nM – 10 nM), drug-free human off-the clot serum (Scantibodies), and 50 mM sodium phosphate buffer, pH 7.7. For medication samples, an antipsychotic was added in place of atropine (Table 3.2). The remainder of the assay is as reported in detail in section 1.7 *In vitro* AA results are reported as picomoles of atropine equivalents per milliliter (pmol/mL). Assay values were standardized to pmol/mL by multiplying by 5: 1 pmol/0.2 sample = 5 pmol/mL based on the amount of ^{3}H-QNB displaced by drugs as compared to the atropine standard curve (Figure 3.1). The acceptable assay range is 0.5–250 pmol/mL, above which results are not reproducible. Thus, *in vitro* AA values <0.5 pmol/mL are reported as 0 and values >250 pmol/mL are reported as

>250 pmol/mL. All standard curve and drug samples were run in triplicates. Previously made controls of low and medium atropine concentrations were run with each assay. Oxotremorine sesquifumarate (100 μM, Sigma-Aldrich) was used to assess for nonspecific binding.

Figure 3.1. Atropine standard curve



An atropine standard curve was run on each assay day. The *in vitro* anticholinergic activity (AA) of a drug was based on the decrease in ^{3}H-QNB muscarinic receptor binding in the presence of this drug. Change in radioligand binding with a drug was related to the amount of atropine needed to displace the same amount of ^{3}H-QNB

Analysis

Atropine standard curve and *in vitro* AA were calculated using weighted logit-log regression. Values reported are averages of two assays, run on separate days. Concentrations versus *in vitro* AAs were plotted for each medication. For the medications which demonstrated AA the average peak concentrations (C_{max}) at steady-state following oral administration of clinically relevant

daily doses were estimated (see below). Interpolations of the concentration-AA plots were used to determine AAs at a given C_{max}. These doses and AAs were then plotted to generate dose-AA curves.

The following pharmacokinetic principles were used to estimate the average C_{max} at each dose for clozapine, olanzapine, and quetiapine:

(1) Clozapine: The average clozapine C_{maxs} for both younger and older adults were determined based on nomograms developed from therapeutic drug monitoring data from 3782 (10% greater than 50 years) patients.[124] The results of this study are consistent with the findings of other studies. For this current report, C_{max} was estimated for male and females separately, given the sex-associated differences in the pharmacokinetics of clozapine (Table 3.3). To be conservative, a positive smoking status was assumed, given the high prevalence of smoking in persons with schizophrenia and the demonstrated decrease in serum clozapine concentrations in smokers. We estimated trough concentrations for older adults to be 25% higher than the average predicted trough concentrations provided in the nomograms (e.g., the model predicted concentrations to increase 4% every 5 years beyond the referenced 40 years). The equation $C_2 = C_1 * e^{-kt}$ was used to calculate C_{max} from trough concentrations. The parameters k and t were estimated using half-life ($t_{½}$) and T_{max} (time at which C_{max} is reached) published values (Clozapine, package insert 2005).

(2) Olanzapine: The average olanzapine C_{maxs} for both younger and older adults were calculated based on data collected from 250 patients (42 [17%] were older than 59 years).[119] The results of this paper approximate findings from other studies. C_{max} was estimated for male and females separately, given the sex-associated differences in the pharmacokinetics of olanzapine

(Table 3.4). Again, a positive smoking status was assumed, given the demonstrated decrease in serum olanzapine concentrations in smokers. We estimated trough concentrations for older adults to be approximately 27% higher than trough concentrations for younger adults based on the values reported by Gex-Fabry and colleagues.[119] The equation $C_2 = C_1 * e^{-kt}$ was used to calculate C_{max} from trough concentrations. The parameters k and t were estimated using $t_{½}$ and T_{max} values reported in the literature.[126]

(3) Quetiapine: The average quetiapine C_{maxs} for both younger and older adults were calculated based on a study that reported C_{max} at steady-state with administration of 250 mg, three-times daily (Jaskiw et al., 2004). Linear pharmacokinetics was assumed in estimating C_{max} at additional doses. There are changes in quetiapine pharmacokinetics with age. However, peak plasma concentrations are similar in younger and older adults.[121, 127] Thus, one dose-AA curve was generated for all ages.

Table 3.3. Estimated C_{max} values, based on total daily dose of clozapine

Clozapine	C_{max} (ng/mL)			
Dose (mg/day)	Adult male	Adult female	Elderly male	Elderly female
50	95	112	107	139
100	164	190	203	234
200	302	345	374	428
300	388	474	481	588
400	500	586	621	728
600	690	819	855	1016
800	862	1035	1069	1283

Table 3.4. Estimated C_{max} values, based on total daily doses of olanzapine

Olanzapine	C_{max} (ng/mL)			
Dose (mg/day)	Adult male	Adult female	Elderly male	Elderly female
2.5	5.5	6.1	6.4	7.1
5	11	12.2	12.8	14.2
10	22	24.4	25.6	28.4
15	33	36.6	38.4	42.6
20	44	48.8	51.2	56.8
30	66	73.2	76.8	85.2

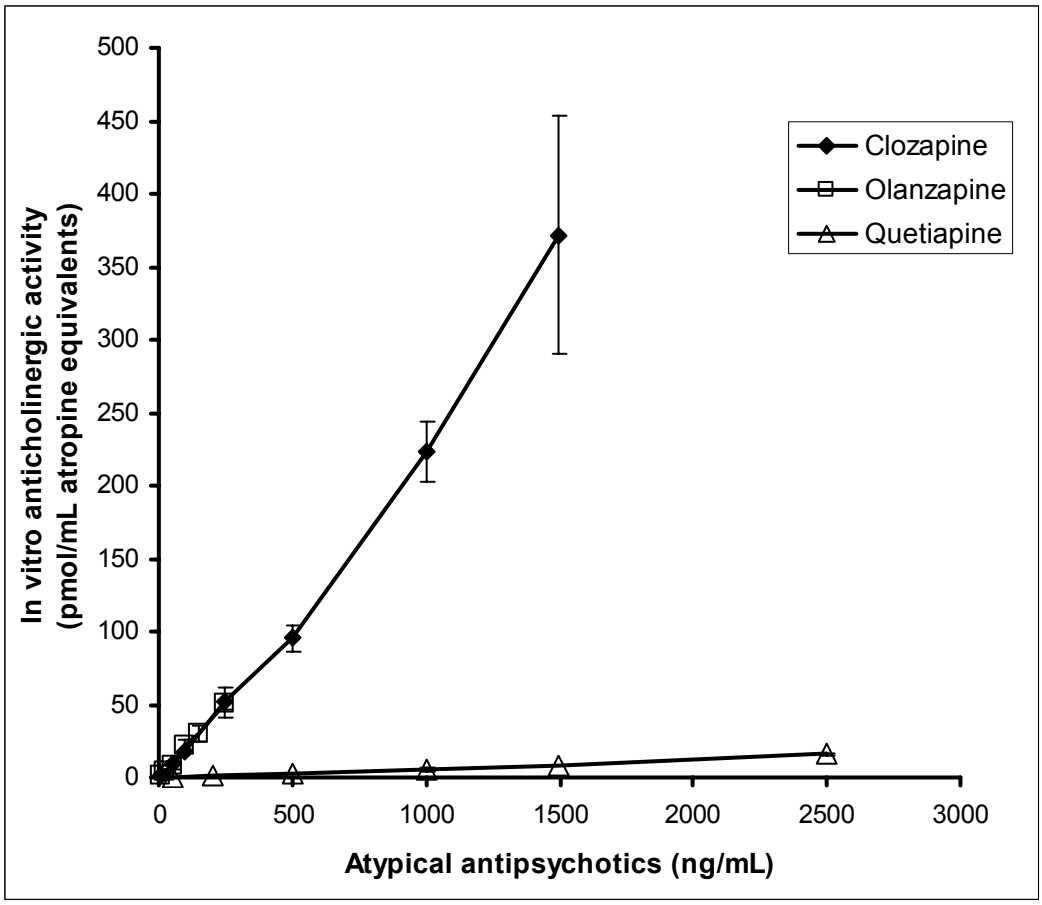
3.1.3 Results

Table 3.2 presents the typical dose and concentration range for each antipsychotic tested. Clozapine, olanzapine, and quetiapine all showed a concentration-dependent increase in AA (Figure 3.2). The highest concentration of clozapine (1500 ng/mL) resulted in an AA above the acceptable assay range. Aripiprazole, risperidone, and ziprasidone did not demonstrate any AA at concentrations up to 1000 ng/mL (data not shown).

Figures 3.3 to 3.5 show the dose-AA relationship for clozapine, olanzapine and quetiapine. The average C_{max} calculated for each daily dose of clozapine and olanzapine (Tables 3.3 and 3.4) was used to link the *in vitro* concentrations to the daily dose, and subsequently to estimate the relationship between daily dose and *in vitro* AA. For example, the average C_{max} at 600 mg/day of clozapine for an adult male is 690 ng/mL. Using Figure 3.2, the *in vitro* AA for 690 ng/mL of clozapine is approximately 142 pmol/mL. Thus, the estimated AA for a patient taking 600 mg/day of clozapine is 142 pmol/mL. This estimated AA of 142 pmol/mL is then plotted for this dose of 600 mg/day.

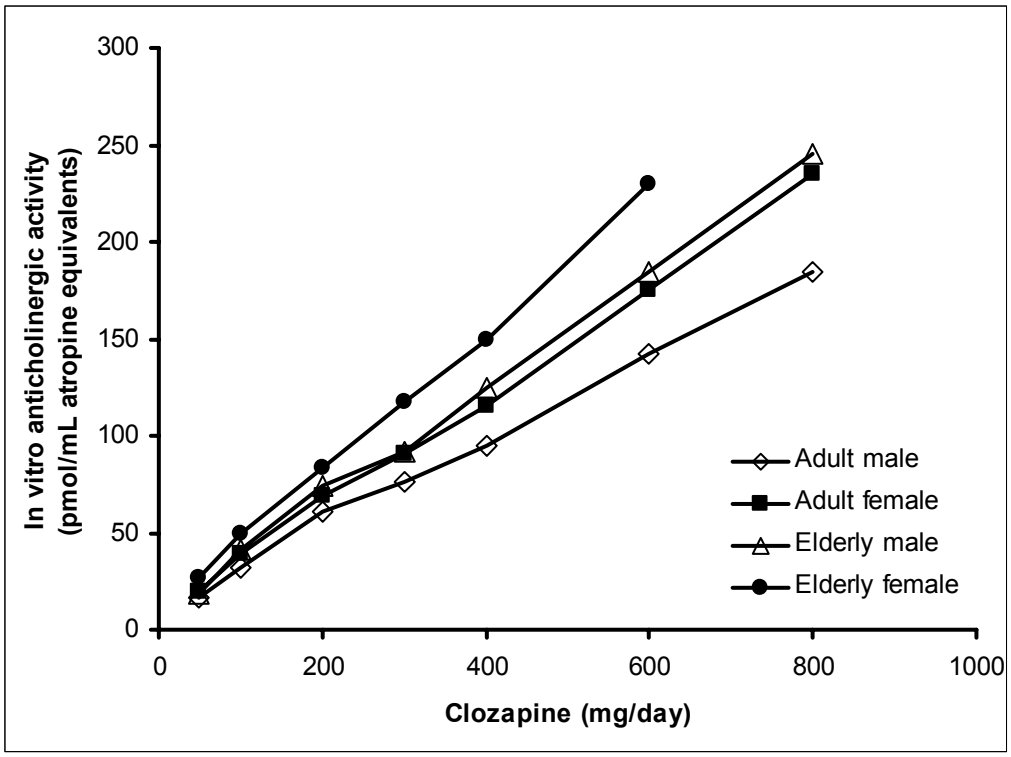
Based on calculated plasma concentrations of each drug at therapeutic doses, AA was estimated to range from 27–250, 1–15, and 0–5.4 pmol/mL of atropine equivalents for clozapine, olanzapine, and quetiapine, respectively (Figures 3.3 to 3.5). At lower doses of quetiapine (less than 100 mg/day), AA was estimated to be 0. At the upper range of daily doses for clozapine and olanzapine, the estimated AA was higher for older adults than for younger ones. The highest dose of clozapine (800 mg/day) resulted in an estimated AA higher than 250 pmol/mL of atropine equivalents, and was therefore not shown in Figure 3.3. For aripiprazole, risperidone, and ziprasidone, the estimated AA for all therapeutic doses was 0.

Figure 3.2. ***In vitro*** **AA of atypical antipsychotics**



In vitro AA for each atypical antipsychotic concentration was tested in triplicates in two assays, run on separate days. The error bars represent the standard deviation of the interassay averages.

Figure 3.3. Dose-AA relationship for clozapine



A dose of 800 mg for elderly females resulted in an *in vitro* AA over 250 pmol/mL. This value was outside the range tested and was therefore not plotted in the graph.

Figure 3.4. Dose-AA relationship for olanzapine

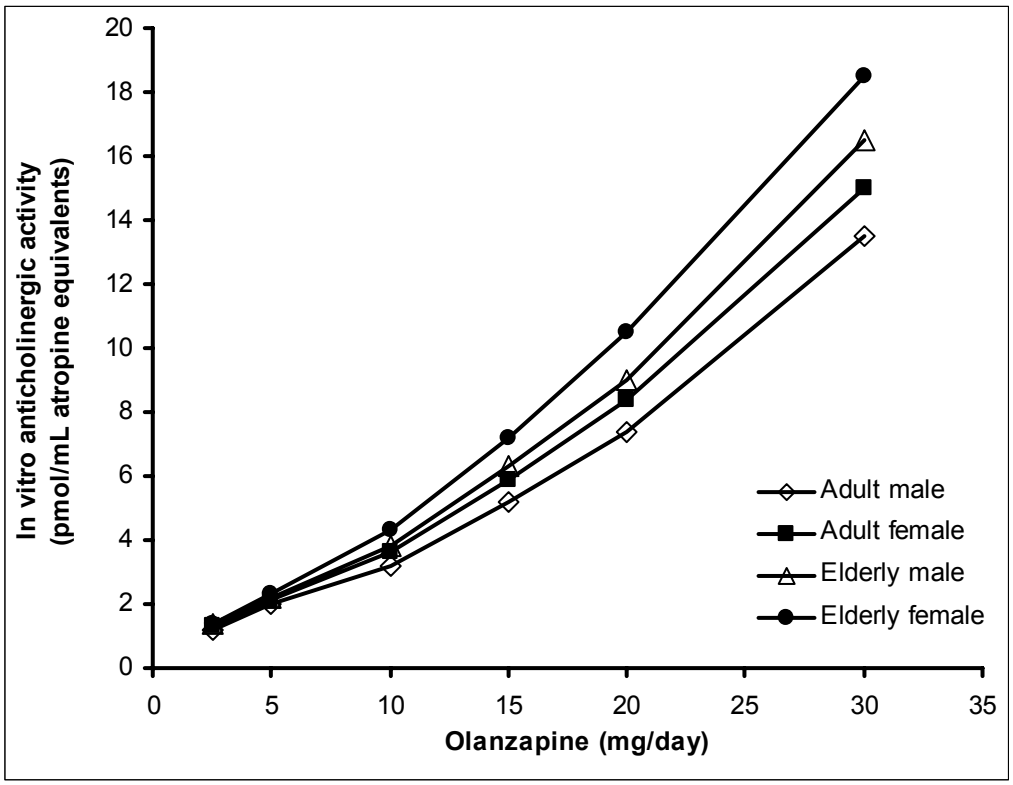
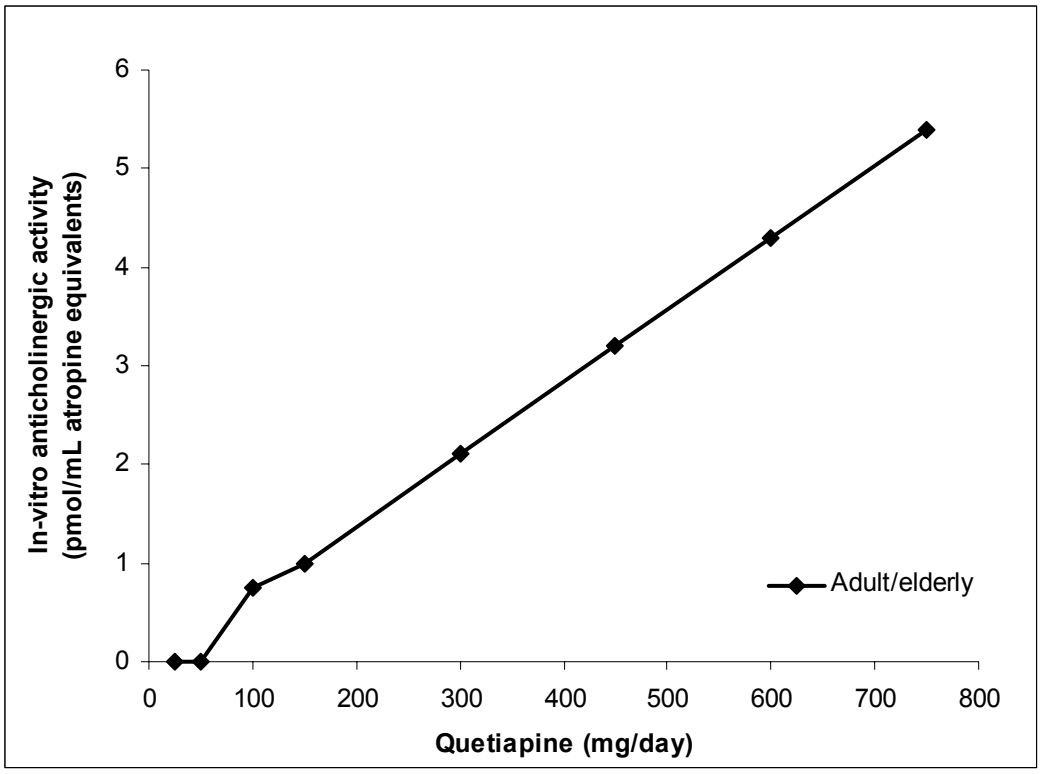


Figure 3.5. Dose-AA relationship for quetiapine



3.1.4 Discussion

We developed a model of anticholinergic activity (AA) for six atypical antipsychotics. With this model, the estimated AA for therapeutic doses of aripiprazole, risperidone, and ziprasidone was 0. In contrast, clozapine, olanzapine, and quetiapine were estimated to have clinically significant dose-dependent increases in AA within their therapeutic range.

Our model of dose-AA relationship has several potential limitations. The estimated dose-AA is a way to assess possible anticholinergic burden. However, the risk of anticholinergic burden needs to be considered within the overall clinical history of each patient (e.g., past sensitivity to anticholinergic agents, extrapyramidal symptoms, effectiveness of an antipsychotic agent,

adherence concerns). For reasons that are not completely understood, some individuals receiving a medication with high AA may not develop clinically apparent anticholinergic effects, while other patients become confused or even delirious on a drug with more modest AA. Our model does not allow one to predict which individuals are at greater risk for anticholinergic toxicity at a given AA level.

In addition, we used average C_{max} estimates that do not take into account inter- or intra-individual variation. As with most medications, there is a large variability in plasma or serum concentrations with a given dose of an atypical antipsychotic due to both environmental and genetic causes. We were conservative and assumed a positive smoking status for individuals receiving clozapine or olanzapine. Nonsmokers have on average 48% and 12% higher serum concentrations at a given dose of clozapine[124] and olanzapine,[119] respectively.

Both risperidone and ziprasidone were analyzed using pill forms purchased from our hospital pharmacy. Impurities or lower concentrations of the drug of interest might have biased the results. However, our results for both risperidone and ziprasidone are comparable to what has been reported previously (Table 3.1).

We used rat forebrain and striatum homogenate for the source of muscarinic receptors. Similar to human forebrain and striatum, all 5 muscarinic receptor subtypes are represented in these brain regions, with M1 and M4 and to a lesser extent M2 being predominantly expressed.[128] Thus, the assay is less sensitive for binding of medications that may have specificity for M3 or M5.

Furthermore, although there is considerable homology between human and rodent muscarinic receptors, there may be a discrepancy in ligand binding between species.

Our assay is designed to optimize the binding of antagonists.[7, 65, 66] However, *in vitro* AA may reflect binding of agonists and thus may not purely be a measure of AA. Some studies have shown that clozapine and olanzapine act as partial agonists under specific *in vitro* conditions.[94, 111, 129] However, it is thought that both of these agents function as antagonists *in vivo*.[94] Thus, we believe that the values reported here for clozapine and olanzapine represent almost exclusively antagonist activity.

Finally, each medication was assessed independently in an *in vitro* system. Although we used human serum in each sample, this *in vitro* assay may not take into account possible interaction of medications and endogenous substances with muscarinic receptors.[74] Future studies need to address possible AA of metabolites of the atypical antipsychotics. Norclozapine, an active metabolite of clozapine, is thought to be a muscarinic agonist and it may mitigate the anticholinergic effect of clozapine. Hence, when clozapine is taken *in vivo*, the overall anticholinergic effect may be less than what would be expected based on its relatively high *in vitro* AA.

For older patients, with or without dementia, any AA is considered detrimental (for a review, see section 1.6.3 and Table 2.1). For younger patients, it is not as clear whether any AA is detrimental or whether there is a threshold AA over which adverse cognitive effects occur. However, multiple studies have shown a relationship between AA and specific areas of cognition

known to be impaired by anticholinergic medications (Table 3.5). Perlick and colleagues[81] and Tune and colleagues[82] independently investigated cognitive performance in patients with schizophrenia taking conventional antipsychotics. Both reported an inverse correlation ($r = -0.5$) between serum AA and verbal learning scores. Tracy and colleagues examined serum AA and cognitive performance in patients with schizophrenia taking clozapine, olanzapine, or risperidone.[103] Higher AA was associated with impairment in verbal learning and executive control. Using an anticholinergic index (based on published reports of medication affinity at muscarinic receptors), Minzenberg and colleagues found an association between anticholinergic load and performance on multiple cognitive tests including verbal learning, visual memory, and praxis.[130] Moreover, anticholinergic load accounted for approximately 10% of the variance in measures of specific cognitive tests and was suggested to contribute up to 30–60% of the memory deficits seen in some patients with schizophrenia.

In conclusion, these data support that clozapine, olanzapine, and quetiapine have a dose-AA relationship, while aripiprazole, risperidone, and ziprasidone, do not show AA at any dose within their clinical range. This is supported by literature findings that clozapine, olanzapine, and to a lesser extent quetiapine, are associated with anticholinergic adverse events.[93, 103, 131] Our model may be a useful tool to determine the dose-AA relationship of newer atypical antipsychotics and other psychotropic medications that might have anticholinergic effects related to their activity at muscarinic receptors.

Table 3.5. Published studies of *in vivo* anticholinergic activity in patients with schizophrenia

Study	Setting; N	Age mean (SD) or range	Design	Mean (SD) or [range] serum AA (atropine equivalent)	Relationship between serum AA and cognition or other clinical outcomes
Chengappa et al., 2000[132]	Outpatients; 24	39	Clozapine Olanzapine Serum AA samples were taken after morning medication dose	27.4 (16.5), [4.5–65.5] 4.8 (3.0), [1.2–10.6]	No association between AA and MMSE scores
de Leon et al., 2003[133]	Inpatients; 40	Not reported	Clozapine 100 mg/day 300 mg/day 600 mg/day	1.4 (1.1) 1.9 (1.2) 2.8 (1.6)	Higher AA associated with constipation
Hitri et al., 1987[134]	Inpatients; 15	28–60	Patients receiving typical antipsychotics and amantadine, benztropine, or trihexyphenidyl. Serum AA samples were taken 12 hours post AC drug administration.	[0–1.2]	AA increased 13-fold following initiation of benztropine and 2.5-fold following trihexyphenidyl. No change in AA following initiation of amantadine
Katz et al., 1985[135]	Outpatients; 22 (12 with schizophrenia)	51 (15)	14 patients receiving AC drugs	3.8 (5.4)	Mean MMSE score: 22/30. No association between AA and verbal learning
Perlick et al., 1986[81]	Inpatients; 17	33 25–49	Patients receiving neuroleptics; 4 taking AC drugs. Serum AA samples taken ≥4 hours post medication	10.6 (8.3), [0–28]	Inverse correlation between AA and verbal learning ($r = –0.54$, $p = 0.01$). No association between AA and recognition or nonverbal recall memory
Strauss et al., 1990[136]	Outpatients; 10	31 21–43	Patients taking AC drugs for EPS. Patients assessed twice, before and after a 2 week period. Time of medication administration not controlled; compliance questionable	Of two samples, higher AA: 3.61 (2.1); lower AA: 1.06 (1.2).	AA associated with verbal learning scores
Tracy et al., 1998[137]	Inpatients; 22	45 (8) 31–58	Clozapine Risperidone	21.8 (12.0), [8.5–46.5] 1.4 (1.5), [0–4.0]	No association between AA and MMSE scores

			Serum AA samples were taken after morning medication dose		
Tracy et al., 2001[103]	Inpatients; 38	40 (10)	Clozapine or olanzapine Risperidone Patients were not taking any other AC medication, or medications known to affect cognition. Serum AA samples were taken after morning medication dose	16.0 (13.0) 1.0 (1.0)	Higher AA associated with impaired verbal learning and executive control
Tune and Coyle, 1980[66]	Inpatients (80%) and outpatients; 35 (31 with schizophrenia)	35 21–65	Patients receiving neuroleptic and AC drugs	[0–50]	Higher serum AA associated with lower EPS
Tune and Coyle, 1981[89]	Inpatients (82%) and outpatients; 109 (95 with schizophrenia)	36 18–81	Patients receiving neuroleptic and AC drugs	[0–50]	Higher serum AA associated with lower EPS
Tune et al., 1982[82]	Outpatients; 24	36 20–58	Patients receiving neuroleptics; 15 taking AC drugs.	12 (2.5), [0–38]	Inverse correlation between AA and verbal learning ($r = –0.51$, $p < 0.01$)

AA: anticholinergic activity; AC: anticholinergic; EPS: extrapyramidal side–effects; MMSE: Mini-Mental State Examination

[a]Various units have been used to report AA leading to some inconsistencies: some investigators have used pmol/0.2 mL sample and pmol/mL interchangeably. Others use a conversion factor of 5 (e.g., 1 pmol/0.2 mL = 5 pmol/mL). Given the uncertainty of some published data, all values in this Table are presented exactly as published in the original reports without regard to units.

3.2 ANTICHOLINERGIC ACTIVITY OF 106 MEDICATIONS COMMONLY USED BY OLDER ADULTS

3.2.1 Introduction

Cognitive decline in nondemented older adults is typically attributed to age-related changes in the central nervous system. A portion of this decline, however, may actually be due to the anticholinergic effect of medications. Studies conducted in older adults have shown that drugs that block muscarinic receptors cause impairment in various cognitive functions including working memory, episodic memory, processing speed, and praxis.[2, 3, 7, 42] Moreover, use of anticholinergic medications has been shown to be a significant predictor of overall performance on general daily activities, mild cognitive impairment, and delirium.[58, 69, 138, 139]

Unfortunately, it is difficult to predict who is at risk for anticholinergic toxicity based on medication use alone. It is not known how most prescribed and over-the-counter drugs and their metabolites affect the cholinergic system. To complicate matters, central anticholinergic effects depend both on the agent and on its dose. Thus even knowing whether or not a medication is anticholinergic may not be sufficient. For instance, amitriptyline and paroxetine are both anticholinergic medications, but they have greatly different potencies at muscarinic receptors. Administration of amitriptyline to an older adult will most likely result in greater memory deficits than that of paroxetine. In addition, a higher therapeutic dose of an anticholinergic agent would be expected to produce greater central effects than that of a lower dose, but not necessarily in a linear fashion.

A radioreceptor assay has been used in research to quantify a person's overall anticholinergic burden, referred to as anticholinergic activity (AA).[7, 65, 66] Serum AA is the measure of binding of all of the compounds present in a person's serum (e.g., medications, metabolites, and possibly endogenous substances) to muscarinic receptors. Up to 90% of older adults living in the community have detectable serum AA.[7, 67] Thus up to 90% have compounds (e.g., medications, metabolites, and possibly endogenous substances) present in their sera that block muscarinic receptors. Several studies have shown that serum AA is associated with global cognitive performance, verbal memory, self-care capacity, and presence of delirium in older nondemented or mildly demented elderly.[7, 67-70] Moreover, serum AA was found to be a stronger predictor of cognitive impairment than age or the total number of all medications an individual is taking (e.g., both prescription and over the counter).[7]

Tune and colleagues have previously reported on the *in vitro* AA of some commonly used medications. [140, 141] However, they only investigated AA at a single concentration of 10^{-8} M [141] that may not be clinically relevant for many of the drugs studied. Building on Tune's original findings, we assessed the *in vitro* AA of 106 prescription and over-the-counter medications at various therapeutic concentrations representative of dose ranges commonly used in the elderly. To make these data more useful to clinicians, we used published pharmacokinetic data from elderly patients to translate the concentration/AA relationship into an estimated dose/AA relationship.[92] Clinicians can use this information to assess the risk : benefit ratio of a particular medication, as well as to estimate the overall anticholinergic burden for a patient.

3.2.2 Methods

Materials/Procedure

We studied the *in vitro* AA of 106 medications using a method previously described.[92] One hundred and three of these agents were selected because they were classified as the 103 medications most frequently dispensed to residents of long-term care facilities in 2003 by a pharmacy provider (Omnicare®). Three additional medications (diazepam, duloxetine, and L-hyoscyamine) were selected based on reports of possible anticholinergic effects. When available, medications were purchased in their pure form (U.S. Pharmacopeia; Sigma-Aldrich; Sequoia Research Products, Pangbourne, UK). Medications purchased in tablet form were crushed or sonicated, dissolved in dilute acid (0.1 N HCl), and in some instances centrifuged (e.g., when excipients would not dissolve into solution). The Appendix lists the solvents in which medications were dissolved. Solvents were chosen based on the solubility and stability profile of each medication. On the day of the assay, drug solutions were aliquoted into 0.2 ml of drug-free (blank) human off-the clot serum (Scantibodies, Santee, California).

Each solvent was tested for possible interference with the assay. At high concentrations, all solvents resulted in a change of ^{3}H-QNB binding to muscarinic receptors (data not shown). Acetone, dimethyl sulfoxide (DMSO), and ethanol were particularly likely to alter binding and produce false positives. For this reason, these solvents were only used when other solvents failed to dissolve a medication and only at concentrations that were shown not to interfere with the assay.

The method followed for assessment of *in vitro* AA is outlined in section 3.1.2 under Procedure.

Analysis

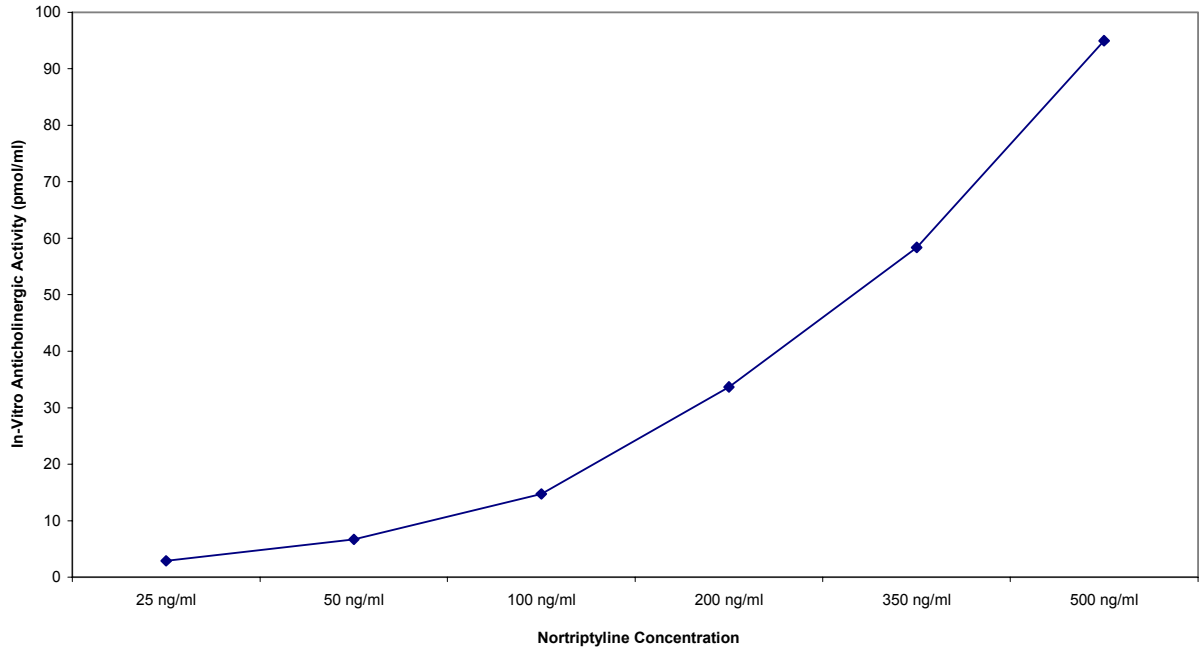
Atropine standard curve and *in vitro* AA were calculated using weighted logit-log regression. For medications that showed detectable AA, we estimated the relationship between daily dose and *in vitro* AA as follows. First, average peak concentrations (C_{max}) at steady-state after oral administration of therapeutic doses were estimated using published pharmacokinetic data and basic pharmacokinetic principles. [92] C_{max} was estimated based on pharmacokinetic data from older adults for all drugs except for atropine, chlorpromazine, doxepin, and L-hyoscyamine for which appropriate data were not available. Based on published data, nonlinear pharmacokinetic principles were used for clozapine (nomogram), fluoxetine, paroxetine, and phenytoin. Linear pharmacokinetics were assumed for the remaining medications because either available data support linear pharmacokinetics or there are no data to suggest nonlinearity. Interpolations of the concentration/AA plots were than used to determine AA at a given C_{max}. Finally, doses and AA were plotted to generate dose/AA curves (Figures 3.6 and 3.7, Table 3.6). Doses reported are total daily doses that were calculated assuming once-daily administration or divided doses as appropriate. The dose/AA relationship for dicyclomine could not be calculated given the lack of appropriate pharmacokinetic studies.

Table 3.6. Estimated AA at therapeutic doses of nortriptyline at estimated mean C_{max} in geriatric patients

Total Dose of Nortriptyline per Day (mg)	C_{max} (ng/mL)	*In vitro* AA (pmol/mL)
10	12	0.8
25	29	3.5
50	59	8.2
100	117	18.0
150	175	29.0

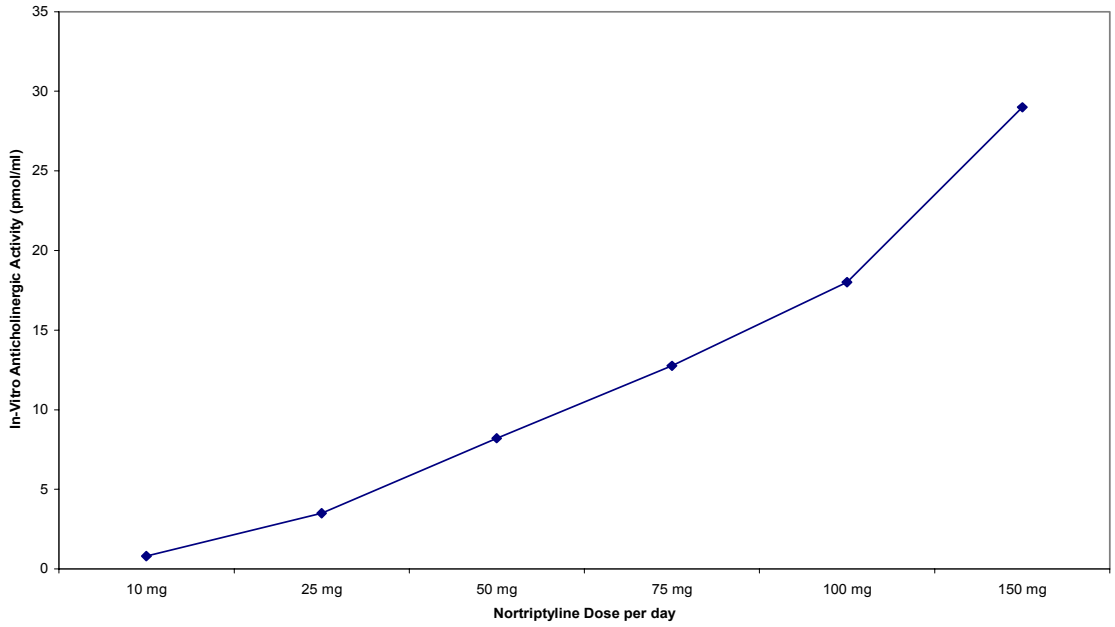
Estimated dose/AA relationships were used to classify the possible AA of each medication (Table 3.6). Classification was determined based on the highest AA estimated for typical doses administered to older adults. Categories range from 0 (e.g., indicating the absence of AA) to +++ (e.g., AA greater than 15 pmol/mL of atropine equivalents). Medications classified as 0/+ have an estimated AA of 0 at therapeutic doses. For these medications, AA was not detectable at average serum concentrations obtained with therapeutic doses. However, some older patients receiving these medications may demonstrate AA if they are receiving supratherapeutic doses or if they have above average C_{max} concentrations.

Figure 3.6. Nortriptyline concentrations (ng/mL) by *in vitro* AA (pmol/mL)



In vitro AA was assessed at six concentrations of nortriptyline. This information was then used to estimate AA at specific therapeutic doses of nortriptyline (see Figure 3.7).

Figure 3.7. Nortriptyline doses (mg) by *in vitro* AA (pmol/mL)



Illustrating how each dose/AA relationship was calculated. The average C_{max} for a total dose of 50 mg/day of nortriptyline for an older adult is 59 ng/mL (Table 3.5). Using Figure 3.6, the *in vitro* AA for 59 ng/mL of nortriptyline is approximately 8.2 pmol/mL. Thus, the estimated AA for an older adult taking 50 mg/day of nortriptyline is 8.2 pmol/mL, which is then plotted for a dose of 50 mg/day. A similar procedure was followed for each of the drugs demonstrating anticholinergic activity.

3.2.3 Results

Table 3.7 presents the *in vitro* AA of each of the 106 medications tested, grouped by drug classification. Thirty-eight of the medications examined demonstrated detectable AA. Of these, 21 medications had dose-dependent AA and 17 demonstrated AA only at the highest (e.g., supratherapeutic) concentrations examined. Figures 3.8 to 3.13 presents the dose/AA relationship for medications that demonstrated AA at representative doses prescribed to older patients. For instance, the average steady-state C_{max} for a total dose of 50 mg/day of nortriptyline for an older adult is 59 ng/mL (Table 3.5). Using Figure 3.6, the *in vitro* AA for 59 ng/mL of nortriptyline is approximately 8.2 pmol/mL. Thus the estimated AA for an older adult taking 50 mg/day of nortriptyline is 8.2 pmol/mL which is then plotted for this dose of 50 mg/day. A similar procedure was followed for each of the drugs demonstrating anticholinergic activity.

Table 3.7. Anticholinergic activity of 106 medications in older adults*

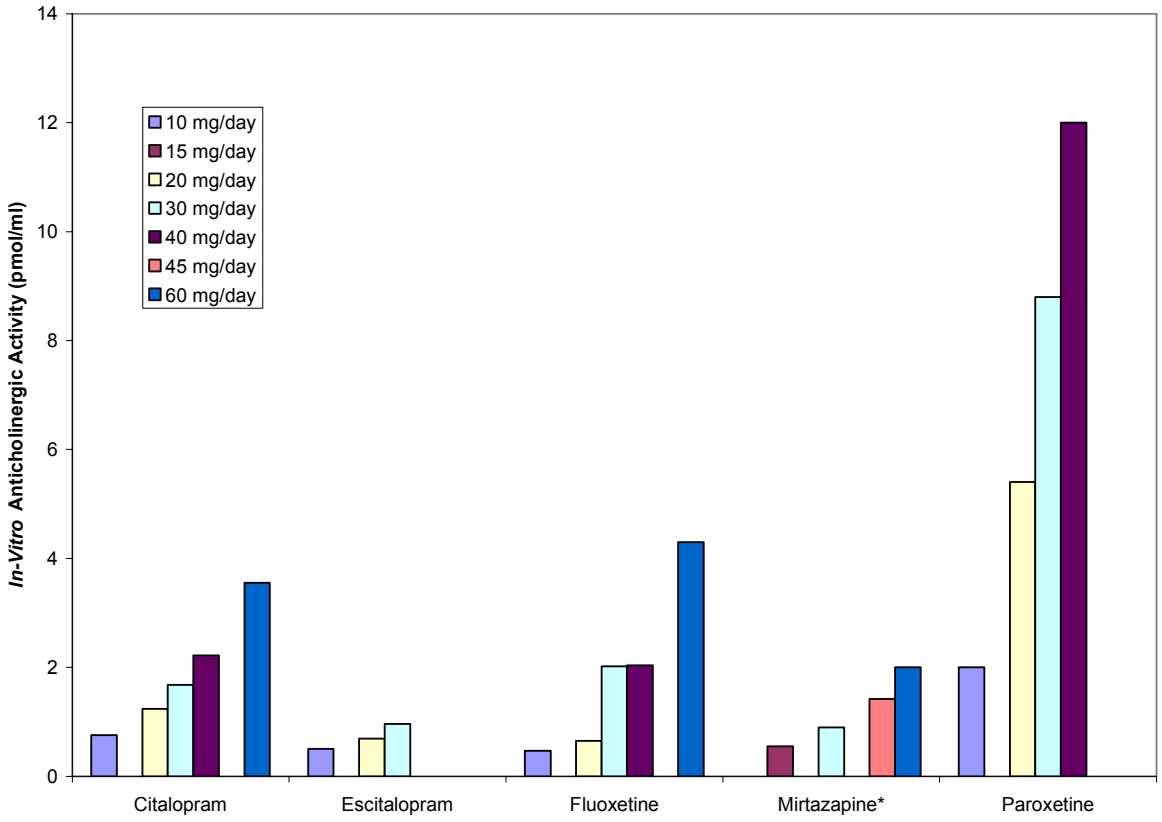
Category	0	0/+ (No or minimal)	+ (0.5–5 pmol/mL)	++ (5–15 pmol/mL)	+++ (> 15 pmol/mL)
Analgesic/anti-inflammatory agents	Acetaminophen Aspirin Codeine Ibuprofen Morphine Tramadol	Celecoxib Fentanyl Hydrocodone Propoxyphene	—	—	—
Antidepressants	Bupropion Sertraline Trazodone Venlafaxine	Duloxetine	Citalopram Escitalopram Fluoxetine Mirtazapine	Nortriptyline Paroxetine	Amitriptyline Doxepin
Antidiabetic agents	Glipizide Pioglitazone Rosiglitazone	Metformin	—	—	—
Antihistamines	Cetirizine Fexofenadin Loratadine	—	—	Diphenhydramine	—
Anti-infectives	Ciprofloxacin Sulfamethoxazole Trimethoprim	Amoxicillin Cephalexin Levofloxacin	—	—	—
Antipsychotics	Aripiprazole Haloperidol Perphenazine Risperidone Ziprasidone	—	Quetiapine	Chlorpromazine Olanzapine	Clozapine Thioridazine
Anxiolytic/sedative-hypnotics	Alprazolam Buspirone Lorazepam Oxazepam Zaleplon Zolpidem	Diazepam	Temazepam	—	—

Category	0	0/+ (No or minimal)	+ (0.5–5 pmol/mL)	++ (5–15 pmol/mL)	+++ (> 15 pmol/mL)
Cardiovascular agents	Amlodipine Atenolol Atorvastatin Diltiazem Enalapri Hydrochlorothiazide Lisinopril Losartan Lovastatin Metoprolol Nifedipine Nitroglycerin Propranolol Simvastatin Valsarta	Digoxin Furosemide	—	—	—
Cognitive-enhancing agents	Galantamine Memantine Rivastigmine	Donepezil	—	—	—
Gastrointestinal/bowel agents	Bisacodyl Famotidine Loperamide Omeprazole Pantoprazole Rabeprazole	Diphenoxylate Lansoprazole	Ranitidine	—	Atropine Dicyclomine L-hyoscyamine
Miscellaneous	Baclofen Carbidopa Clopidogrel Darbepoetin Dipyridamole Epoetin Levodopa Levothyroxine Megestrol Warfarin	—	—	—	—
Mood stabilizers/ anticonvulsants	Carbamazepine Gabapentin Lamotrigine Valproate	Phenytoin Topiramate	—	—	—

Category	0	0/+ (No or minimal)	+ (0.5–5 pmol/mL)	++ (5–15 pmol/mL)	+++ (> 15 pmol/mL)
Urinary incontinence Agents	—	—	—	Oxybutynin	Tolterodine

*Classification was determined based on the highest estimated for typical doses administered to older adults. 0 = no AA at therapeutic doses; 0/+ = no or minimal AA (no AA at doses across the therapeutic range; however, patients with above average C_{max} or receiving supratherapeutic doses may show some AA); + = low AA (0.5-5 pmol/mL across the therapeutic range); ++ = moderate AA (5-15 pmol/mL); +++ = high AA (>15 pmol/mL).

Figure 3.8. *In vitro* AA for clinically relevant doses of five newer antidepressants



*The above dose/AA relationship for mirtazapine is based on the mean steady-state peak concentrations (C_{max}) in older women (sex-associated differences in mirtazapine pharmacokinetics are reported).

Figure 3.9. *In vitro* AA for clinically relevant doses of three antidepressants

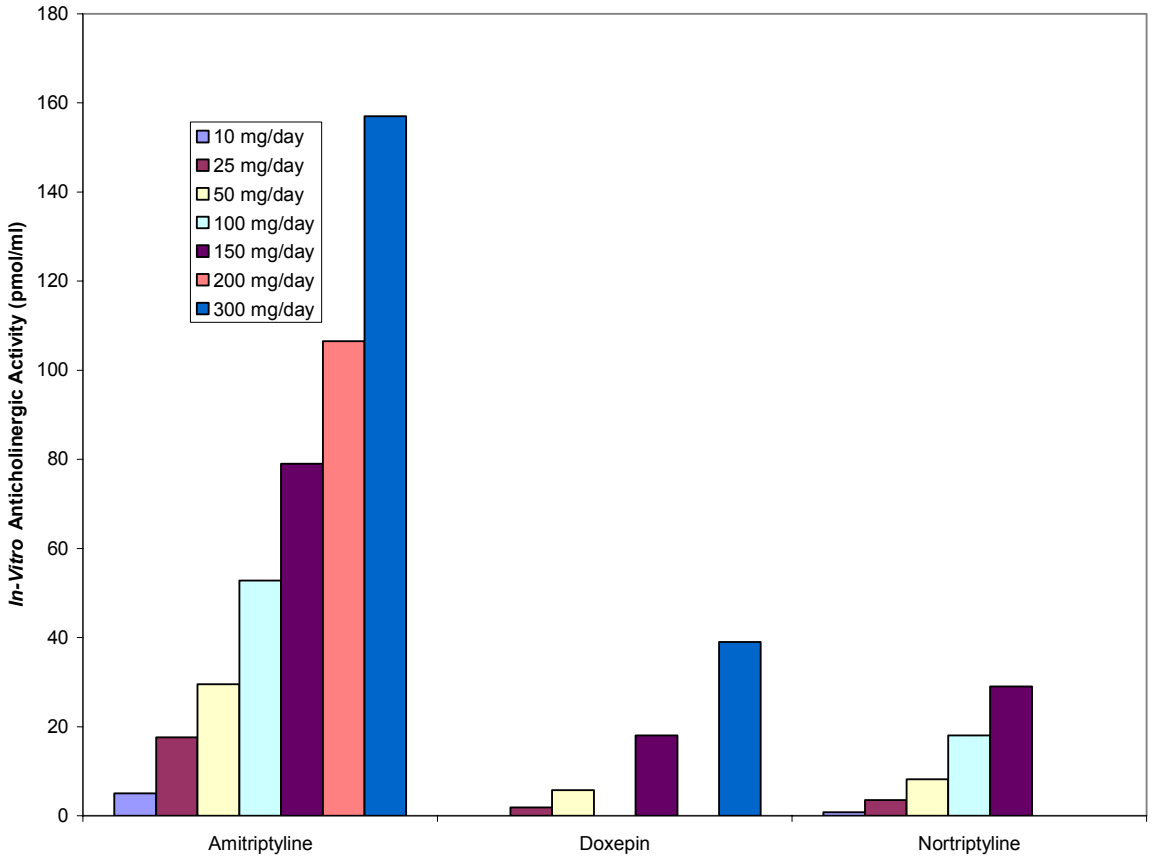


Figure 3.10. ***In vitro*** **AA for clinically relevant doses of three atypical antipsychotics**

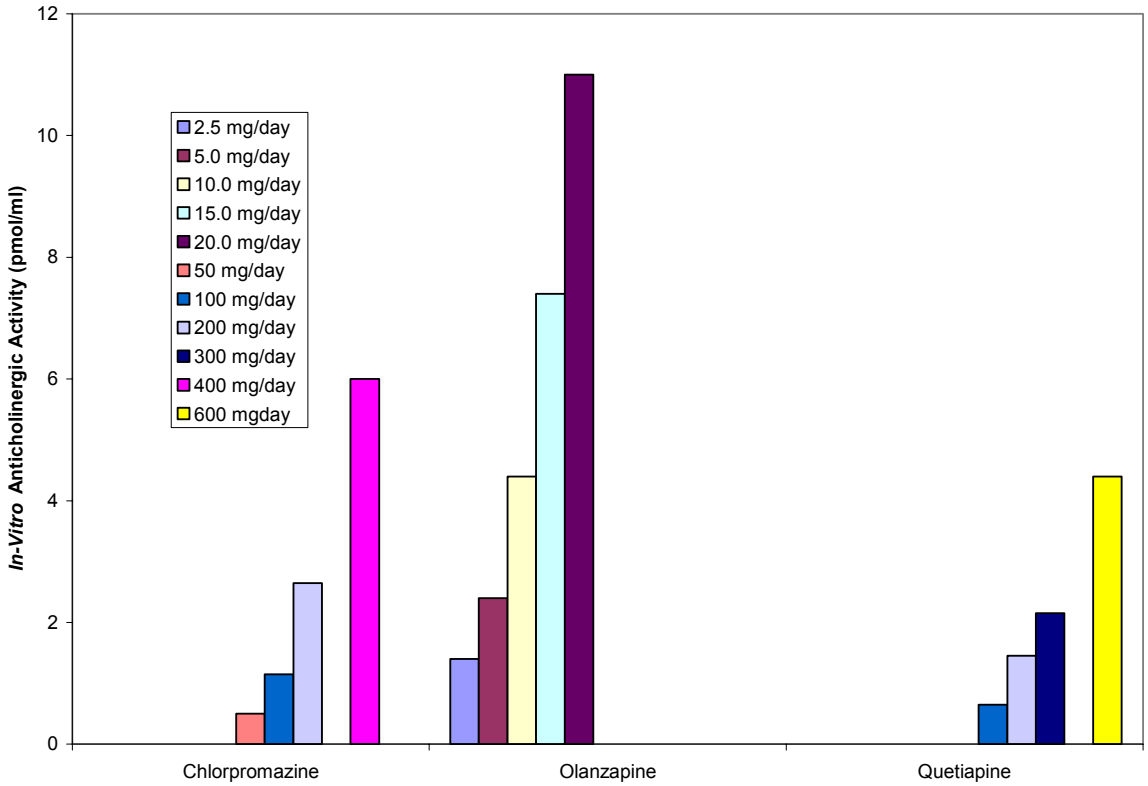
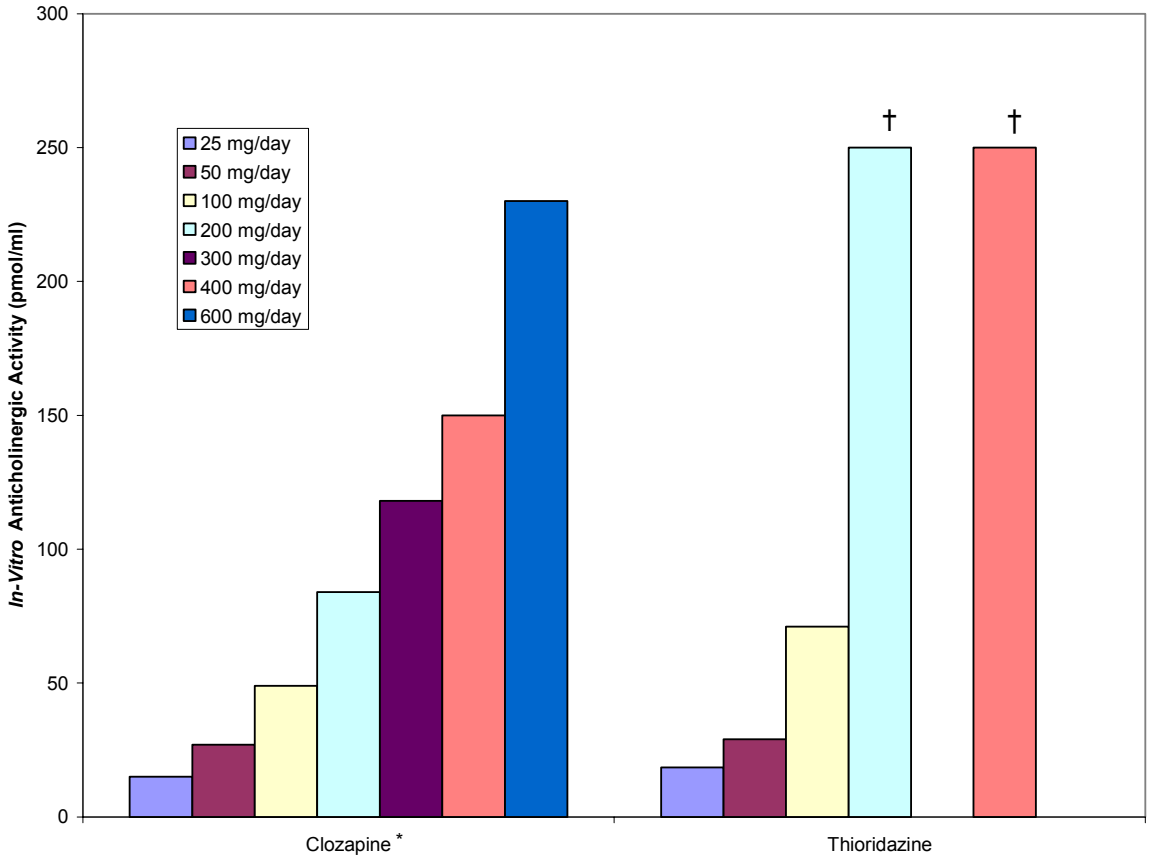
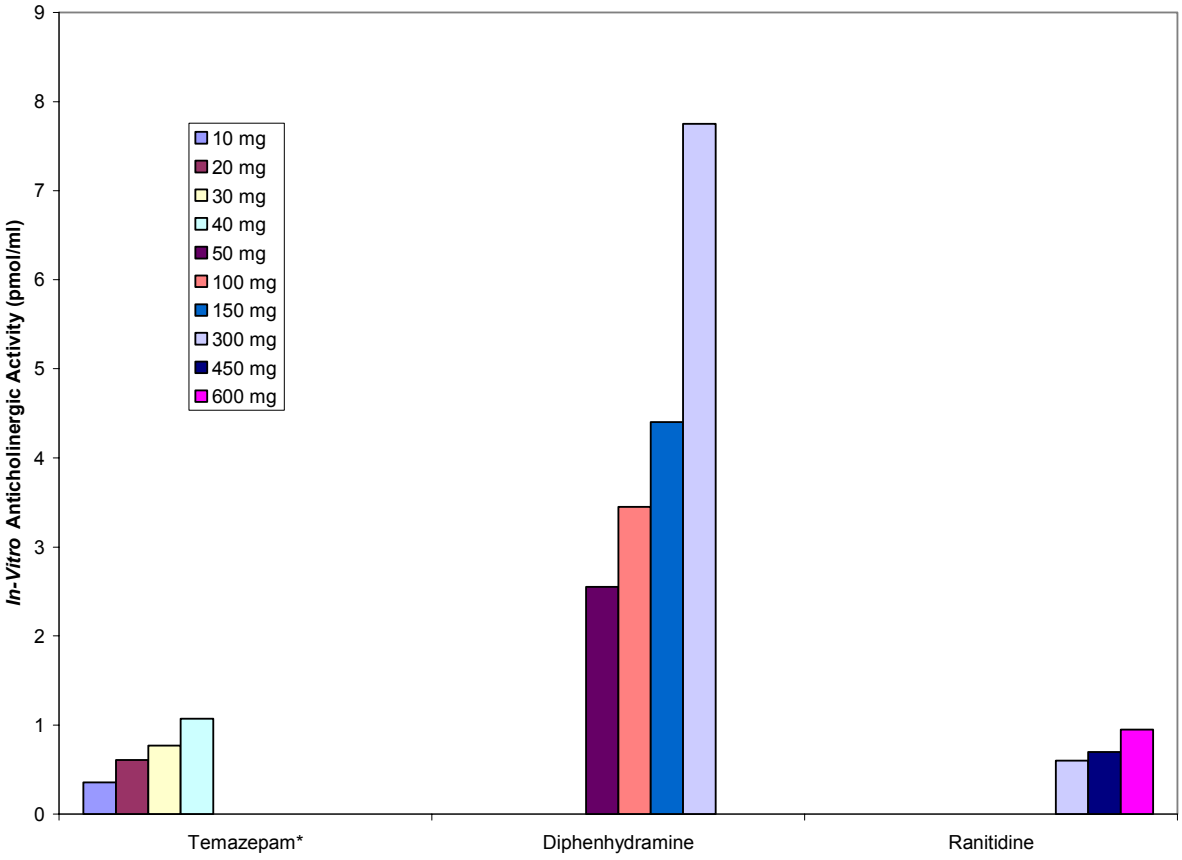


Figure 3.11. ***In vitro*** **AA for clinically relevant doses of two antipsychotics**



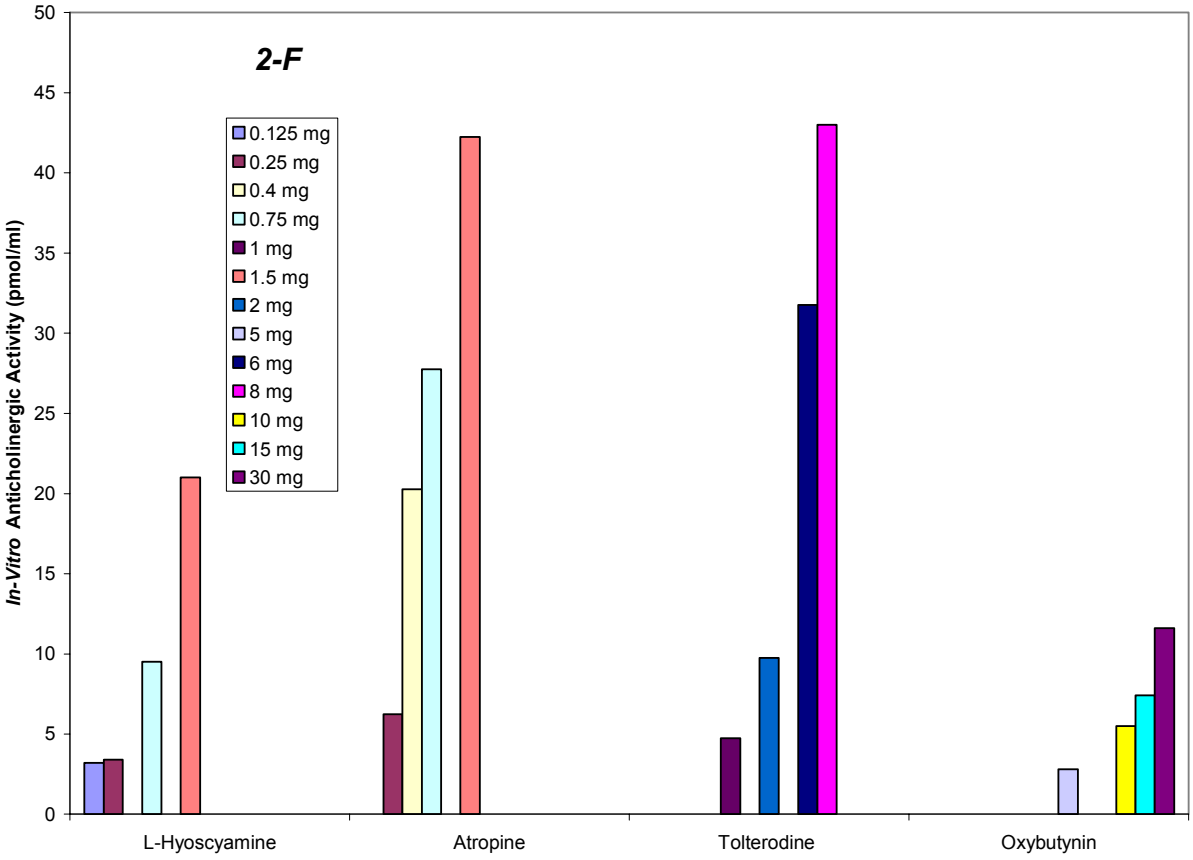
*The above dose/AA relationship for clozapine is based on the mean steady-state peak concentrations (Cmax) in older women (sex-associated differences in the pharmacokinetics of clozapine are reported).
†The AA concentrations are >250 pmol/mL at 200 and 400 mg/day of thioridazine).

Figure 3.12. ***In vitro*** **AA for clinically relevant doses of three agents**



*The above dose/AA relationships for temazepam are based on the mean steady-state peak concentrations (C_{max}) in women (sex-associated differences in the pharmacokinetics of temazepam are reported).

Figure 3.13. ***In vitro*** **AA for clinically relevant doses of four agents**



3.2.4 Discussion

Using an established radioreceptor assay, we examined the AA of 106 medications commonly prescribed to older adults. We then estimated the dose/AA relationship for each medication using available pharmacokinetic data. Twenty-one of the medications tested demonstrated dose-dependent AA. In addition, 17 other medications demonstrated AA at the highest concentrations tested (i.e., above-average concentrations showed AA). The remainder of the medications investigated did not demonstrate AA at any of the concentrations examined.

Our measures of *in vitro* AA are useful to assess the potential of specific medications to cause central anticholinergic effects. They take into account the apparent affinity (or Kd) of a medication, as well its dose-dependent relationship at muscarinic receptors. Moreover, these AAs were estimated based on an atropine standard curve, making the comparison between medications relatively straightforward. Thus, estimated AA may be a more useful measure of risk for medication-induced cognitive impairment than the dichotomous classification of anticholinergic or nonanticholinergic.

These results are important because of the growing body of evidence suggesting that anticholinergic medications contribute to memory impairment in older adults (see sections 1.3 thru 1.6).[4–9] Acute administration of the anticholinergic agent scopolamine produces cognitive deficits similar to those seen in Alzheimer's disease. Patients receiving scopolamine show impairment in multiple cognitive domains including attention, verbal and visiospatial learning, processing speed, and praxis.[2, 3, 42] Furthermore, older adults receiving anticholinergic agents are more likely to experience global cognitive impairment. In one study, 80% of elderly patients

receiving at least one anticholinergic medication were classified as being mildly cognitively impaired compared with only 35% of nonusers.[58] In another study, community-dwelling elderly persons with elevated serum AA were 13 times more likely to suffer from global cognitive impairment than persons without detectable serum AA.[7] Even very low AA has been associated with specific cognitive deficits. In one study, depressed elderly subjects with any detectable serum AA performed more poorly on verbal learning measures than did those without AA.[67] The relationship between AA and peripheral adverse effects (e.g., constipation and dry mouth) has not been well studied and therefore is not a focal point of this discussion. However, we would expect higher AA levels to be associated with greater peripheral effects.[133]

Our model can be used clinically to estimate the possible anticholinergic burden associated with a specific medication or combination of medications. In turn, such an estimate can be used to predict which older patients are at risk for anticholinergic toxicity. However, the risk of anticholinergic burden needs to be considered within the overall clinical history of each patient (e.g., past sensitivity to anticholinergic agents, memory complaints, effectiveness of an agent, disease state, adherence concerns). Establishing the presence of a risk cannot be equated with the presence of anticholinergic toxicity as the exact relationship between serum AA and cognitive performance is still not known. Mulsant and colleagues reported a nonlinear relationship between serum AA and MMSE (e.g., a measure of global cognition) performance in older community-dwelling persons.[7] In contrast, in two separate studies, a linear relationship ($r = -0.5$) was found between serum AA levels and verbal learning in persons with schizophrenia under age 60 years.[81, 82] However, all of the studies published to date have used 1) a relatively small number of subjects, 2) a specific patient population, 3) and limited cognitive assessments.

The association between AA and performance most likely varies depending on the population examined, which cognitive domain is being assessed (e.g., verbal learning, working memory, global cognition), and possible covariates such as age, sex, level of education, and disease state. In addition, the presence of AA does not imply that a noticeable cognitive deficit will be present. For instance, in a study of older community-dwelling persons, high AA was associated with a 6-fold increase in the prevalence of cognitive impairment. However, only 30% of older persons with high AA demonstrated cognitive impairment (versus 5% of those with no detectable AA), .defined in this study as scoring less than 25 on the MMSE [7]

Our model of dose/AA relationships has several technical limitations.[92] Only parent drugs were assessed for this report. Future investigations also need to address the possible AA of metabolites. Many of the medications examined (e.g., clozapine, oxybutynin, and tolterodine) have metabolites that are known to bind to muscarinic receptors. However, the AA of these metabolites were not investigated and thus do not contribute to the relationships reported here. Moreover, even medications with no reported AA may have metabolites which have affinity at the muscarinic receptors (for example, desloratadine the metabolite of loratadine).[142]

Some medications were not available commercially in their pure form. The extraction process used may have removed some of the drug of interest, thus reducing the amount of drug that was tested. Although no evidence is available suggesting it happens, it is also possible that excipients themselves could interfere with the assay causing a false positive or negative. The radioreceptor assay used for this study is designed to optimize the binding of antagonists.[7, 65, 66] Nevertheless, it

is possible that AA also reflects binding of agonists and may not be purely a measure of anticholinergic activity.

Our model is based on average C_{max} values in relatively healthy older adults. Frail elderly or more medically compromised older adults are usually not represented in published pharmacokinetic studies, yet typically have higher average C_{max} values. For instance, the maximum (±SD) peak plasma concentrations after a single dose of 5 mg of immediate-release oxybutynin were 16.7 ± 7.5 ng/mL in healthy older adults and 32.0 ± 22.2 ng/mL in "frail" elderly subjects.[143] Thus, given the large pharmacokinetic variability of most medications in older adults, a person may have considerably higher or lower AA than is estimated. Frail or medically compromised elderly would be expected to have significantly higher estimated AA than is reported in Table 3.7.

With the exception of oxybutynin, all of the dose-AA relationships reported in Table 3.7 were based on the pharmacokinetics of an immediate-release formulation. However, medications administered as an extended-release formulation, such as fluoxetine, paroxetine, quetiapine, or tolterodine, may have considerably altered pharmacokinetics.

There is significant overlap between our findings and those reported by Tune and colleagues.[140, 141] For example, in both studies hydrochlorothiazide, ibuprofen, and propranolol are identified as not being associated with anticholinergic activity, while amitriptyline, diphenhydramine, and ranitidine are identified as being potentially associated with clinically significant anticholinergic activity. There are also some discrepancies. For instance, Tune and colleagues identified digoxin,

furosemide, and warfarin as being potentially associated with clinically significant anticholinergic activity, while our results suggest that digoxin and furosemide are associated with AA only in atypical cases and that warfarin is not associated with AA. Reasons for these differences are difficult to determine given the brevity of the methods description in the reports of Tune and colleagues. It is possible that the solvents used to dissolve medications may have resulted in false positives (e.g., acetone is a common solvent employed for the dissolution of warfarin and at higher concentrations it interferes with the AA assay). One probable reason for some of these differences is that we report AA based on therapeutic doses and drug concentrations, whereas Tune et al. reported the AA of each medication only for one fixed concentration of 10^{-8} M. For instance, in our *in vitro* assay, digoxin would also demonstrate AA at a concentration of 10^{-8} M (i.e., 10 nmol per L or 8 ng per mL). However, we classified digoxin as having no AA at therapeutic doses (Table 2) given that the current recommendation for target serum concentrations is between 0.5 and 1.0 ng per mL (e.g., 0.6–1.3 nmol per L) and we did not identify AA below 6 ng/mL.

Our results related to donepezil and tolterodine deserve additional comments. Donepezil, a cholinesterase inhibitor, demonstrated AA at supratherapeutic concentrations. Our results are in accordance with the work of Snape and colleagues who have reported that donepezil is functioning as an antagonist at muscarinic receptor subtype 1.[149] These findings seem counterintuitive, given that donepezil is indicated for the treatment of dementia of the Alzheimer's type. Pharmacologically, however, this is not unexpected since acetylcholine itself interacts with both muscarinic receptors (of which it is the neurotransmitter) and acetylcholinesterase (of which it is the substrate). Of note, our model confirms that donepezil can bind to muscarinic receptors, but it does not predict that donepezil is associated with clinically

significant anticholinergic activity. Tolterodine, a potent anticholinergic agent, is thought to have relatively low distribution into the CNS. However, as stated above, the blood-brain barrier may change with age, disease state, presence of dementia, as well as other environmental factors such as medication use.[144-146] Moreover, although formal cognitive testing is lacking, many reports suggest that memory impairment can occur with tolterodine administration.[147, 148, 150]

4.0 ANTICHOLINERGIC MEDICATIONS AND OCULAR RESPONSE

4.1 PUPIL

As discussed in section 1.8, one of the limitations of serum AA is the possible disconnect with central anticholinergic activity. An optimal tool would be one that assesses possible risk (e.g., serum AA) and is a good indicator of central anticholinergic activity. The pupil has several unique characteristics which make it an appealing target of exploration. Chapter 4 reviews basic nervous control and anatomy of the pupil, as well as discusses why ocular response may be useful as a tool to assess central anticholinergic activity.

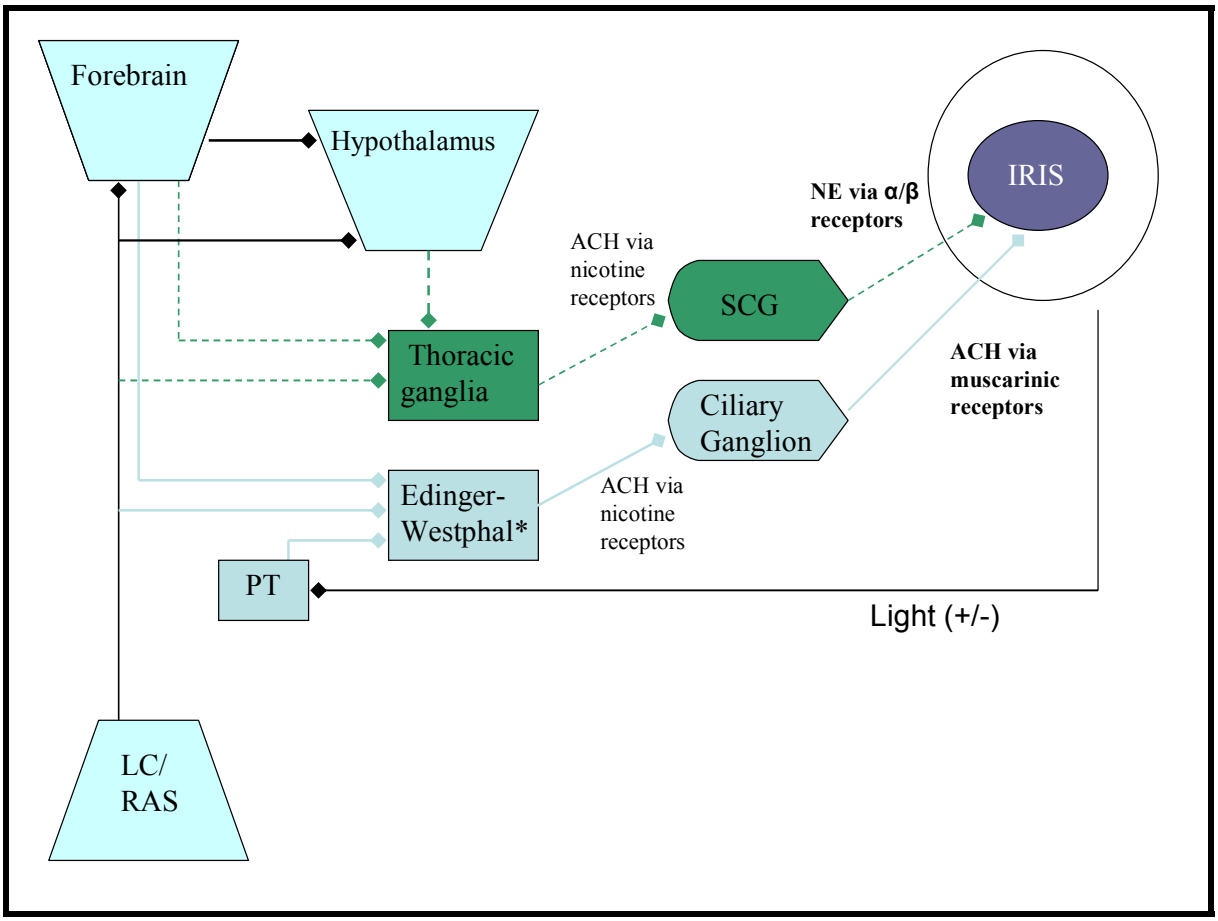
4.1.1 Basic Anatomy

Pupillary diameter and response is controlled by the smooth musculature present within the iris tissue. The sphincter muscle has fibers which are arranged in a circle surrounding the pupil, while dilator muscle fibers run radially. The sphincter is predominantly under parasympathetic control, with contraction producing miosis (i.e., a decrease in pupil size). The dilator muscle is primarily under sympathetic control, with contraction producing mydriasis (i.e., dilation).[151] Multiple central ascending and descending inputs contribute both directly and indirectly to parasympathetic and sympathetic tone. Figure 4.1 offers a simplified outline of autonomic innervation of the iris. Norepinephrine and acetylcholine are the principal neurotransmitters involved in ocular sympathetic and parasympathetic nerves, respectively. Other neurotransmitters possibly involved in pupillary response include angiotensin II, dopamine, neuropeptide Y, prostaglandin E, serotonin, substance P, vasoactive intestinal polypeptide, vasopressin, and the opiate peptides dynorphin A and α-neo-endorphin.[152] Pupil diameter

exhibits a large interindividual and some intraindividual variation in both dark and lighted conditions, and tends to decline with age (Table 4.1).[153-156]

The color of the iris is mainly due to the yellowish-brown to black pigment called melanin, which is found in the connective tissue and epithelial cells of the iris. Darker colored eyes (e.g., brown or black) have greater levels of melanin than do lighter colored eyes. Eye color may be an important factor in the efficacy of an ocular medication, as many drugs bind to melanin (see Section 4.2.2).

Figure 4.1. Autonomic innervation of the iris



A simplified schematic of parasympathetic and sympathetic input to the iris (modified from White and Depue).[157-160] Dark green represents the sympathetic system, while the darker blue signifies the parasympathetic system.
ACH = acetylcholine, NE = norepinephrine; LC = locus coeruleus; PT = pretectum; RAS = reticular activating system; SCG = superior cervical ganglion
*Edinger-Westphal is part of the oculomotor nucleus in the midbrain.

Table 4.1. Average pupil diameter across age*

Age (years)	# eyes	Diameter in Light (mm)** Average (SD)	Diameter in Dark (mm) Average (SD)
18-29	42	3.0 (2)	6.2 (2.7)
30-39	34	3.0 (1.5)	5.7 (2.8)
40-49	46	3.0 (1.7)	5.5 (3.0)
50-59	30	2.8 (1.7)	5.2 (2.5)
60-75	26	2.7 (1.5)	4.4 (2.5)

*Subjects participating in this study were "healthy" individuals. Thus, the interindividual variation reported here may not reflect the additional variability which occurs with medication use and various disease states such as diabetes.
**Authors did not specifically state luminance used in light condition

4.1.2 Muscarinic Receptors in the Iris

Muscarinic receptors are the predominant receptors involved in parasympathetic control at the postganglionic level. All 5 subtypes are present in the iris.[161-163] M3 is the most abundant post-junctional receptor and is thought to be the most important subtype for mediating pupil contraction.[98, 99, 164, 165] Choppin and colleagues reported that M3 ligands produced greater contraction of the sphincter muscle *in vitro* than ligands with minimal affinity for M3.[164] In addition, M3 knockout mice have approximately 5-fold larger pupils in bright light than do wild-types. However, additional dilation occurred in the pupils of knockout mice with topical administration of atropine (i.e., an antimuscarinic agent), indicating that the remaining subtypes must play some role in pupil miosis.[98] Evidence has suggested that M2 is a heteroreceptor, present on prejunctional sympathetic nerve terminals. In other words, stimulation of the M2

receptor can cause an inhibition of norepinephrine release. Minimal research has been conducted with the remaining muscarinic receptor subtypes.

4.2 ANTICHOLINERGIC MEDICATIONS AND PUPIL DIAMETER

Many anticholinergic drugs increase pupil diameter, although with different potencies, despite similar affinities at muscarinic receptors. The anticholinergic drugs atropine and scopolamine have similar affinities and comparable dose-related peripheral effects such as reduced salivation and gastrointestinal motility. However, scopolamine has approximately 7-10 fold greater potency on pupillary dilation than does atropine.[23] Moreover, some high affinity anticholinergic medications such as glycopyrrolate, have minimal effects on the ocular system (e.g., pupil diameter and near-point of accommodation) when administered orally or systemically.[166, 167] Much of the variability in ocular response with different antagonists is most likely due to differences in medication diffusion rates across blood-ocular barriers (BOBs).

4.2.1 Blood-Ocular Barrier

The blood-ocular barrier (BOB), which shares similar characteristics to the blood-brain barrier (BBB; e.g., the presence of tight junctions, efflux transporters such as P-glycoprotein, and minimal pinocytotic vesicles), may be limiting the amount of medication that reaches the eye (Table 4.2). Distribution of medications into ocular tissues is associated with distribution of medication into the CNS. Steuer et al reported that drugs in an *in vitro* ocular blood-barrier

system had similar permeability coefficients as an *in vivo* BBB system.[168] *In vivo*, atropine has similar potency on both pupillary response, accommodation, and memory impairment.[23] Glycopyrrolate, a quaternary ammonium compound (which does not cross the BBB well), has very potent peripheral anticholinergic effects, but minimal ocular and cognitive effects.[166, 167, 169] Tolterodine, an agent used for the treatment of incontinence, has limited distribution into the CNS,[170, 171] minimal reported cognitive effects in a nondemented population,[172] and minimal effects on ocular response at therapeutic doses.[173] Given the correlation between ocular and CNS effects, ocular response may be a useful tool to characterize how readily a medication enters the CNS following acute administration.

Table 4.2. Characteristics of endothelial cells in brain, iris, and skeletal tissues

	Brain	**Iris**	**Skeletal**
Tight Junctions	Yes	Yes	No
Permeability of Urea (cm/sec-5)*	0.44	2.8	9.7
P-glycoprotein	Yes	Yes	No
Fenestrations	No	No	Yes
Vesicles (#/μM^2 cytoplasm)	10.8	15.5	103.6

*Urea is a polar compound

4.2.2 Effect of Eye color

Many studies have shown that topically administered anticholinergic drugs produce a reduced, but prolonged effect in individuals with brown eyes. Although less well studied, there is evidence indicating that both iris color and race influence pupillary dilation with nontopical administration as well. Garde et al reported that the mean peak change in pupil diameter following 0.4 mg/70 kg intramuscular administration of scopolamine was 2.6 and 1.6 mm in Caucasian and African American subjects, respectively.[174] Moreover, the time of onset of significant mydriasis occurred within 15 to 30 minutes in Caucasians, compared to 45 to 90 minutes in the African American group. In an *in vitro* system, the apparent affinity of the anticholinergic agent zamifenacin for muscarinic receptors in ocular tissue taken from blue eyes was approximately 50 times greater than the apparent affinity for muscarinic receptors in brown eye tissue.[164] Thus, having brown eyes and being nonCaucasian may decrease and alter the timeline of ocular response to a medication.

4.2.3 Discussion

Measurement of ocular response and serum AA *before and after* an *acute* administration of a medication may provide an indication of anticholinergic effects, as well as possible central activity. For instance, most quaternary medications (e.g., positively charged) do not readily distribute across the BOB, and thus would not cause a significant change in pupil diameter. An agent which does not cross the BOB, most likely is not distributed into the CNS. In other words, following a single administration of a quaternary agent, an increase in serum AA levels would be seen, with minimal or no central activity or ocular effects.

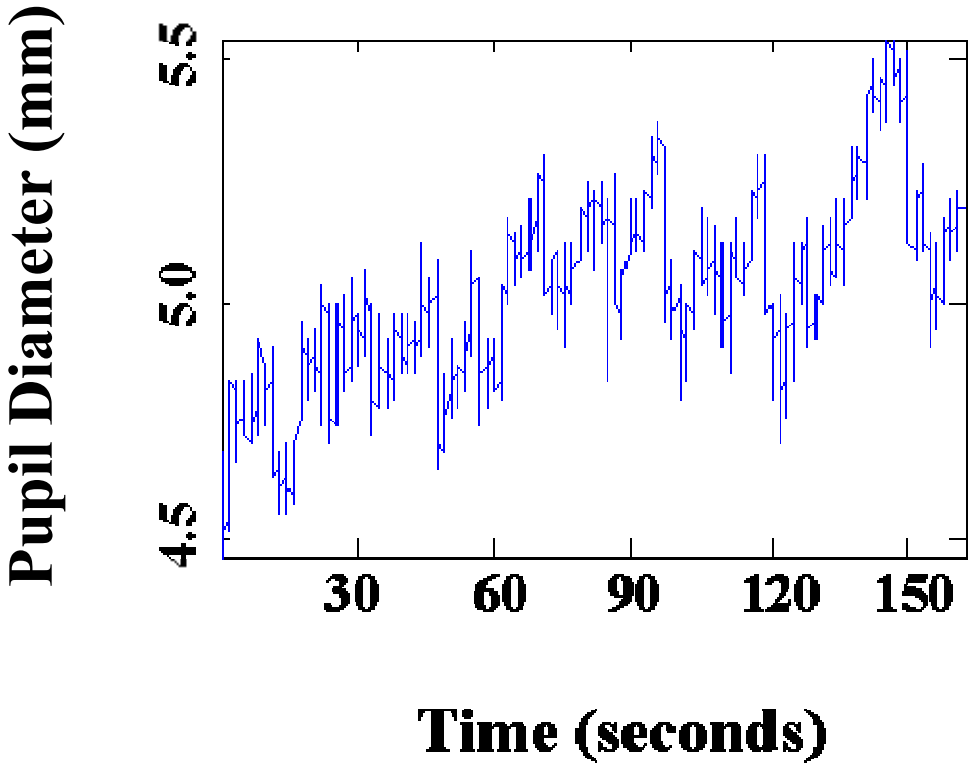
There are possible limitations associated with using pupil diameter for the above mentioned example. Many antimuscarinic medications have affinity at additional receptors which may alter pupillary or central responses. For instance, amitriptyline, a potent anticholinergic agent, causes minimal changes in pupil diameter due to competing alpha-adrenoceptor blockade. In addition, tiredness manifests itself in many individuals with a decrease in pupil size. A medication which causes significant fatigue may result in a distortion of the pupillary response as centrally mediated fatigue changes compete with the dilation at the peripheral level. Finally, older adults have a higher incidence of disease states and medication usage with possible ocular effects (e.g., diabetes, stroke, beta-blockers).

Although acute measurement (e.g., following a baseline measurement) may have some utility for assessing specific medications, it is doubtful that pupil diameter measurement alone will be useful in a naturalistic setting to detect those individuals who may be at risk for medication-induced impairment. As shown in table 4.1, relatively "healthy" older adult have a large interindividual variability in pupil diameter. It is expected, although not well studied, that this variability would be increased in a more representative population (e.g., including older adults with a variety of disease states, medication regimens, as well as frail elderly). Given all of the possible confounding variables (e.g., additional medications, disease states, anxiety, tiredness, diurnal rhythms, parasympathetic and sympathetic tone), pupil diameter at a single point in time most likely would not provide any additional information.

4.3 PUPILLARY UNREST

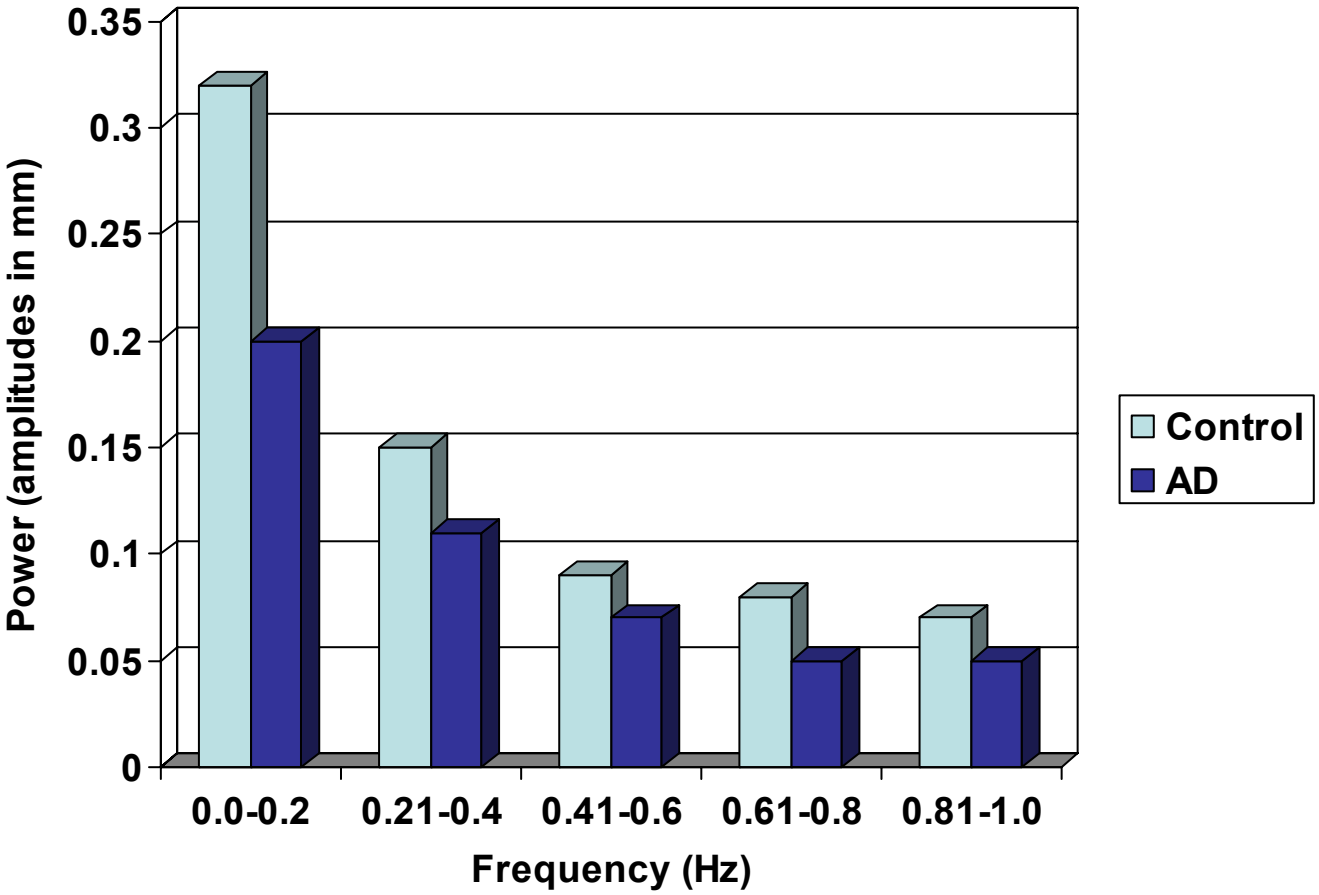
Pupil size is constantly changing as a result of the dynamic input from a variety of sources including the sympathetic and parasympathetic systems (Figure 4.2). These irregular pupillary oscillations or unrest are thought to be centrally mediated.[160, 175-180] There is a large interindividual difference in the speed, extent, and the general pattern of these movements with a reduction of unrest occurring with age.[160] All individuals display to a varying extent an increase in the number and amplitude of oscillations with an increase in light intensity.[160] Interestingly, identical but not fraternal twins, have almost indistinguishable oscillatory patterns, indicating a strong genetic influence. Moreover, the pattern of unrest is reproducible in repeated tests done months apart.[160, 181] Multiple disease states with cognitive effects (e.g., Alzheimer's disease, multiple sclerosis, and alcohol dependency) are associated with a decrease in amplitudes of pupillary oscillations.[179, 182, 183] Specifically, Grunberger and colleagues reported that patients with AD showed lower oscillatory amplitudes at specific frequencies (e.g., 0.0 to 0.2, 0.21 to 0.4, 0.61 to 0.8, and 0.0 to 1.0 Hz; Figure 4.3) then aged matched controls.

Figure 4.2. Pupil diameter across time in a single individual



The above figure shows data from a single subject from the clinical trial outlined in chapters 5 and 6. Pupil motility was measured for three minutes

Figure 4.3. Comparison of Alzheimer's Disease patients and aged-matched controls: Power of oscillations at varying frequencies



Pupil diameter was measured over 25.6 seconds. Fourier analysis was used to calculate the total power at each of the frequency bands listed above. Higher power is indicative of greater amplitude changes in pupil diameter across the measurement period. Modified from Grunberger and colleagues.[179]

The specific effects of anticholinergic medications on pupillary unrest are unknown. However, it has been suggested that pupillary response is correlated to other autonomic systems such as heart rate, due to similar central ascending or descending inputs.[184, 185] Furthermore, multiple investigators have shown a decrease in power in high frequency heart rate oscillations (e.g., centrally mediated) with anticholinergic administration.[186, 187]

4.3.1 Pupillary unrest as tool?

Given that unrest is centrally mediated and that diseases with cognitive deficits are associated with a decrease in oscillatory amplitudes, measurement of pupillary unrest may provide some indication of central anticholinergic toxicity. However, use of pupillary oscillations may have the same limitations outlined in section 4.2.3 (e.g., possible confounding effects of medication and disease states). The interindividual variability in older adults and possible covariates associated with this variability are not known. The diurnal rhythm and the stability of measurements across time are not known for older adults. Moreover, as discussed further in section 6.7, tiredness may affect the dynamics of oscillations.

4.4 SUMMARY

Serum AA may be a useful tool to help predict which individuals are at risk for medication-induced cognitive impairment. However, this peripheral measure may not always be a good indicator of central AA when it is composed of agents which do not readily distribute into the CNS. An optimal tool would be one that can also estimate drug distribution into the CNS. The blood-ocular barrier (BOB), which shares similar characteristics with the blood-brain barrier (BBB), limits the amount of exposure that the eye receives to many endogenous and exogenous substances.[188-190] Furthermore, there is limited ocular response with anticholinergic agents which are not readily distributed into the CNS.[166, 167, 191] Therefore, measurement of ocular response and serum AA following acute administration of a medication may provide an

indication of anticholinergic effects, as well as possible central activity. However, given the very large interindividual variability in pupillary diameter in "healthy" elderly and the possible confounding effects of medication, disease state, tiredness, and diurnal rhythms, use of pupil diameter alone (e.g., without a baseline measurement) may not be a feasible measure. Measurement of pupillary unrest may be a useful tool in conjunction with serum AA to measure possible central AA effects with chronic administration of agents. Pupillary unrest is centrally mediated and is associated with the presence of cognitive decrements. However, more information about oscillations occurring in older adults is needed to assess possible feasibility. Use of pupillary oscillations may have the same limitation as use of pupil diameter (e.g., possible confounding effects of medication and disease states). Chapters 5 and 6 of this doctoral dissertation address a small clinical study investigating the possible utility of pupillary unrest in conjunction with serum AA for determination of anticholinergic-induced cognitive impairment.

5.0 METHODS: RELATIONSHIP BETWEEN SERUM ANTICHOLINERGIC ACTIVITY, COGNITION, AND OCULAR RESPONSE

Specific Aims

The clinical project outlined below was a pilot study conducted to determine the feasibility of using oxybutynin (an anticholinergic medication with some CNS distribution) as a probe to investigate the relationship between serum AA, pupillary response, and cognition. We had hypothesized that oxybutynin would cause mydriasis, a decrease in resting pupillary unrest, and a decrease in performance on specific cognitive assessments. The long-term goal of this project is to investigate ocular and cognitive responses in a larger cohort. We hypothesize that in a larger sample, serum AA and oscillatory changes, *together,* will better describe cognition than serum AA or ocular response *alone.*

Specific Aim: Determine serum AA levels, ocular response, and cognitive changes in older adults receiving a single dose of an anticholinergic medication. A randomized, placebo-controlled, cross-over pilot study was conducted in nine older adults to investigate the effects of orally administered oxybutynin. Information gathered was used to determine the feasibility of a larger scale study. Some of the items which were examined include 1) whether oxybutynin is an adequate medication for this proposed project (e.g., is there sufficient cognitive and ocular response and is this medication well tolerated in older adults with acute administration); 2) time period in which cognitive and ocular effects are seen post administration of oral oxybutynin; 3) practice effects which occur with brief testing intervals of cognitive assessments; and 4) tolerability of study procedures.

Secondary Aim: Characterize serum AA levels over time following placebo and oxybutynin administration. The relationship between serum AA, time, and R-oxybutynin/R-desethyloxybutynin (gathered as part of the primary specific aim) was investigated using linear and nonlinear models with various statistical forms for interindividual and residual variability.

5.1 OXYBUTYNIN

5.1.1 Pharmacodynamics

R,S-Oxybutynin is a potent and specific anticholinergic medication (dissociation constant (k_d) range 0.5 - 11 nM; Table 5.1).[192] Its primary metabolites, the racemates, R and S, N-desethyloxybutynin, also have antagonistic properties (k_d range 2 – 16 nM). The antimuscarinic activity for both the parent and the metabolite predominantly reside in the R-enantiomers.[193-195] Oxybutynin and/or its active metabolite are thought to have some distribution into the CNS. In healthy, young males, 3 successive doses of 5 mg oral oxybutynin immediate release (IR), resulted in CNS effects as measured by power decreases in 4 out of 6 frequency bands (theta, alpha1, alpha2, and beta1).[171] The maximum effect on frequency bands was reached 1 to 2 hours following each of the 3 consecutive doses, with a cumulative effect of multiple-dose administration evident. Cognitive effects have also been observed following single and multiple doses of oxybutynin in older adults.[63, 196] Katz and colleagues conducted a double-blind, placebo controlled randomized cross-over study investigating the effects of oxybutynin on cognition in older adults.[63] A single dose of 5 or 10 mg oxybutynin IR caused verbal learning impairments and deficits in attention.

An increase in mydriatic symptoms has been reported in humans with multiple-dose administration of oxybutynin. Unfortunately, most studies conducted in humans have focused on measurement of only a few adverse effects, such as dry mouth. Only one systematic study has been conducted investigating the pupillary effects of oxybutynin in humans. In a small female Turkish cohort, pupil diameter was measured at baseline and one month following treatment of 5 mg, three times daily, oxybutynin.[191] No significant difference in pupil diameter was found between baseline and post-drug measurements. However, the study had multiple limitations including use of a ruler and a slit-lamp (e.g., increased inaccuracy as compared to use of a pupillometer) to measure pupil diameter. In animals, oxybutynin produces significant mydriasis at oral doses similar to those which cause dry mouth (a well documented anticholinergic adverse effect of this medication).[194, 197] Near-point accommodation changes (e.g., resulting from blockade of ocular muscarinic receptors) have also been shown with oxybutynin. A single dose of 2.5, 5.0, or 7.5 mg oxybutynin IR caused dose-dependent changes in accommodation of 13, 20, and 29%, respectively.[173]

Table 5.1. Affinity of racemic R,S-oxybutynin and R,S-desethyloxybutynin*

Compound	M1	M2	M3	M4	M5
R,S-oxybutynin	8.7	15.9	6.9	3.1	30.6
R,S-desethlyoxybutynin	4.49	11.5	2.4	1.5	9.9

*Values reported are equilibrium inhibitory (K_i) constants in nanomolar (nM) units. K_i reflects affinity; smaller values indicate greater receptor affinity.[86]

5.1.2 Pharmacokinetics

R,S-oxybutynin IR is rapidly absorbed from the gastrointestinal tract with detectable levels typically evident within 0.25 hours and peak concentrations occurring within 0.5 to 1.5 hours.[143, 198] Oral oxybutynin has a low systemic availability of approximately six percent, with a large portion of the drug being metabolized to the non-antimuscarinic metabolite N-oxide and to the racemates R and S desethyloxbutynin.[198] Desethyloxybutynin levels are also rapidly seen with detectable levels occurring within 0.25 hours, and peak concentrations seen within 0.5 to 2.0 hours. In healthy older adults (n=10), a large interindividual variability in pharmacokinetic parameters is seen. Following a single oral, 5 mg dose, the mean C_{max}, area under the curve (AUC), distribution half-life, and elimination half-life for oxybutynin in this population is approximately 16.7 (7.5) ng/ml, 31.8 (9.0) ng*hr/ml, 0.29 (0.11) hours, and 2.3 (1.0) hours, respectively. Mean C_{max}, AUC, and elimination half-life for desethyloxybutynin is approximately 57.9 (20.8), 236.2 (87.2) and 2.3 (1.2), respectively.[143]

Specific enantiomeric concentrations in older adults have not been reported. Following a single dose of oxybutynin IR to younger adults, the C_{max}, t_{max}, and AUC for the R and S enantiomers is approximately 2.85 (1.5) and 5.19 (2.97) ng/ml; 1.20 (0.57) and 0.93 (0.44) hours; and 14 (6) and 20 (9) ng*ml/h, respectively. Desethyl values for the R and S enantiomers are approximately 30.29 (7.28) and 15.24 (4.55) ng/ml; 1.11 (0.39) and 1.04 (0.30) hours; and 217 (82) and 100 (43) ng*ml/hr, respectively.[199]

Both oxybutynin and desethyloxybutynin are over 99% bound in plasma with the majority of drug binding via albumin or alpha-acid glycoprotein. Stereoselectivity is also present with protein binding. The unbound fraction of R-oxybutynin is approximately twice greater than that of its S counterpart; whereas the unbound fraction of R-desethyloxybutynin is lower than S-desethyloxybutynin.[200] Cytochrome 3A4 is thought be the predominant metabolizing enzyme for both R and S oxybutynin.[200-202] However, CYP2C9, CYP2C19, CYP2D6, and CYP3A5 contribute to the *in vitro* metabolism of both enantiomers. The *in vitro* kinetics are slightly different for each enantiomer with the R/S ratios of the elimination rate 0.77, 0.96, 0.088, 0.67, and 2.35 for CYP2C9, CYP2C19, CYP2D6, CYP3A4 and CYP3A5, respectively. For the desethyl metabolite, CYP2D6 is thought to be the primary metabolizing enzyme with an *in vitro* R/S ratio of 0.18. Similarly to oxybutynin, CYP2C9, CYP2C19, CYP2D6, and CYP3A5 also contribute to the metabolism of both metabolite enantiomers with a stereoselectivity for each isomer evident (4.60, 1.23, 0.78, and 0.88, respectively).[200]

5.2 RESEARCH DESIGN AND METHODS

5.2.1 Overview

This experimental study is a double-blind, placebo-controlled, randomized, cross-over investigation of oxybutynin IR (5 mg, oral) on serum AA levels, ocular response, and cognitive performance in older adults. Serum AA and serum drug levels, cognitive performance, and ocular response were measured at baseline and for 6 hours following administration of medication or placebo.

5.2.2 Screening

Individuals were initially screened briefly over the telephone (see Appendix B). On average, the telephone screening took approximately 20 minutes. Individuals that did not fail any of the inclusion/exclusion criteria based on the telephone screening were invited to participate in a more formalized screening visit. The screening visit (see Appendix C) was conducted at Western Psychiatric Institute and Clinic (WPIC). Each subject was screened for medical conditions which may be contraindicated for anticholinergic medications (e.g., myasthenia gravis, glaucoma-see Inclusion/Exclusion criteria). In addition, subjects were screened for medical or psychiatric conditions that may affect cognitive or ocular response (Section 5.4, Inclusion/Exclusion criteria). Specifically, screening included a medical and psychiatric history (including prescription and over-the-counter medications), smoking status, vital signs, an electrocardiogram (EKG), a visual acuity test (Snellen exam), and biochemical tests (blood urea nitrogen (BUN), serum creatinine, sodium, potassium, thyroid stimulating hormone (TSH), aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin, hematocrit, and hemoglobin levels). Approximately 20 mL of blood were taken for biochemical tests. BUN, serum creatinine, sodium, and potassium were measured to ensure healthy kidney functioning; AST, ALT, and albumin were measured to ensure healthy liver functioning; hematocrit and hemoglobin were measured to test for anemia (given that blood draws were a part of this study); TSH levels were measured because an altered thyroid system may make an individual more susceptible to anticholinergic-induced elevated body temperature. In addition, the Geriatric Depression Scale (GDS) and the Mini-Mental State Exam (MMSE) were administered.

Typical caffeine and alcohol use, drug use, native language, years of education, dominant hand, and demographic data including age, weight, height, sex, eye color, and ethnicity were recorded. During the screening visit, subjects were introduced to all of the cognitive assessments which were to be performed during the study session. The digit symbol substitution test (DSST), the verbal learning assessment, and the letter-number sequencing test were practiced three times. One trial was completed for the both the N-back task and the logical memory paragraph. When a medical or psychiatric concern was discovered during screening, subjects were referred to the appropriate care.

Geriatric Depression Scale (GDS): The GDS is a self-reporting 30 item questionnaire that assesses for depression symptoms.[203] This scale was chosen because it can be administered with minimal clinician training and only takes 5 to 10 minutes to complete. The GDS scale was used in conjuction with open ended questions from the clinician in order to assess for depression. A cut-off score was not used for the inclusion criteria, as all rating scales, including the GDS, have a relatively high false positive.[204]

Mini-Mental State Exam (MMSE): The MMSE is a 30 point, clinician administered screening tool for cognitive status (e.g., assesses orientation, immediate and short-term recall, language, and the ability to follow simple verbal and written commands).[205] This scale was chosen because it can be administered with minimal clinician training and takes only 5 to 10 minutes to complete. The median MMSE scores for individuals 65 to 79 years of age are 27 to 29. A score of less than or equal to 24 is typically classified as being cognitively impaired.[206, 207]

Visual Acuity Assessment: A hand-held minimized Snellen eye-chart (lines of letters decreasing in size) was held in front of subjects' eyes. Subjects were requested to read the smallest line of letters possible with both eyes open. The appropriate corrective eyewear was worn during this task.

5.2.3 Study Session

This was a double-blind, placebo-controlled, randomized, cross-over investigation of oxybutynin immediate release (5 mg, oral, rapid release) on serum AA levels, ocular response, and cognitive performance. Each subject participated in 2 study visits separated in time by at least 7 days. It was predicted that oxybutynin and its active metabolite would be cleared from the body within 24 to 48 hours.[143] However, a minimum of a 7 day separation was chosen to avoid possible treatment order effects due to unknown variables such as tolerance or a discrepancy between central and peripheral pharmacokinetics. For instance, many medications bind to the melanin present in ocular tissue, and this often prolongs the pharmacodynamic effects of the agent.

The only difference between study session 1 and 2 was the study medication received. For study session 1, subjects received in a double-blind, randomized fashion, either oxybutynin or placebo. On study session 2, subjects received the alternate study treatment. All study session measures were conducted in the Neuroscience Clinical and Translational Research Center (N-CTRC-housed within WPIC).

Subjects were requested to try to maintain their usual medication regimen throughout the entire study and to avoid alcohol, nicotine, and caffeine products for 48 hours before each study session, as well as on the day of study. Subjects were requested to avoid these products because they can interfere with ocular and/or cognitive responses. Medications were taken as normal on the day of the study (this information was recorded) and a light breakfast (at home) was permitted. Special exceptions were made (e.g., bisphosphonates and antacids). Subjects were requested not to take antacids on the morning of or during the study session. This is because antacids and similar such medications may interfere with the absorption and bioavailability of oxybutynin. Subjects were requested not to take a bisphosphonate on the morning of the study due to the possibility of increasing the risk of gastrointestinal distress. Subjects were called the day before the study to inquire if there had been a change in medication usage or health status since the last visit. Study visit was rescheduled if subject was suffering from an acute medical condition (e.g., cold).

Baseline evaluations (Appendix D): Subjects arrived at WPIC at approximately 8:00 AM. Any medical or medication changes (including over-the-counter and herbal products) since the last visit were recorded. Baseline blood pressure, pulse, temperature, right and/or left eye resting pupil diameter were measured. A member of the staff who had been trained and certified in blood draws, placed an intravenous catheter in a forearm for multiple blood sampling. Subsequently, a blood sample (approximately 10 ml) was collected for baseline serum AA and serum drug levels. Cognitive assessments (i.e., verbal learning, letter-number sequencing task, N-back test) were administered while concurrently measuring pupillary response (e.g., pupil diameter and oscillations) using the ISCAN pupillometer. The DSST was always performed

directly following the immediate recall portion of the verbal learning task. This was to limit the subjects from practicing the word list for the delayed recall portion of verbal learning. All cognitive assessments at baseline were completed in the same order across sessions and subjects, with the exception of the N-back task. For the N-back task, 5 subjects always completed 1N-back first. The remaining subjects always completed 2N-back first. Parallel versions of cognitive tasks were administered in an approximate latin square design. Baseline measurements took approximately 1.5 to 2 hours.

After baseline assessments, 5 mg of oral oxybutynin IR or oral placebo (dispensed by WPIC pharmacy) was administered. Blood samples and pupil diameter were collected (ocular response was recorded and then blood sample drawn) at approximately 20, 40, 60 minutes and 1.5, 2, 4, and 6 hours after study medication administration. Sampling times and blood volume were chosen so as to limit the risk placed on older adults and to optimize possible pharmacokinetic modeling of both oxybutynin and desethyloxybutynin. The majority of blood samples occurred in the first two hours following study medication administration when absorption and distribution were prominent.

All cognitive assessments were completed in the same order at each time point and across subjects. The verbal learning assessment and the DSST were administered at approximately 1, 2, and 4 hours after the administration of medication or placebo. The letter-number sequencing test was administered at approximately 2 and 4 hours and the N-back test at 90 minutes post-medication. Immediately before and during verbal learning, immediate recall, letter-number sequencing, and the N-back tasks, pupillary response using the ISCAN pupillometer was

measured. Some assessments were not completed at all time points due to the intensive study schedule the first 3 hours following study medication administration.

At approximately 90 minutes post study medication, subjects were read two logical memory paragraphs from the Wechsler Memory Scale-Revised (WMS-R). Subjects were telephoned the day after the study session at their convenience (approximately 24 hours post administration of this assessment) and asked to recite as much of the paragraph that they can remember.

Subjects were requested to remain seated for the first 3 hours following study medication administration (e.g., with the exception of escorted restroom breaks). Vital signs and temperature were recorded at approximately 20 min, 40 min, 1, 2, 3, 4, and 6 hours following study medication administration for safety reasons only. Adverse events were assessed by means of spontaneous reports, observation, and a standardized questionnaire (see Appendix E). Fluids were encouraged throughout the study session. Lunch was provided approximately 3 hours following study medication administration; however snacks were available throughout the study session. At the end of the study, subjects were asked what medication he/she thinks was received.

5.2.4 Assessments

Digit Symbol Substitution Test (DSST): The digit symbol substitution test was used as a measure of attention.[208] A similar version to that used in the Wechsler Memory Scale-Revised (WMS-R) was employed. Subjects were requested to write as many figures corresponding to a series of numbers as possible. The total score is the number of correct choices completed within 90

seconds. Higher scores indicate better performance. The same version was used for each time point. The DSST has been shown to be impaired with more potent anticholinergic agents or at higher doses of low potent agents. However, the DSST was predominantly chosen for use to serve as a consistent task to be performed after the immediate verbal learning assessment in order to limit practice of word lists.

Letter-number sequencing test: The letter-number sequencing task, taken from the Wechsler Adult Intelligence Scale-3rd edition (WAIS-III) was used as a measure of attention and working memory.[209] Parallel, alternate forms were used for each time point and administered in an approximate latin squares design. Subjects were played an audio recording of a combination of numbers and letters and asked to recall the numbers first in ascending order and then the letters in alphabetical order. No time limit was given for how long the subject had to respond. The first three trials presented two digits (e.g., a letter and a number). If the subject correctly responded for at least one of the three trials, the subject would be advanced to the next tier which consisted of three trials of three digits each. This would continue until subject missed all three in a tier or until after 8 items was reached. The maximum score possible was 21 items with higher scores indicating better performance. Initial versions did not include the letter O. Approximately half way through the study, the letters C, D, L, and S were also removed due to subjects consistently recalling back a similar sounding letter.

Logical Memory Paragraph: Logical memory paragraphs from the WMS-R were used to examine long-term memory.[208] Parallel, alternative forms of this assessment were used for each time point. Subjects were read two short paragraphs from the WMS-R and were instructed to

remember the paragraphs as closely as possible. Subjects were telephoned approximately 24 hours after administration of this assessment and asked to repeat the story. These instructions deviate from the immediate/delayed recall instructions given in WMS-R. The majority of studies on anticholinergic-induced cognitive impairment have investigated episodic memory using a delayed recall of 20-45 minutes. We wished to explore the effects of oxybutynin on long-term memory using a delayed recall of 24 hours. Maximum score possible was 50 with higher scores indicating better performance.

N-back test: To examine working memory, participants completed a "1N-back" and a "2N-back" task in which single stimuli (e.g., numbers) were presented serially at 0.5 Hz. For the 1N-back task, subjects were told to push the space button when the current stimulus matched the one presented immediately before it (e.g., a---a). For the 2N-back task, subjects were told to push the space button when the current stimulus had been presented two stimuli back (e.g., a---b---a). The 1N-back and the 2N-back each consisted of 100 stimuli with approximately 23 possible correct positives. Scores are presented as percent correct (e.g., how many times the subject appropriately hit the space button divided by the total possible correct). Subjects were not penalized for false positives.

Only a few studies have examined working memory and anticholinergic medication. In younger adults, results have been inconsistent (e.g., not consistent across different anticholinergic medications, doses, and working memory assessments). Older adults have been shown to be impaired on the N-back test following a single dose of the anticholinergic medication

scopolamine (personal communication, Paul Newhouse). Parallel, alternative forms of this assessment were used for each time point.

Verbal Learning Assessment: The verbal learning assessment used for this study is a modification of the selective reminding task (SRT) of Buschke.[2] A different, parallel list of 15 words were used for each of the testing points, with no word used more than once. Words across test versions were matched for frequency/imagery ratings, meaningfulness, the number of syllables, and the number of letters using Pavio's word list generator (http://www.math.yorku.ca/SCS/Online/paivio/).[210] A total of 13 word lists were created (e.g., 2 alternatives, 3 for screening, and 8 for study sessions). The 8 versions for the study session were administered in an approximate latin square design. The words were presented at a rate of 1 word every 2 seconds using an audio recording. Subjects were instructed to remember as many words as possible for an immediate and delayed recall test. After the last word was read, subjects were given 3 minutes to verbally recall as many words as possible. The score was the number of words correctly recalled. Recall was again evaluated after a 30 minute delay. This test took approximately 5 to 10 minutes to complete. Verbal learning was chosen as a parameter to study because it has been shown to be impaired by oxybutynin. Moreover, verbal learning has consistently been shown to be impaired with various anticholinergic medications (e.g., low and higher doses, low and higher potency).

5.2.5 Additional measurements

Oxybutynin serum levels: Oxybutynin serum levels were analyzed using a previously published method, slightly modified.[211] Serum levels of R- and S-oxybutynin, as well as the enantiomers of the active metabolite, R- and S-desethyloxybutynin were measured using LC-MS/MS. Stock

standards of oxybutynin and desethyloxybutynin (1 mg/mL) were prepared in methanol. A combined standard (1000 ng/mL of each agent) was prepared in serum and frozen in aliquots. For each analysis, a serum standard was thawed and further diluted in blank serum in order to generate eight standards from 0.5 to 100 ng/ml (i.e., 0.25 to 50 ng/ml of each enantiomer). The limit of quantitation for each enantiomer was 0.25 ng/mL. The sample volume for both the standard curve and the subject samples was 450 µL. The standard curve was extracted with subject samples and a zero standard was included with each analysis. Trihexyphenidyl was used as the internal standard (i.e., 50 µL of 1 ug/ml).

Ten percent perchloric acid was used to facilitate protein precipitation. The supernatant was alkalinized with 100 µL of 5N ammonium hydroxide and then extracted twice with 6/4 hexane/ethyl acetate. The extract was dried under nitrogen and reconstituted with mobile phase for injection into the LC/MS.

A chiral AGP column (100 x 0.46 mm) was used on a ThermoFisher TSQ Quantum triple quadrapole. The mobile phase was made up of 92 percent of 0.1% formic acid (pH adjusted up to 3.5), 6 percent methanol, and 4 percent of acetonitrile for desethyloxybutynin; and 90 percent of 0.1% formic acid (pH adjusted up to 3.5), 6 percent methanol, and 4 percent of acetonitrile for oxybutynin.

Pupillary Measurement Analysis: The right eye was the preferred eye to measure pupil diameter/response because of evidence suggesting that parasympathetic tone is enhanced in the right eye as compared to the left.[156] All ocular measurements were recorded in dim light

(approximately 2.5 foot-candles). Subjects were facing the light source for ISCAN pupil measurements. Subjects were facing away from the light during handheld measurements. Difference in positioning may contribute to variability between measurements.

The NeuroOptics^{TM} hand-held pupillometer (resolution approximately 0.1 mm) was used to measure right eye pupil diameter at baseline and 20, 40, 60 minutes and 1.5, 2, 4 and 6 hours post study medication. This pupillometer captures 14 pupil positions per second over approximately an 8 second scanning period. We measured pupillary response at least three times at each time point. At least 1 minute lapsed in between replicates. Values at each time point for a given subject were averaged. Not all subjects have 3 successful pupil values at each time point (e.g., due to blinking). For this task, subjects were requested to focus on a neutral colored object greater than 1 meter away. Positioning of the subject and the focus object remained the same for all right eye measurements. In the one subject with left eye measurement, subject was placed approximately 2.0 feet to the left (closer to the light source) and maintained this position for all left eye measurements during both study sessions.

An ISCAN pupillometer, model RK 406, (resolution >0.05 mm) with an infrared light source was used to measure pupillary oscillations. Head position was maintained by a head and chin rest. For these measurements, subjects were requested to focus on fixation point approximately 1.2 meters away. Lens accommodation, or near response, does not contribute to pupillary unrest at this distance. Positioning and the fixation point remained the same for all subjects. A remote control system was used to keep the eye within recording limits. Horizontal eye diameter and pupillary unrest was measured for 3 minute periods as well as during the specific cognitive

assessments listed above (i.e., verbal learning, letter-number, and the N-back tasks). The analog output was digitized and stored.

Off-line, individual trial data was filtered using a 6.73 Hertz (Hz-cycle per second) two-pass digital filter. A blink algorithm to remove blinks was then applied. Oscillations were assessed at lower frequencies of 0.0 to 0.20; 0.21 to 0.40; 0.41 to 0.60; 0.61 to 0.80; 0.81 to 1.0; 1.0 to 2.0; 0.0 to 2.0. Wavelet analysis was used to assess the power (e.g., reflects the cumulative changes in amplitude) at each frequency across time. The higher the power, the greater the oscillations and/or amplitude changes which occur. Average power for each 30 seconds of measurement was examined (e.g., 0.0 to 30 seconds, > 30 to 60 seconds, etc).

Serum Anticholinergic Activity: See section 2.3.

5.3 ANALYSES

5.3.1 Descriptive

Given the small n, descriptive analyses were an important component of the analyses performed. Mean and standard deviation were calculated for all cognitive scores, pupil diameter, and serum AA at each time point. In addition, data was summarized using various graphical approaches.

5.3.2 Additional Analyses

A mixed model with repeated measures was used to examine ocular and cognitive responses with medication and time as within factors (e.g., an ANOVA was performed with verbal leaning scores as the dependent variable, a separate ANOVA was performed with logical memory scores as the dependent variable, etc). Appropriate transformation of the data was performed prior to analyses. Post-hoc tests were conducted using Tukey's.

The relationship between cognitive performance, serum AA levels and ocular response with oxybutynin was investigated using nonlinear, mixed-effects modeling using the software NONMEM® (ICON, Elliot City, Maryland). Forward and backward step-wise regression was performed to examine potential covariates affecting change in cognitive performance including serum concentrations, serum AA levels, and power of pupil oscillations.

5.3.3 Practice effects

Retest reliability and practice effects have not been well examined with brief testing intervals in older adults. It has been shown that the majority of practice effects for older adults occurs within the first 1-2 administrations of the cognitive assessments (parallel, alternative forms) being performed for this protocol when the tests are separated in time by approximately one week or more. There is some evidence to suggest that for verbal learning assessments (e.g., Hopkins verbal learning test and California verbal learning test), interference from one list to the next may occur with brief testing intervals. In younger adults, prevention of this interference can eventually be learned with repeated administration. It is not known if or when older adults will

learn prevention of list interference. We have attempted to limit interference by having only one immediate recall trial for each word list at each testing time point (as opposed to the majority of the verbal learning measures which employ 3-6 trials per list) and having subjects practice the assessments 3 times on the screening session day.

5.4 SUBJECT POPULATION

Only non-brown eyes, male or female Caucasians, 65 years or older were invited to participate in this study. Having brown eyes and being nonCaucasian may decrease and alter the timeline of ocular responses to specific medications (Section 4.2.2). A homogenous group of individuals was targeted in order to minimize the possible pupillary response variability associated with race and eye color.

Nicotine from tobacco can influence both cognitive and ocular responses. Because nicotine withdrawal (e.g., abstaining from tobacco) may also result in memory and ocular changes, only nonsmokers were invited to participate in this study. The majority of older adults screened consumed at least 1 or more products containing caffeine or alcohol. Subjects who consumed these products were allowed to participate in the study; however, they were requested to avoid these items for 48 hours prior to and during study sessions. Subjects were excluded if they would not or felt that they could not comply with abstinence from both caffeine and alcohol.

A cutoff for corrected vision was specified in the inclusion/exclusion criteria because of multiple computer or paper tasks which required reading or writing. Individuals with an MMSE score of

less than 25 were also excluded from participation in this study as we were targeting nondemented individuals.

The following inclusion/exclusion criteria were used to guide subject selection:

Inclusion Criteria

1) Caucasian
2) non-brown eyed
3) nonsmokers
4) age 65 years or older
5) corrected vision of at least 20/30
7) MMSE score of > 24

Exclusion Criteria

Individuals with specific contraindications or precautions to anticholinergic medications were excluded from study participation. Examples include:

1) glaucoma
2) interstitial cystitis
3) intestinal atony
4) uncontrolled hypertension
5) cardiac arrhythmias, other than occasional (≤ 1/6 beats) asymptomatic premature ventricular contractions (PVCs)
6) angina

7) history of (h/o) a documented myocardial infarction

8) myasthenia gravis

9) current esophagitis

10) ulcerative colitis

11) heart rate of less than 50 or greater than 90 beats per minute

12) h/o an allergic reaction to oxybutynin

13) abnormal value(s) for biochemical tests obtained at screening (as determined by the study physician using the UPMC adult reference ranges as a guideline)

Individuals were also excluded if they had a medical or psychiatric conditions which may have interfered with cognitive or ocular responses. Examples include:

1) macular degeneration with significant vision impairment (e.g., preventing completion of visual cognitive exams)

2) cataracts with significant vision impairment (e.g., preventing completion of visual cognitive exams)

3) h/o stroke

4) h/o traumatic brain injury resulting in a continuing CNS deficit (e.g., neurocognitive deficit)

5) major depressive disorder (current or within the past 1 year)

6) anxiety disorder (e.g., panic disorder, generalized anxiety disorder, obsessive compulsive disorder)

7) bipolar disorder

8) alcohol or drug abuse/dependency

9) schizophrenia

10) Parkinson's disease

11) Dementia

12) use of illicit drugs

13) taking medications known to be potent anticholinergic agents (e.g., scopolamine, atropine, tolterodine) or suspected to significantly affect cognitive or ocular responses (e.g., benzodiazepines, opiates, antidepressants)

14) cannot refrain from the consumption of caffeine and/or alcohol for 2 days before and day of each study visit

5.5 SUBJECT RECRUITMENT

Subjects were recruited from several sources. Robert Nebes, PhD (a co-investigator for this study) maintains a pool of normal older volunteers (e.g., individuals who have participated previously in his protocols or who have previously contacted him about participating in studies) who have expressed willingness to be contacted about potential aging studies. These individuals were informed of this new research study by Dr. Nebes. In addition to Dr. Nebes's pool of volunteers, an advertisement was placed in a newspaper geared towards older adults.

6.0 RESULTS: RELATIONSHIP BETWEEN SERUM ANTICHOLINERGIC ACTIVITY, COGNITION, AND OCULAR RESPONSE

6.1 DEMOGRAPHICS

6.1.1 Recruitment

A recruitment letter was sent out to 142 individuals (89 females). Over twenty individuals never responded to the letter. Approximately 48 responded either via mail or over the telephone that they were not interested in participating. The primary reason volunteered for lack of interest was that the study involved taking a medication. Of the interested individuals, approximately 18 were excluded over the telephone because of brown eyes; 27 due to one or more medical issues, and 9 for other reasons (e.g., smoking, inability to refrain from caffeine). A small subset of individuals was recruited via newspaper advertisement or by word of mouth referral. Of these, approximately 3 were excluded over the telephone due to brown eyes, 6 for medical reasons, and 4 for other.

Nineteen individuals (five obtained via newspaper advertisement or word of mouth referral; twelve females) passed the telephone interview and were brought in for formal screening. Of these, nine passed the formal screening and participated in at least one study session. Three subjects were excluded from participating in the study sessions (i.e., two for specific EKG abnormalities and one for a medication that was not disclosed during the telephone screening). The remaining subjects had either a medical issue which had not resolved prior to the study conclusion (e.g., hypertension) or necessitated a repeat EKG and/or biochemical testing (e.g., mild dehydration). Repeat biochemical or EKG testing was not included in the initial wording of the IRB protocol or consent. IRB modification approval was not received until after study conclusion.

6.1.2 Summary of subjects completing at least 1 study session

Nine subjects (8 females) participated in at least one study session between December 2006 and March 2007. Subject 11 (male) was withdrawn from the study early during his first study session (placebo) due to a serious adverse event (see table 6.2) and was excluded from all of the following analyses. Subject 5 withdrew from the study after session 1 (active) citing discontentment with the blood draws and lack of time as the primary reasons. Subject 15 only had blood draws performed during study session 1 (placebo) since she was only agreeable to continued participation if further blood draws were excluded.

The average age, height, and weight of the 8 female subjects was 70 (67-76) years, 63 (61.5-64.75) inches, and 163 (137-213) pounds, respectively. All subjects had some coursework following high school with one completing a 4 year degree and three obtaining a Masters degree. Five subjects had previously participated in at least one study which involved some memory or cognitive testing.

Table 6.1 lists the medical history and current medications for each subject. Given that older adults are on an average of up to 8 medications a day, it was a challenge to recruit subjects that were not on any medication with possible ocular or cognitive influences. Given such, we loosened the criteria to allow specific nonanticholinergic medications (e.g., metoclopramide, fluticasone, and salmeterol) which may have possible ocular or cognitive effects. However, we provided firm instructions for all subjects to take their medications the same exact way and time for each study session. Despite these instructions, there was a discrepancy between medication use in multiple subjects for study session 1 and 2. Also, given the prevalence of cataracts in an

older population (i.e., up to 80% of older adults eventually develop cataracts),[212, 213] a decision was made to allow individuals to participate in the study if he/she had only mild progression of the disease or a history of cataract removal of at least two years prior. Multiple sources indicate that ocular effects (e.g., blood-ocular barrier disruption or pupil size) in these instances are mild or not present.[214-216]

Table 6.1. Medical and medication history

Subject	Medical History	Medication the day before placebo study session*	Medication the day of placebo study session*	Medication the day before active study session*	Medication the day of active study session*
1	Asthma, treated Allergies, environmental Arthritis	fluticasone 250 µg/salmeterol 50 µg: 8:00 loratadine 10 mg: 8:00 fluticasone 50 µg: 23:00	fluticasone 250 µg/salmeterol 50 µg: 6:30	fluticasone 250 µg/salmeterol 50 µg: 07:05 loratadine 10 mg: 07:05 Multivitamin: 07:05 fluticasone 50 µg: 23:59	No medications taken
2	Allergies, environmental Arthritis HTN, mild Osteoporosis	Vitamin D: 10:45 G, C, MSM: 10:45, 15:00, 20:30 Ca, Mg, Vit D: 10:45, 15:00, 20:30 Multivitamin: 10:45 Vitamin C: 10:45 Vitamin E: 10:45	Vitamin D: 07:00 Ca, Mg, Vit D: 07:00 G, C, MSM: 07:00 Multivitamin: 07:00 Vitamin C: 07:00 Vitamin E: 07:00	Vitamin D: 9:00, 19:30 Ca, Mg, Vit D: 9:00, 19:30 G, C, MSM: 09:00, 19:30 Multivitamin: 09:00, 19:30 Vitamin C: 9:00, 19:30 Vitamin E: 09:00, 19:30 Estradiol Vaginal Tablet (extended release inserted 1 x weekly)	Vitamin D: 07:00 Ca, Mg, Vit D: 07:00 G, C, MSM: 07:00 Multivitamin: 07:00 Vitamin C: 07:00 Vitamin E: 07:00 Estradiol Vaginal Tablet (extended release inserted 1 x weekly)
4	ALT elevated (47 IU/L)	Ca, Vit D: 08:00 Vitamin C: 08:00 Multivitamin: 08:00	Ca, Vit D: 08:10 Vitamin C: 08:10 Multivitamin: 08:10	Ca, Vit D: 08:30 Vitamin C: 08:30 Multivitamin: 08:30	No medications taken
5	Allergies, environmental h/o breast cancer Hypothyroid, treated HTN, mild, treated Osteoarthritis	Subject did not participate in placebo arm	Subject did not participate in placebo arm	Enalapril 10 mg: 09:00 AM Synthroid 0.1 µg 09:00	Enalapril 10 mg: 07:00 AM Synthroid 0.1 µg 07:00
11	Cataracts, R/L eyes, mild progression EKG-findings of	Atorvastatin 10 mg: 22:00	No medications taken	Subject did not participate in active arm	Subject did not participate in active arm

	left axis deviation HTN, mild Hypercholesterolemia, treated				
12	Arthritis Cataract removal, R/L eyes, 6 years h/o breast cancer HTN, mild Hypercholesterolemia, treated NIDDM R leg 2 inches shorter than L due to car accident (no head trauma)	Ibuprofen 400 mg: 09:30 Metformin 500 mg: 09:30 Multivitamin: 09:45 B-complex: 09:45 Vitamin C: 17:00 Vitamin E: 17:00 Aspirin 81 mg: 17:00 Simvastatin 40 mg: 22:00	Ibuprofen 400 mg: 06:00 Metformin 500 mg: 06:00 Multivitamin: 06:00 B-complex: 16:00 Vitamin C: 16:00 Vitamin E: 16:00 Aspirin 81 mg: 16:00	Ibuprofen 400 mg: 09:30 Metformin 500 mg: 09:30 Multivitamin: 09:30 Vitamin C: 13:30 Vitamin E: 13:30 Aspirin 81 mg: 13:30 Lisinopril 5 mg: 17:15 Simvastatin 40 mg: 21:30	Ibuprofen 400 mg: 08:30 Metformin 500 mg: 08:30 Multivitamin: 08:30 B-complex: 08:30 Vitamin C: 14:15 Vitamin E: 14:15 Aspirin 81 mg: 14:15 Lisinopril 5 mg: 16:45¥
15	Allergies, environmental Arthritis Collagenous colitis EKG, L bundle branch block Goiter/nodes on thyroid, benign Hiatal hernia/esophagitis, treated h/o squamous skin cancer Osteopenia Scoliosis	Esomeprazole 40 mg: 08:00, 21:15 Diclofenac 75 mg: 11:00, 20:00 0.3 mg conjugated estrogen/1.5 mg medroxy progesterone: 11:00 aspirin 650 mg: 13:00, 17:00 calcuium: 20:00	Esomeprazole 40 mg: 06:10 Diclofenac 75 mg: 07:00 0.3 mg conjugated estrogen/1.5 mg medroxy progesterone: 07:00 calcuium: 07:00	Esomeprazole 40 mg: 08:00, 17:00 Diclofenac 75 mg: 09:30, 20:15 0.3 mg conjugated estrogen/1.5 mg medroxy progesterone: 09:30 aspirin 650 mg: 16:00 calcuium: 09:30, 20:15	Esomeprazole 40 mg: 06:00 Forgot to take remaining morning medictions
16	Arthritis Cataracts, R/L mild progression Sinuses	Multivitamin: 06:45 Fish oil 1000 mg: 06:45 Flax oil: 06:45 Aspirin 81 mg: 06:45 Ca, Mg, selenium: 06:00 Fluticasone: 100 µg (or 200?): 06:45 Acidopholus: 22:00 Aspirin 325 mg q hs	Multivitamin: 06:45 Fish oil 1000 mg: 06:45 Flax oil: 06:45 Aspirin 81 mg: 06:45 Ca, Mg, selenium: 06:00 Fluticasone: 100 µg (or 200?): 06:45	Multivitamin: 07:40 Fish oil 1000 mg: 07:40 Flax oil: 07:40 Aspirin 81 mg: 07:40 Ca, Mg, selenium: 07:00 Fluticasone: 100 µg (or 200?): 06:48 Aspirin 325 mg:18:00	Multivitamin: 06:50 Fish oil 1000 mg: 06:50 Flax oil: 06:50 Aspirin 81 mg: 06:50 Ca, Mg, selenium: 06:10 Fluticasone: 100 µg (or 200?): 06:47

18	Anemia, mild Arthritis Cataract removal, R/L, 3 years GERD, treated Osteoporosis	Celecoxib 200 mg: 06:30 C, G, MSM: 06:30 Fish oil 1000 mg: 06:30 Multivitamin: 06:30 Esomeprazole 40 mg: 21:00 Metoclopramide 10 mg: 21:00 Aspirin 81 mg: 21:00 Raloxifene 60 mg: 21:00	Celecoxib 200 mg: 06:20 C, G, MSM: 06:20 Fish oil 1000 mg: 06:20 Multivitamin: 06:20 0	Celecoxib 200 mg: 06:50 C, G, MSM: 06:50 Fish oil 1000 mg: 06:50 Multivitamin: 06:50 Esomeprazole 40 mg: 20:30 Metoclopramide 10 mg: 20:30 Aspirin 81 mg: 20:30 Raloxifene 60 mg: 20:30	Celecoxib 200 mg: 06:35 C, G, MSM: 06:35 Fish oil 1000 mg: 06:35 Multivitamin: 06:35

*Underlined items indicate that there is a discrepancy between study session 1 and study session 2.

[¥] Subject initiated this medication in between session 1 (placebo) and session 2 (active)

ALT=alanine transferase, Ca=calcium, C=chondroitin, G=glucosamine, GERD=gastroesophogeal reflux disease, h/o=history of, HTN=hypertension or high blood pressure, Mg=magnesium, MSM= Methyl Sulfonyl Methane, NIDDM-Type 2 diabetes, Vit=vitamin

6.2 ADVERSE EVENTS

Adverse events were determined by a 4 point questionnaire (Appendix E), open-ended questions, and objective review. Table 6.2 summarizes all of the adverse events recorded during the study sessions. One of the aims of this study was to assess the tolerability of oxybutynin in an older population. As can be seen in Table 6.2, oxybutynin was well tolerated by this group of subjects with a similar profile of adverse events occurring with oxybutynin as compared to placebo.

We used this 4 point questionnaire (Appendix E) successfully in previous studies when administered at a single time point during one study session. We chose it for this study because it was simple to use and took minimal time to complete. However, this questionnaire alone did

not prove to be very useful for following the change in adverse events over a short period of time. For instance, subjects verbally reported having an increase in fatigue, dry mouth, or dry eyes, yet this was not reflected on the adverse event questionnaire.

Table 6.2. Adverse events recorded during study sessions

Subject	Placebo	Oxybutynin
1	• ↓ Urinary urgency (mild), 01-31-07; *Causality-not probable.* • ↑ in tiredness (mild), 01-31-07; *Causality-possible* • ↑ in irritability (mild)-objective, 01-31-07; *Causality-possible*	None reported-02-08-07
2	• ↓ in blood pressure throughout session (from 180s/90s when first arrived to 150s/90s), 01-18-07; *Causality-not probable* • Mild headache by the end of the day, 01-18-07; *Causality-possible* • Increase in urine flow, increase in output, mild-subjective, 01-18-07; *Causality-not probable* • ↓ in tiredness (mild), 01-18-07; *Causality-not probable* • ↓ in dry eyes (mild), 01-18-07; *Causality-not probable* • ↓ in weakness/lack of strength (mild), 01-18-07; *Causality-not probable* • ↓ in tension/nervous restlessness (mild), 01-18-07; *Causality-not probable* • ↓ in feelings of thirstiness (mild), 01-18-07; *Causality-not probable* • ↓ in concentration difficulties (moderate), 01-18-07; *Causality-not probable*	• ↓ in blood pressure throughout session (From 150s/80s when first arrived to 120s/70s), 01-25-07; *Causality-not probable* • Some blood loss at site of catheter placement post catheter removal; resolved by applying pressure to the wound and holding arm up for approximately 5 minutes (mild) 01-25-07; *Causality-Definite* • Brief, mild, Neck/head pain, 01-25-07; *Causality-probable*
4	• ↓ in feelings of dry mouth (mild), 01-04-07; *Causality-not probable* • ↓ in feelings of dry eyes (mild), 01-04-07; *Causality-not probable* • weakness/lack of strength (mild), 01-04-07; *Causality-not probable* • Tiredness (mild); 01-04-07, *Causality-possible*	• ↓ in dry mouth (mild); 12-19-06, *Causality-not probable* • ↓ in increased salivation (mild), 12-19-06; *Causality-possible* • ↓ in feelings of thirstiness (mild), 12-19-06; *Causality-not probable* • weakness/lack of strength (mild), 12-19-06; *Causality-possible* • concentration difficulties (mild), 12-19-06; *Causality-probable*
5	***This subject only came in for 1 study session**	• ↓ in blood pressure (from 120s/80s to 100s/70s) from 40 minutes post study medication to 3 hours post study medication (mild), 12-18-06; *Causality-possible--This decrease in blood pressure could correspond to Cmax of enalaprit (active metabolite of prodrug enalapril) or study medication.*

		• Mild tenderness at site of catheter placement (reported 24 hours post study session), 12-19-06; *Causality-Definite* ***This subject has only come in for 1 session**
11	• ↓ in tiredness (mild), 02-05-07; *Causality-not probable* • vasovagal episode in response to blood draw attempt, 02-05-07; *Causality-probable* [The subject felt faint and experienced a decrease in heart rate (~38) and blood pressure (70s/30s). The subject maintained consciousness and maintained a fully alert mental status during this event. There was no chest pain or discomfort, but some mild nausea. No change noted on the EKG. Symptoms spontaneously resolved]	Study unblinded early to AE which occurred during study session 1 (placebo)
12	• ↑ in tiredness (mild), 02-22-07; *Causality possible* • ↓ in feelings of dry mouth (mild), 02-22-07; *Causality-not probable* • ↑ in salivation (mild), 02-22-07; *Causality-not probable* • ↑ in weakness/lack of strength (mild), 02-22-07; *Causality-not probable* • ↑ in tension/nervousness (mild); 02-22-07; *Causality-possible* • ↓ in tremor/shaking (mild); 02-22-07; *Causality-not probable* • ↓ in concentration difficulties; 02-22-07; *Causality-not probable* • ↓ in nocturnal urgency; 02-22-07; *Causality-not probable*	• During a catheter attempt at 20 minutes post study medication, a needle infiltrated subject's vein. At 2 hour post study medication, subject noted that site of infiltrated vein was swollen, bruised, and had an itchy/stingy sensation (mild). Ice and then a cold compress were placed at site of infiltrate. Swelling was significantly down by the end of the study session, however a bruise remained, 03-01-07; *Causality-Definite* • ↑ in blood pressure from baseline-120s/70s to 140s/80s (mild), 03-01-07; *Causality-Possible* • ↓ in ability to follow commands, mild (objective), 03-01-07; *Causality-Probable* • ↑ tiredness (mild), 03-01-07; *Causality-Probable* • dizziness/lightheadedness (mild), 03-01-07; *Causality- Probable* • ↓ in dry mouth (moderate), 03-01-07; *Causality-Not probable* • ↑ in confusion (mild), 03-01-07; *Causality-Probable* • ↑ in concentration difficulties (mild), 03-01-07; *Causality-Probable*
15	• Mild increase in thirst, 03-21-07; *Causality-possible* • Mild tiredness or fatigue, 03-21-07; *Causality-possible* • Mild headache, 03-21-07; *Causality-possible* • Single episode of subjective mild	• Mild tiredness or fatigue, 03-30-07, *Causality-probable* • Mild headache, 03-30-07, *Causality-probable* • Mild increase in thirst, 03-30-07, *Causality-probable* • Mild brief neck/back discomfort from

	"heart fluttering" (This episode occurred post lunch during relaxation, when no study measures were being conducted. Subject reports that this event occurs occasionally with the most common stimulus being caffeine consumption. No other sxs were noted at the time of "heart fluttering"—subject denied chest pains, denied nausea, denied lightheadedness, and denied shortness of breath. No irregular heart beats were noted at any of the blood pressure time points), 03-21-07; *Causality-not probable* • Mild nasal congestion lasting approximately 1-2 hours, 03-21-07; *Causality-not probable* • Moderate Anxiety, near the end of this study session (session 1), verbally reported (e.g., subject did not list this as an adverse event on any of the questionnaires or during previous open ended prompts) moderate anxiety associated with catheter insertion and blood draws. Subject was agreeable to come back for study session 2 and participate in all cognitive and ocular measurements; however subject was only agreeable to 1 attempt at catheter insertion (as opposed to up to 3 tries), 03-21-07; *Causality-Definite*	the chin/head rest, 03-30-07, *Causality-definite* • Mild watery eyes while staring constantly at a dot for 3 minutes, 03-30-07, *Causality-definite* • Bruising at the site of unsuccessful catheter insertion, 03-30-07, *Causality-definite* • Increase in trips to the restroom and increase in urine output (subjective—subject also noted that she may be drinking more than usual), 03-30-07, *Causality-possible*
16	• Subject c/o mild headache the day before and the day of the study session, 03-28-07 and 03-29-07; *Causality-probable-most likely caffeine withdrawal headache* • Mild increase in headache during study procedures, 03-29-07; *Causality-probable* • At one time point, diastolic blood pressure was 49 (usual was ~60s). Subject reported feeling mild tiredness, denied dizziness or lightheadedness, denied shortness of breath, denied chest pain, and denied nausea. Subject was encouraged to have juice and a snack. Following snack, blood pressure returned to normal (~115s/60s), 03-29-07; *Causality-not probable* • Mild tiredness or fatigue, 03-29-07; *Causality-possible* • Mild concentration difficulties, 03-29-07; *Causality-possible* • Some blood loss at site of catheter	• Subject c/o mild headache the 2 days before, 1 day before, and the day of the study session, 03-11-07 to 03-13-07; *Causality-probable -most likely caffeine withdrawal headache* • Mild increase in headache during study session, 03-13-07; *Causality-probable* • Mild tiredness or fatigue, 03-13-07; *Causality-probable* • Mild increase in concentration difficulties, 03-13-07; *Causality-probable*

	placement post catheter removal; resolved by applying pressure to the wound and holding arm up for approximately 5 minutes, 03-13-07; *Causality-definite*	
18	None reported	• Mild fatigue, 03-19-07, *Causality-probable* • Mild dry eyes, 03-19-07, *Causality-probable*

This study is a double-blind, randomized, cross-over study with two 8 hour study session days. The only difference between study session 1 and study session 2 is the medication received (e.g., placebo or oxybutynin).
*Causality listed above could be due to either study medication or study session procedures.
**All adverse events listed above resolved within 24 hours unless otherwise noted.

6.3 OXYBUTYNIN PHARMACOKINETICS

6.3.1 Blood Collection

Blood samples were taken at baseline and at approximately 0.33, 0.66, 1, 1.5, 2, 4 and 6 hours post study medication administration. Blood samples were available from all 7 of the participating subjects from the placebo session and 7 of the 8 subjects from the active session. In total, there were 52 and 51 available blood samples for the placebo and oxybutynin arm, respectively.

6.3.2 Population Pharmacokinetics of R-oxybutynin and R-desethyloxybutynin

The S-enantiomers for oxybutynin and its metabolite have minimal antimuscarinic activity at the serum levels produced with acute administration of a 5 mg dose.[193-195] Therefore, the

pharmacokinetic profiles of only the R-enantiomers were assessed. Figure 6.1 shows the average concentration time profiles for both the parent and metabolite. Figure 6.2 shows the individual concentration time profiles.

One and two-compartment linear pharmacokinetic models with various statistical forms for interindividual and residual variability were tested during model development using NONMEM-V®. The subroutines ADVAN2 TRANS2 (one-compartment model) and ADVAN4 TRANS4 (two-compartment model) were used. Given the small number of subjects available for analysis, the pharmacokinetic profile of the parent was calculated first, separate from the metabolite. A two-compartment model with additive error, a lag time and interindividual variation on clearance and central volume best fit the data (Figures 6.3 and 6.4). The inter-individual variability (e.g., the unexplained random variability in individual values) of clearance or volume was assumed to be log-normally distributed. The relationship between clearance or volume (e.g., the pharmacokinetic parameter (P)) and its variance was therefore expressed as shown below:

$$\text{Equation 6.1: } P_j = P_{TV} \times e^{\eta_P}$$

where P_j was the value of the pharmacokinetic parameter for the j^{th} individual, P_{TV} was the typical value of P for the population, and η_P denoted the difference between P_j and P_{TV}, independently, which was identically distributed with a mean of zero and variance of ω_P^2. The residual variability, which was comprised of, but not limited to, experimental errors, process noise and /or model misspecifications, was best fit by an additive model error as described below:

$$\text{Equation 6.2: Additive error: } y_{ij} = \hat{y}_{ij} + \varepsilon_{ij}$$

where y_{ij} was the jth observation in the ith individual, $\hat{y}_{ij}$ was the corresponding model prediction, and ε_{ij} was a normally distributed random error with a mean of zero and a variance of σ^2.

The parent parameter values obtained are similar to what has been reported previously in the literature (e.g., when the very low bioavailability of oral oxybutynin is taken into account). The population predicted clearance/f, central volume/f, peripheral volume/f (e.g., f takes into account bioavailability) were 459 (58) L/hr, 235 (98) L, and 463 L.

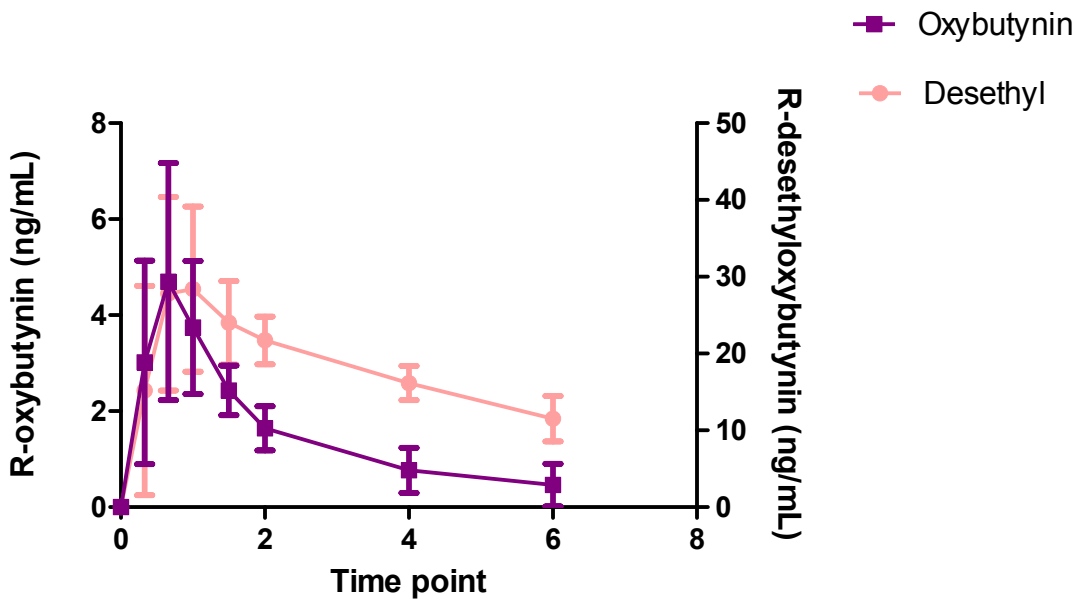
At present, attempts to characterize the pharmacokinetics of R-desethyloxybutynin have been unsuccessful. Two approaches were utilized during model development of the metabolite. In the first approach, the best fit model of the parent with the predicted population parameters was incorporated into the metabolite model. In other words, 1 and 2 compartment models for the metabolite were tested while dictating that the parent had 2-compartment kinetics with lag time and interindividual variability on clearance and central volume (with all parent population means fixed). The subroutine ADVAN TRANS1 was used. A two-compartment model with interindividual variation on the fraction of parent cleared to desethyloxybutynin (FM) best fit the metabolite data (Figures 6.3 and 6.4). Equation 6.1 was used to describe the interindividual variation on FM. A combined additive and proportional error as described below best fit the residual variability:

Equation 6.3: $$y_{ij} = \hat{y}_{ij}(1+\varepsilon_{ij}) + \varepsilon_{ij}'$$

where y_{ij} was the jth observation in the ith individual, $\hat{y}_{ij}$ was the corresponding model prediction, and ε_{ij} (or ε_{ij}') was a normally distributed random error with a mean of zero and a variance of σ^2.

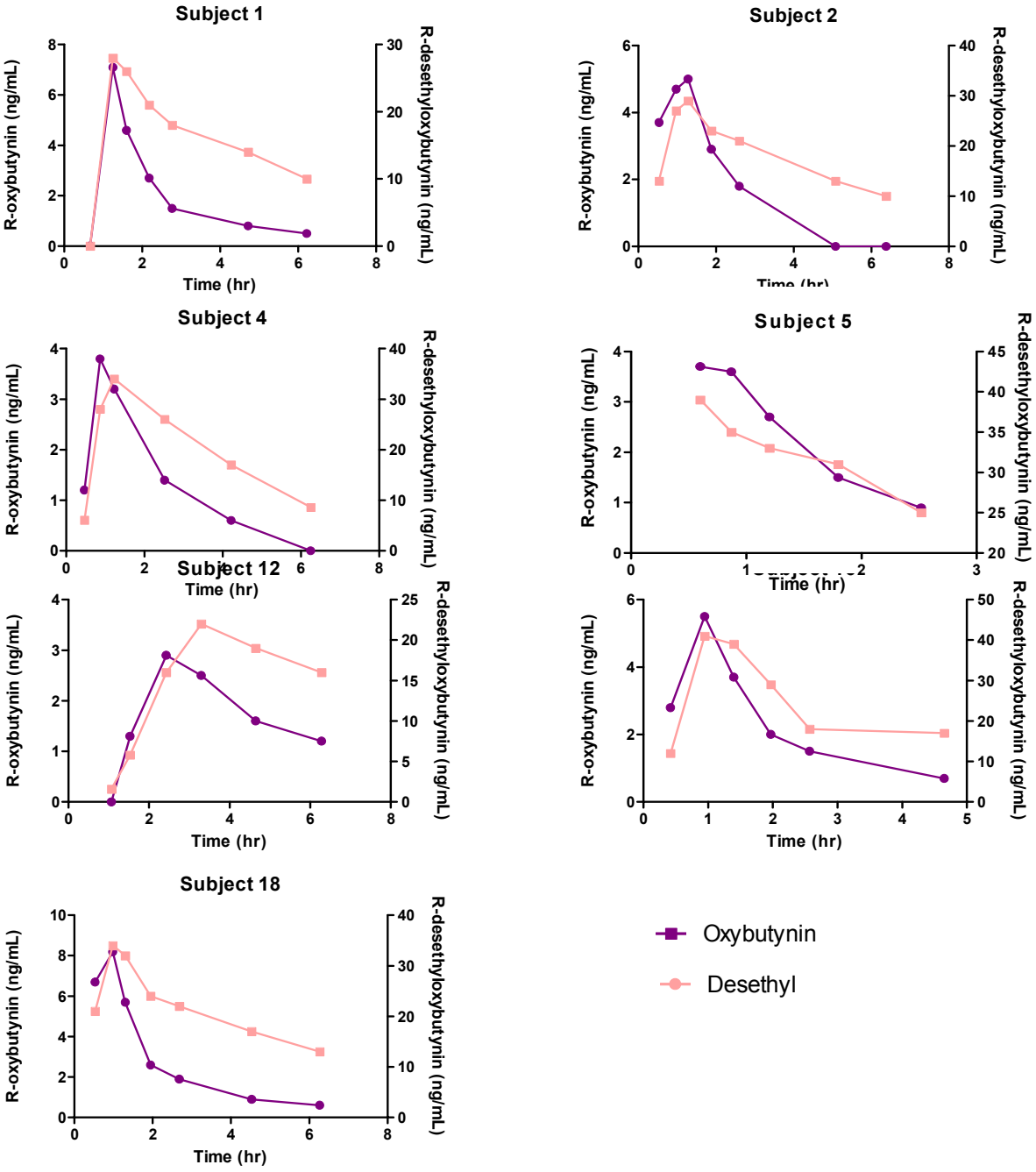
However, the model was not stable as even minor changes in the user defined initial parameter values yielded large differences in metabolite parameter outputs. The predicted parent concentrations however were stable and similar to those estimated when using observed parent values alone. A second approach was to utilize the individual predicted parameter values from the parent model (e.g., as opposed to the first approach where the population mean values were fixed, but individual values were allowed to vary) Again, a two-compartment model with interindividual variation on FM best fit the metabolite data. However, the model was still unstable to changes in the user defined initial parameter values. The available data may be insufficient for adequate characterization of specific metabolite pharmacokinetic parameters. However, the actual observed values of individual parent and metabolite concentrations were well traced by the model outlined above. Hence, the specified metabolite model may be sufficient for incorporation into pharmacodynamic models or for prediction of metabolite values at a given time.

Figure 6.1. Average R-oxybutynin and R-desethyloxybutynin concentrations over time



A single dose of oxybutynin (5 mg) was administered orally after the zero time point blood draw. Serum levels were assessed at approximately 0.33, 0.66, 1.0, 1.5, 2.0, 4.0, and 6.0 hours following oxybutynin administration. Error bars reflect standard deviation.

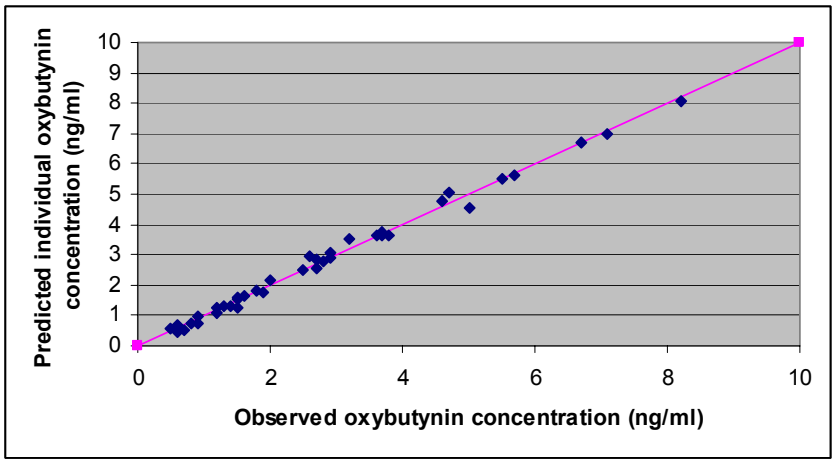
Figure 6.2. Individual R-oxybutynin and R-desethyloxybutynin concentrations over time



A single dose of oxybutynin (5 mg) was administered orally at the zero time point. Serum levels were assessed at approximately 0.33, 0.66, 1.0, 1.5, 2.0, 4.0 and 6.0 hours following oxybutynin administration. Subject 15 did not participate in blood draws for her active session and therefore is not represented here.

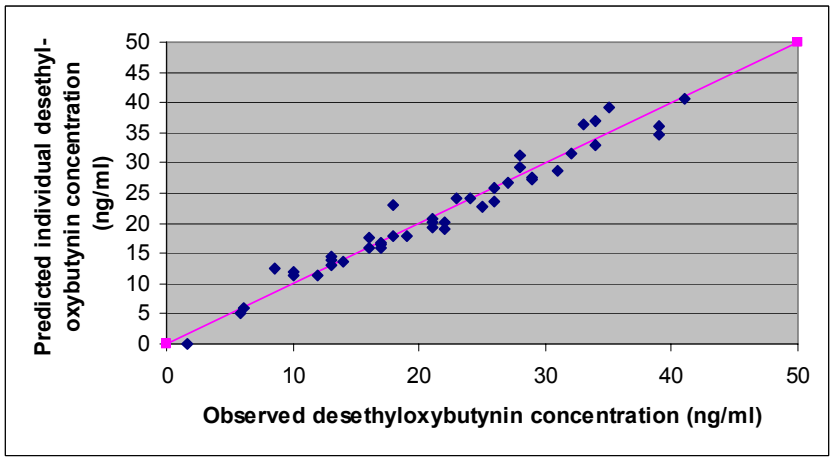
Figure 6.3. Observed versus individual predicted drug concentrations

R-oxybutynin



The predicted individual R-oxybutynin concentrations reported above were generated using observed R-oxybutynin concentrations alone (e.g., without the metabolite data). However, a similar fit of R-oxybutynin concentrations was seen when the combined parent-metabolite model was used. The pink line is a unit line.

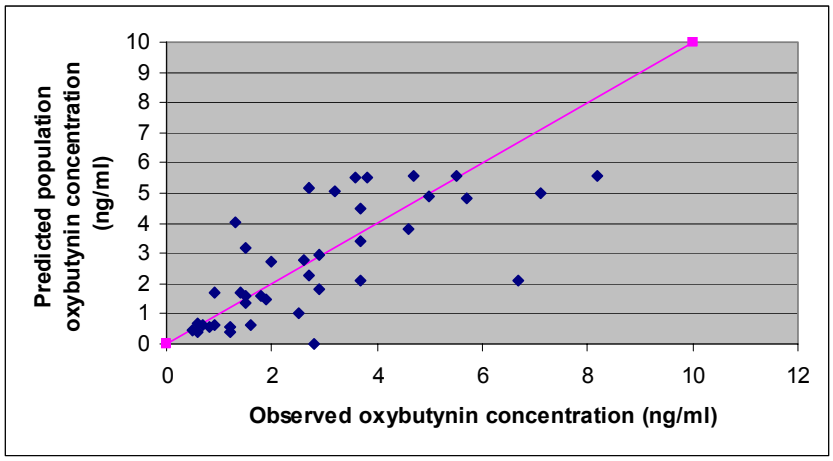
R-desethyloxybutynin



The predicted individual R-desethyloxybutynin concentrations reported above were generated using the combined parent-metabolite model. The pink line is a unit line.

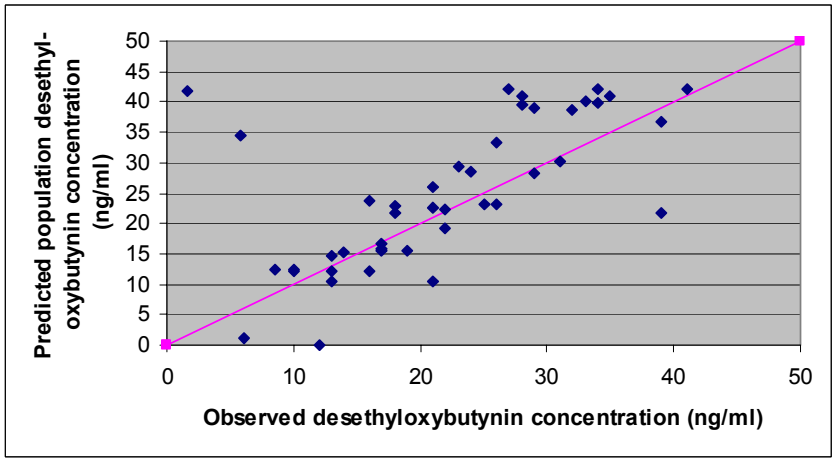
Figure 6.4. Observed versus population predicted drug concentrations

R-oxybutynin



The predicted population R-oxybutynin concentrations reported above were generated using observed R-oxybutynin concentrations alone (e.g., without the metabolite data). The pink line is a unit line.

R-desethyloxybutynin



The predicted population R-desethyloxybutynin concentrations reported above were generated using the combined parent-metabolite model. The pink line is a unit line.

6.4 SERUM ANTICHOLINERGIC ACTIVITY

Blood collection for serum AA is as outlined under section 6.3.1 The average (SD; range) baseline values for placebo and active arm are 2.9 (2; 0 to 6.1) and 1.9 (1.3; 0.7 to 4.4) pmol/mL of atropine equivalents, respectively. The individual without AA at baseline on placebo day showed detectable AA at later time points. Part of the discrepancy between placebo and active baseline serum AA is most likely due to failure by subjects to take their medications exactly the same way for study sessions 1 and 2 (Table 6.1). A change in serum AA over time on placebo day was evident, as would be expected if medication is driving serum AA levels (Figures 6.5 and 6.5).

6.4.1 Relationship between serum AA and drug levels

The relationship between serum AA and R-oxybutynin/R-desethyloxybutynin was investigated using linear and nonlinear models with various statistical forms for interindividual and residual variability using NONMEM-V®. Placebo data was modeled first, using the following base equations (t = time, con = constant, C_0 = initial serum AA with time point 0.33 hr values replaced for baseline for subject 12):

Equations

6.4 F = con

6.5 F = con + m*t

6.6 F = con*exp(-k*t)

6.7 F = con + b*exp(-k*t)

6.8 $F = C_0*\exp(-k*t)$

6.9 $F = a*\exp(-k_1*t) + b*\exp(-k_2*t)$

6.10 $F = a*((\exp(-k_1*t) - \exp(-k_2*t))$

Using baseline values with monoexponential decline over time (e.g., Equation 6.8) and additive error provided the best fit (Figure 6.7); although the findings were similar when a constant was used instead (e.g., Equation 6.6). The estimated population mean for k was 0.0624 hr^{-1}. Interindividual variation on k did not improve the fit of the model. The additive error used is represented in equation 6.2

Data from the active session were then modeled investigating the effects of R-oxybutynin and R-desethyloxybutynin on serum AA. The following base equations were employed (oxy = R-oxybutynin concentrations, des = R-desethyloxybutynin concentrations, t = time, con = constant):

Equations

6.11 F = con

6.12 F = con + m*oxy

6.13 F = con + m*des

6.14 F = con + m*(oxy + des)

6.15 F = con + m*oxy + b*des

6.16 F = con + (emax*(oxy**n))/(ec50**n + oxy**n)

6.17 F = con + (emax*(des**n))/(ec50**n + des**n)

6.18 F = con + (emax*(oxy**n))/(ec50**n + oxy**n) + (emax*(des**n))/(ec50**n + des**n)

In addition, time was also tested with the above listed equations (see equation 6.8 under placebo)

Incorporating both parent and metabolite (no interindividual variation) with a linear model (e.g., Equation 6.15) and proportional error best fit the data (Figure 6.7). The proportional error structure used was:

Equation 6.19: $y_{ij} = \hat{y}_{ij}(1 + \varepsilon_{ij})$

where y_{ij} was the jth observation in the ith individual, $\hat{y}_{ij}$ was the corresponding model prediction, and ε_{ij} (or ε_{ij}') was a normally distributed random error with a mean of zero and a variance of σ^2. The fit was similar if baseline serum AA levels (e.g., C_0) or constant was employed. Time did not improve the model in the presence of oxybutynin and desethyloxybutynin. The estimated slope for oxybutynin was 0.913; the estimated slope for desethyloxybutynin was 0.05. Oxybutynin produced a similar AA slope (i.e., 0.86) when assessed *in vitro* (Section 3.2). At the time of model development, *in vitro* AA of desethyloxybutynin was unavailable for comparison.

Figure 6.5. Average serum anticholinergic activity versus time

Figure 6.5A.
Serum AA over time following placebo

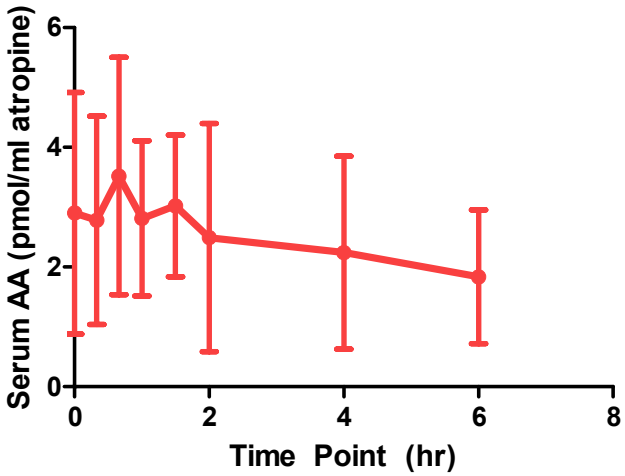
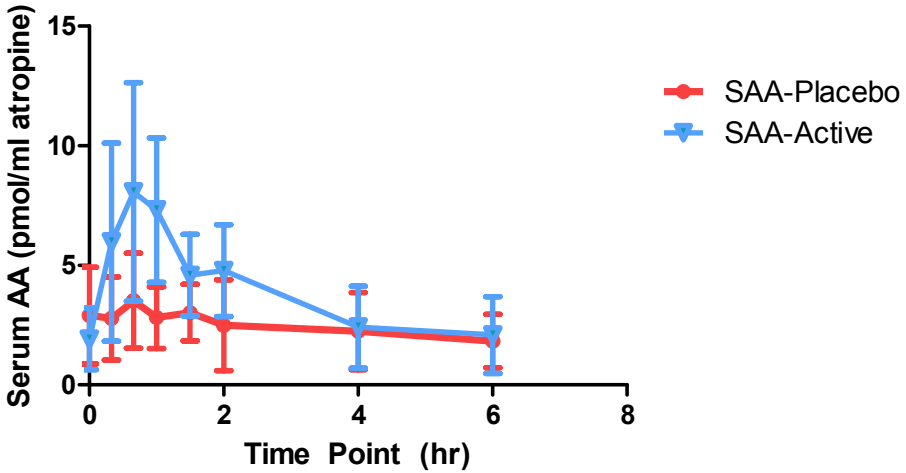


Figure 6.5B.
Serum AA over time following oxybutynin



A single dose of placebo (Figure 6.5A) or oxybutynin (Figure 6.5B) was administered orally at the zero time point. Serum AA was assessed at baseline and at approximately 0.33, 0.66, 1.0, 1.5, 2.0, 4.0, and 6.0 hours following study medication administration. Error bars reflect standard deviation.

Figure 6.6. Individual serum anticholinergic activity versus time

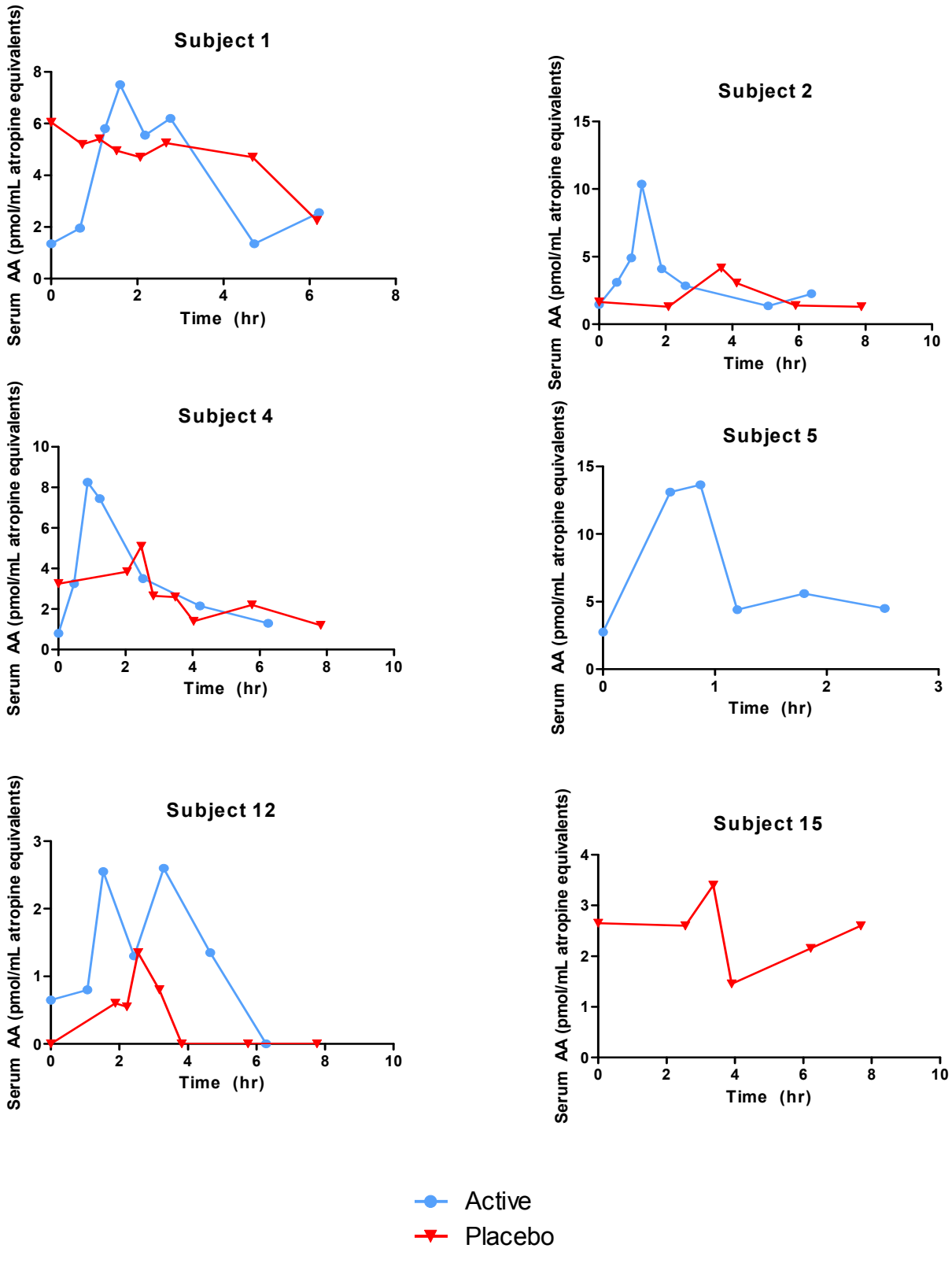
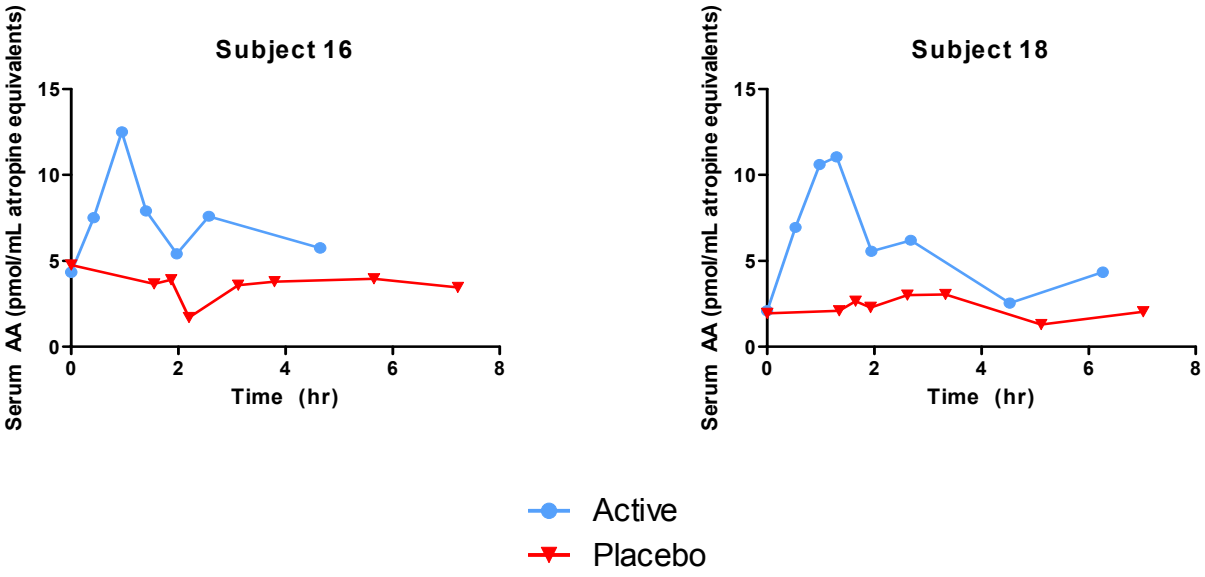


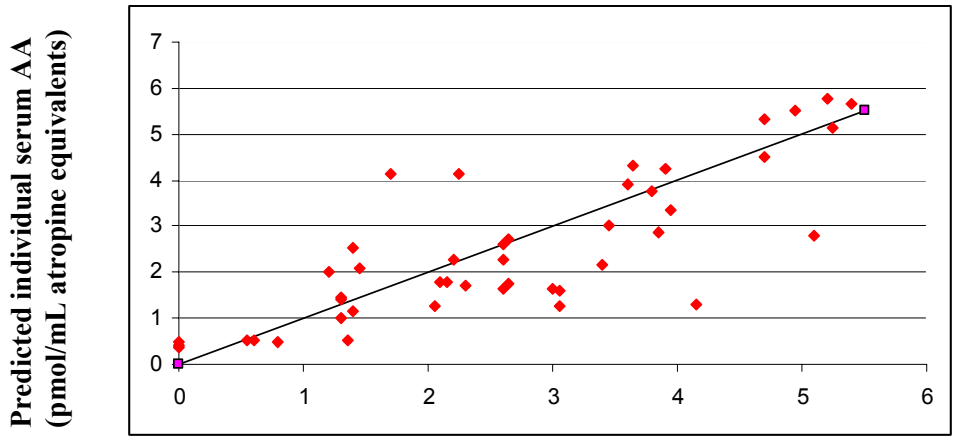
Figure 6.6. Individual serum anticholinergic activity versus time (continued)



Individual serum AA versus time plots following administration of a single dose of placebo (red) or oxybutynin (blue). Serum AA was assessed at baseline and at approximately 0.33, 0.66, 1.0, 1.5, 2.0, 4.0 and 6.0 hours following study medication administration. Subject 5 participated only in the active session and therefore only active data is plotted above. Subject 15 did not participate in blood draws for the active session and therefore only placebo data is plotted.

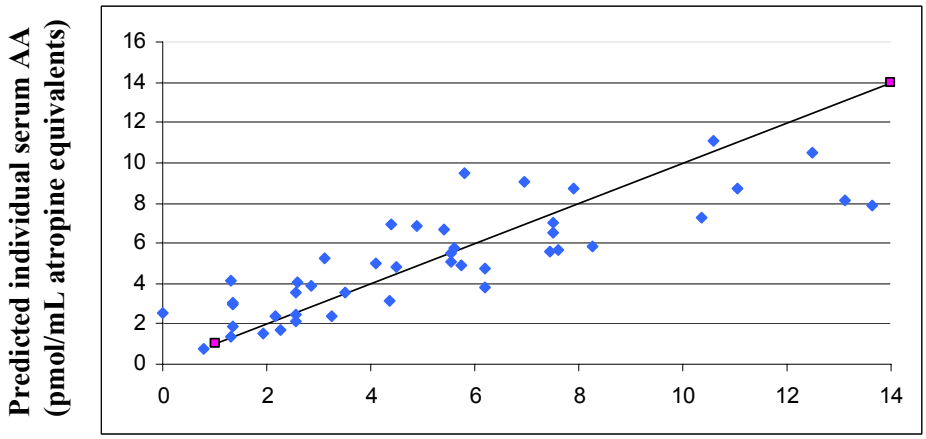
Figure 6.7. Observed versus individual predicted serum anticholinergic activity

Placebo day



The predicted individual serum AA reported above were generated using equation 6.8. The black line is a unit line.

Oxybutynin day



The predicted individual serum AA reported above were generated using equation 6.15. The black line is a unit line.

6.4.2 Discussion

Serum AA over time following placebo administration was characterized by a monoexponential decline, with an "elimination" half-life of approximately eleven hours. Serum AA following oxybutynin administration was well described by a linear model of R-oxybutynin and R-desethyloxybutynin serum levels. This is the first study in which serum AA was measured across time in the absence of the administration of a specific anticholinergic agent. Moreover, serum levels of oxybutynin and its metabolite were strongly associated with serum AA despite the presence of moderate baseline serum AA. Both of these findings support the notion that serum AA represents the cumulative effects of medications and their metabolites.

Baseline serum AA levels were considerably higher than we expected given that we specifically excluded from participation all individuals receiving any known anticholinergic agent. In fact some placebo serum AA values overlapped with those seen following oxybutynin administration (Figure 6.5B). This finding highlights the fact that the muscarinic receptor binding profile of most medications, metabolites and possibly endogenous substances is unknown

6.5 COGNITIVE PERFORMANCE

6.5.1 Verbal Learning

Parallel versions of immediate and delayed verbal learning recall were administered at 4 time points during each study session (i.e., at baseline and approximately 1, 2 and 4 hours post study

medication administration) in an approximate latin square design. A total of 7 subjects during placebo session and 8 subjects during active session completed this task. One baseline placebo immediate/delayed recall and one active delayed recall at the 1 hour time point were not performed due to administrative reasons. The subject with missing baseline data was removed from placebo analysis. A word list generator was used to create different word lists of "equal" difficulty. However, it appeared based on subject performance that some versions may have been easier than others (Table 6.3). Version 2 was replaced with version 9 part way through the study. Given the small n, it cannot be conclusively determined which versions are not equivalent.

Table 6.3. Average verbal learning test scores for each test version*

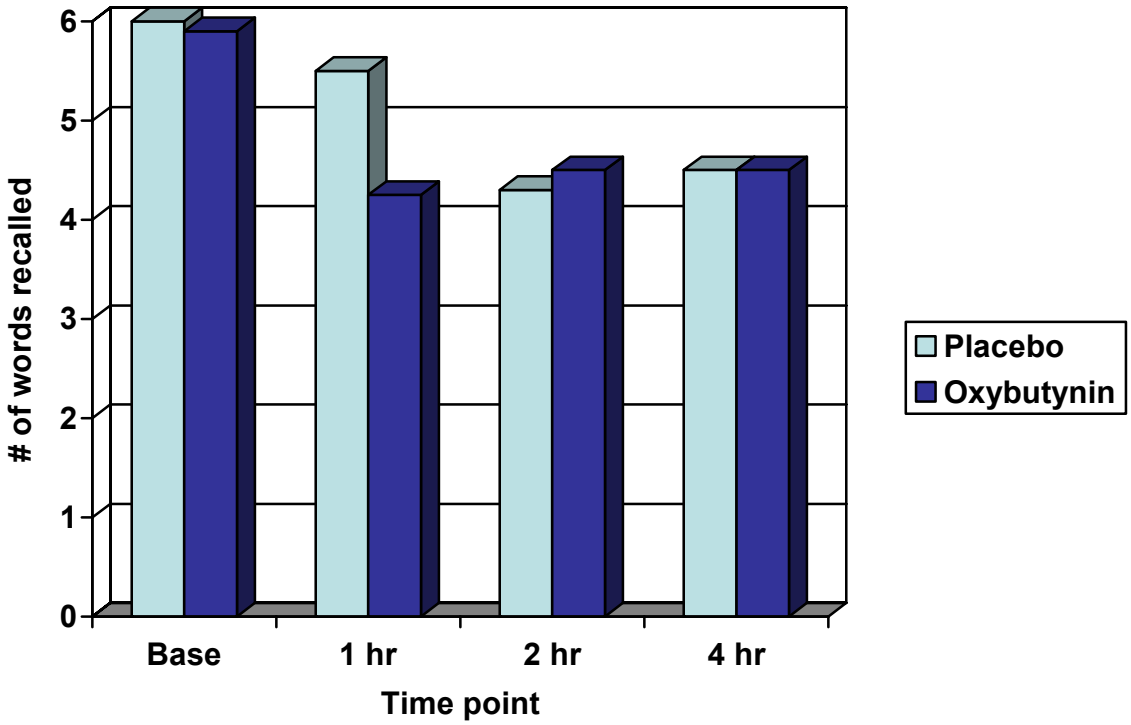
Version of VLT	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Num. of subjects	2	3	6	6	6	4	4	2	1
Average Score	4.5	3.0	6.0	5.3	4.2	5.0	6.3	5.5	8

*Only placebo values were used for the averages reported above

The average baseline immediate recall scores for session 1 and session 2 were 5.5 and 6.8 words, respectively, indicating that some learning may have occurred across study sessions (e.g., including only the individuals who participated in both baseline assessments). Average baseline immediate recall scores were comparable between placebo and active study medication sessions (Figure 6.8). A decrease in immediate recall scores was seen at the 1 hour time point following oxybutynin and at the 2 and 4 hour time points for both oxybutynin and placebo (Figure 6.8).

Time was a significant variable (P=0.041) using a two-way repeated measures ANOVA. However, individual comparisons from baseline were not significant in post hoc analyses.

Figure 6.8. Immediate verbal learning scores following placebo and oxybutynin administration

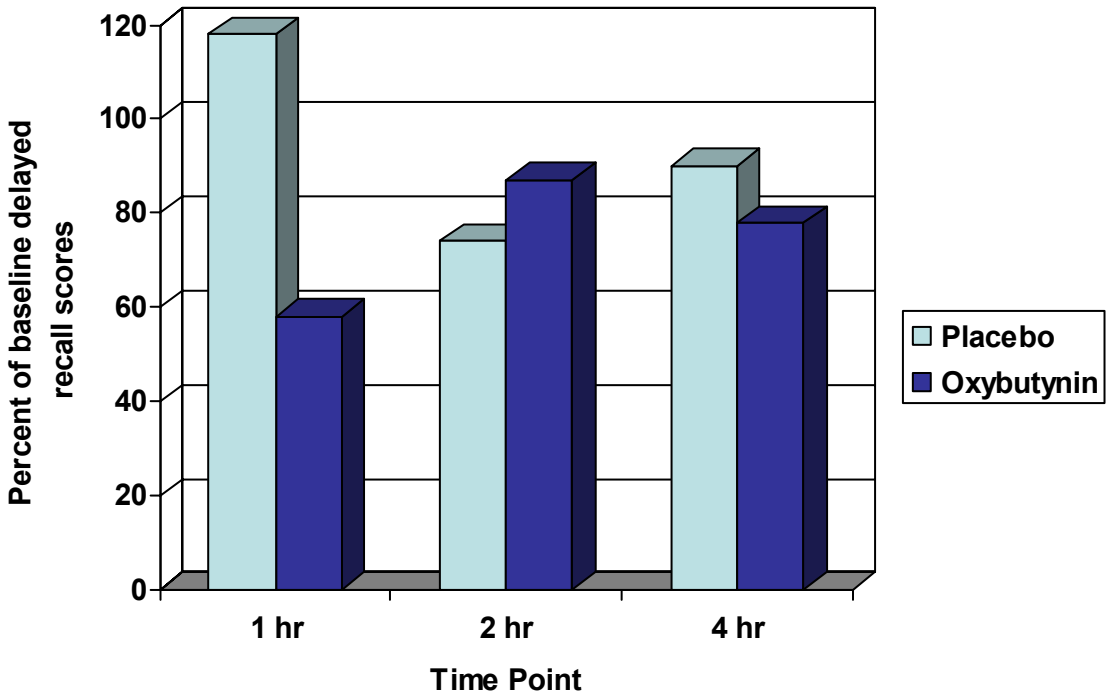


Parallel lists of fifteen words each were administered for assessment of immediate and delayed verbal learning at baseline and approximately 1, 2 and 4 hours following study medication administration. For immediate recall, subjects were asked to recall as many words as possible directly after the words were read a single time. Time was found to be a significant variable (P=0.041) using a two-way repeated measures ANOVA. However, individual comparisons from baseline were not significant in post hoc analyses.

Delayed verbal learning scores were analyzed as the percentage of immediate verbal scores. The average baseline delayed scores for session 1 and session 2 were 48 and 68.5%, respectively, indicating that some learning may have occurred across study sessions (e.g., including only the

individuals who participated in both baseline assessments). Delayed scores at baseline of the active arm were higher than the placebo arm (i.e., 50 and 69%, respectively). Following oxybutynin administration, there was a decrease in delayed recall across all 3 time points (Figure 6.9). Following placebo administration, an increase in scores was seen at 1 hour post placebo and a subsequent decrease at the 2 hour time point. These differences did not reach statistical significance using a two-way repeated measures ANOVA.

Figure 6.9. Delayed verbal learning scores following placebo and oxybutynin administration

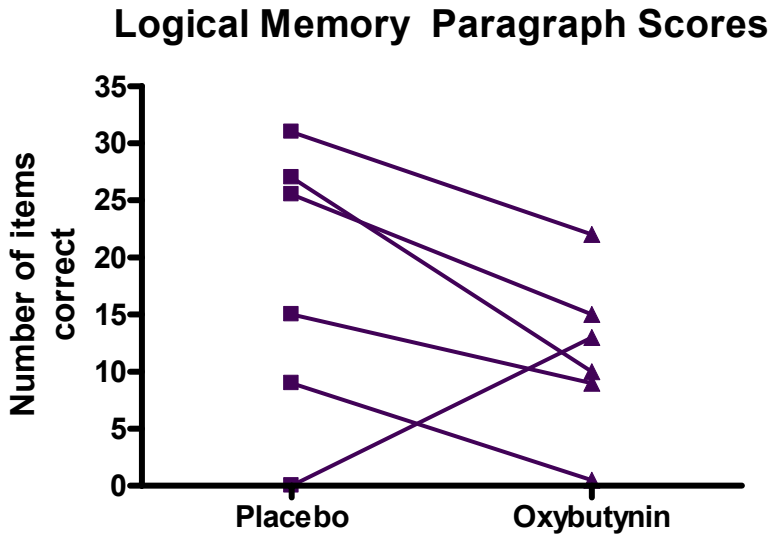


Parallel lists of fifteen words each were administered for assessment of immediate and delayed verbal learning at baseline and approximately 1, 2 and 4 hours following study medication administration. For delayed recall, subjects were asked to recall as many words as possible thirty minutes after the words were read a single time. No statistical differences were seen using a two-way repeated ANOVA.

6.5.2 Logical Memory

Parallel versions of the logical memory paragraph were administered at one time point (i.e., approximately 90 minutes post study medication administration) during each study session. Subjects were called approximately 24 hours following test administration at subjects' convenience (average = 25.2 and 25.5 hours following placebo and oxybutynin, respectively; range = 22 to 29 hours). Seven subjects completed this assessment at both study sessions. One subject was dropped from the analysis because she repeated the story outside of the study session prior to the 24 hour recall. Overall, the remaining six subjects had noticeably lower scores with oxybutynin administration (i.e., an average of 17.9 versus 11.6), with 5 out of 6 subjects demonstrating an impairment in performance (Figure 6.10). However, a paired t-test did not reveal a statistical significance. Interestingly, the subject who scored 0 during the placebo arm had complained of moderate anxiety associated with the blood draws. Subsequently, blood draws were not performed for her during session 2 (active).

Figure 6.10.



Parallel stories were read approximately ninety minutes following study medication administration. Subjects were called within 22 to 29 hours and asked to recite as much of each paragraph and in as great of detail that they could recall. The lines above connect individual subjects across sessions. On the left side of the graph are the exact scores that each subject received following placebo administration (maximum possible items correct was 50). On the right side are the scores that the same subjects received following oxybutynin administration.

6.5.3 Digit Symbol Substitution Test

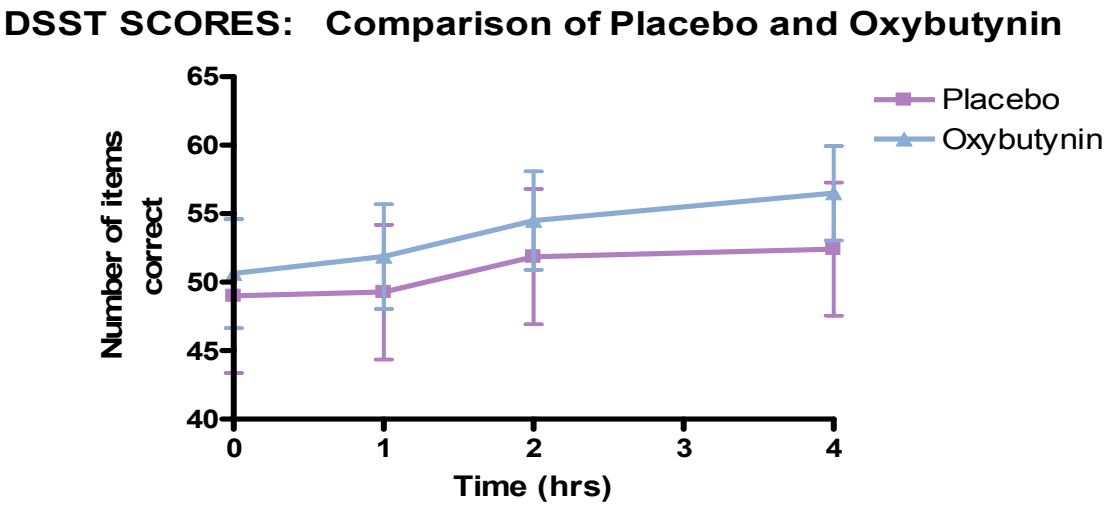
The same version of the DSST was administered at 4 time points during each study session (i.e., at baseline and approximately 1, 2 and 4 hours post study medication administration). A total of 7 subjects during placebo session and 8 subjects during active session completed this task. One placebo baseline was not performed due to administrative reasons. This subject was removed from placebo analysis. The average (SD; range) baseline scores for session 1 and session 2 were 48.3 (12.1; 30 to 65) and 53.2 (14.0; 35 to 68), respectively, indicating that learning had occurred across study sessions (e.g., including only the individuals who participated in both baseline

assessments). In fact, 5 out of the 6 individuals who had completed both baseline assessments, showed an improvement at the second study session.

DSST scores continued to show practice effects across each trial for both study sessions (Figures 6.11 and 6.12). Because of this, we estimated practice effects following both placebo and oxybutynin administration to investigate differences between medications. Raw DSST scores were used. Various linear and sigmoidal models were assessed to determine practice effects using the software NONMEM®. A linear model with interindividual variation on the constant [e.g., F = constant + m*(number of times completing DSST)] and additive error (Equation 6.2) provided the best fit for both placebo and active data (Figures 6.13 and 6.14). The model fit did not improve when interindividual variation was placed on the practice effects slope (i.e., m).

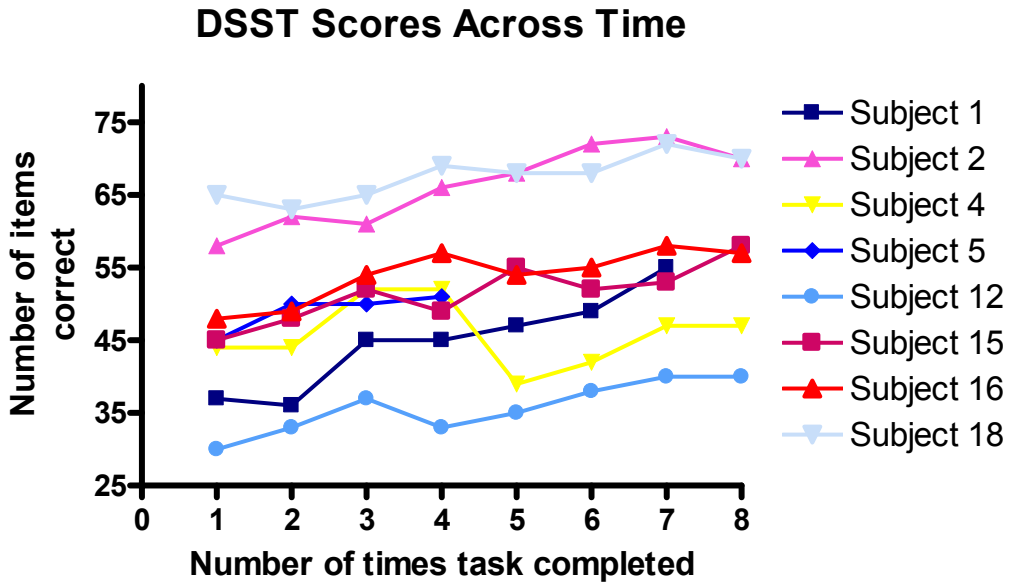
The estimated slope of improvement was similar between occasions, with placebo being 1.89 and active 2.0. Thus, the average increase in score for each repeat of test taking regardless of study medication was approximately 2 items. However, three out of eight subjects showed a decrease (n=2) or no change (n=1) in DSST scores approximately 1 hour following oxybutynin administration, whereas all subjects in the placebo arm showed improvement at this time point. Moreover, the plots of DSST scores across time appeared to be more convex following oxybutynin administration as compared to placebo (Figure 6.15).

Figure 6.11.



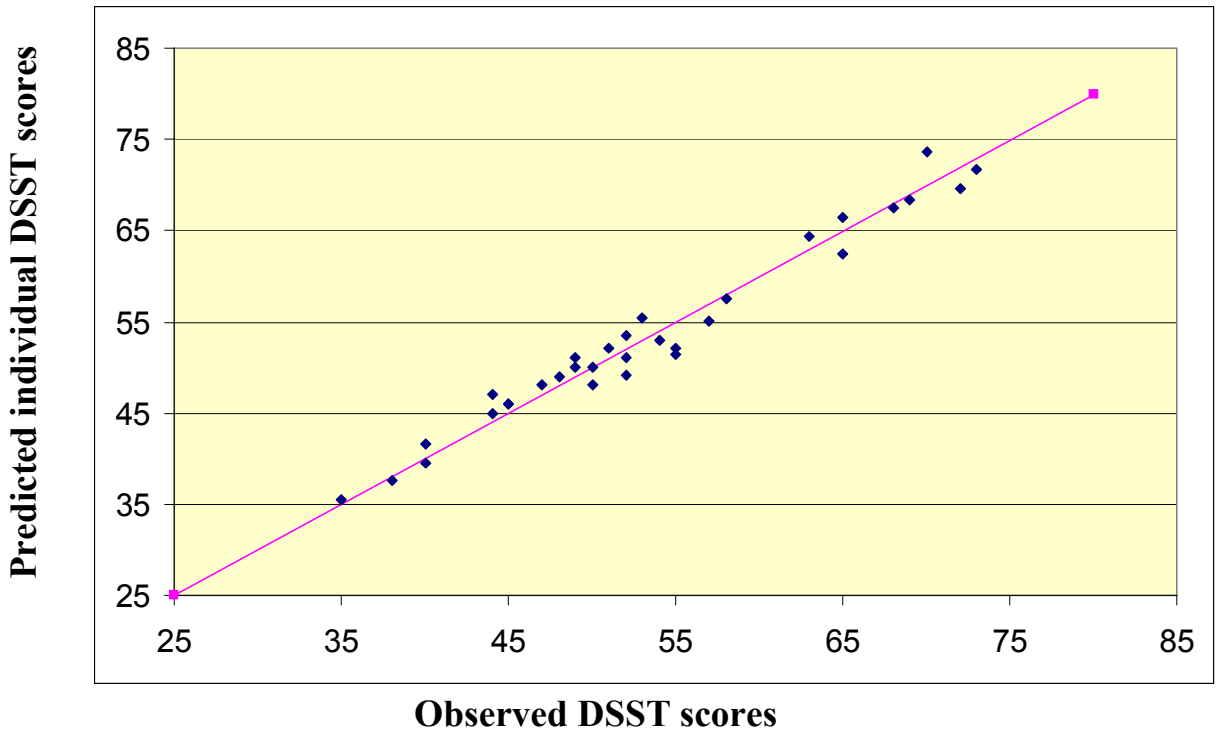
The lines above connect the average scores at each time point across each medication session. The error bars represent standard deviation.

Figure 6.12.



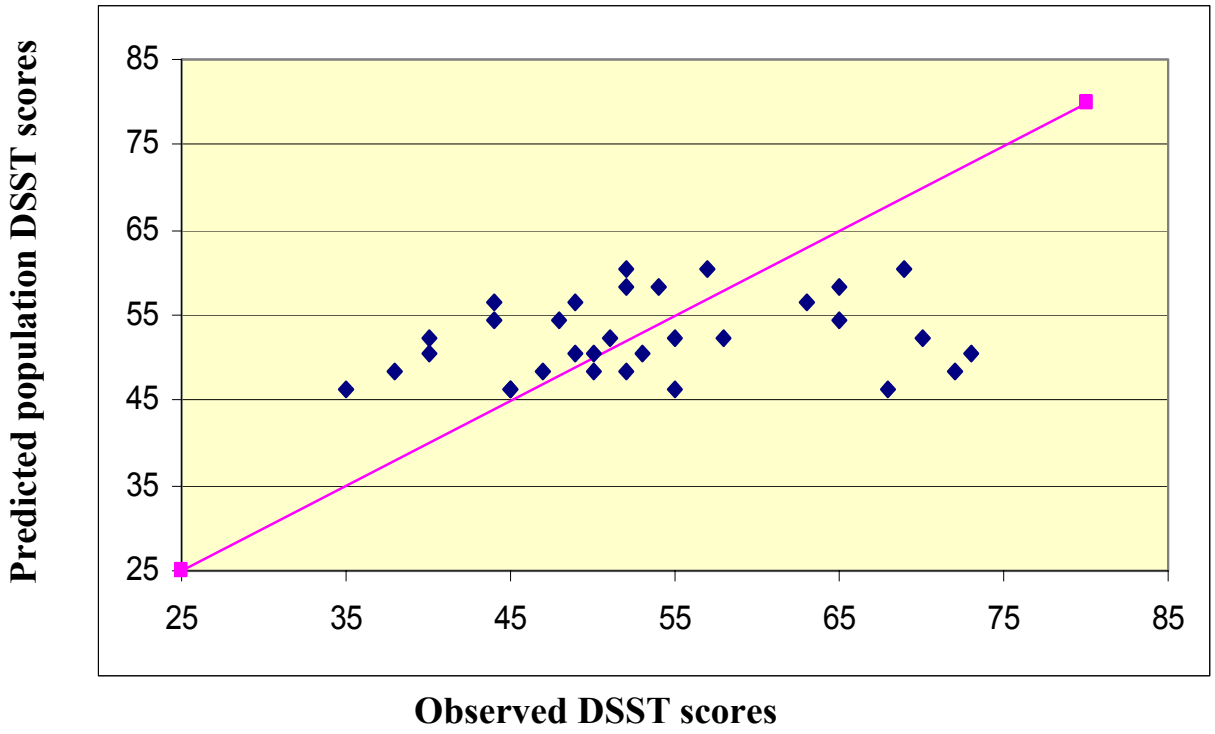
The lines above connect individuals' scores across each time the DSST was administered for both study sessions 1 and 2 (e.g., for some individuals tasks 1 thru 4 were completed during the placebo session, for others during the active session).

Figure 6.13. Observed versus individual predicted DSST scores



The predicted individual DSST scores were determined using the best fit model discussed above [F = constant + m*(number of times completing DSST)]. The pink line is a unit line.

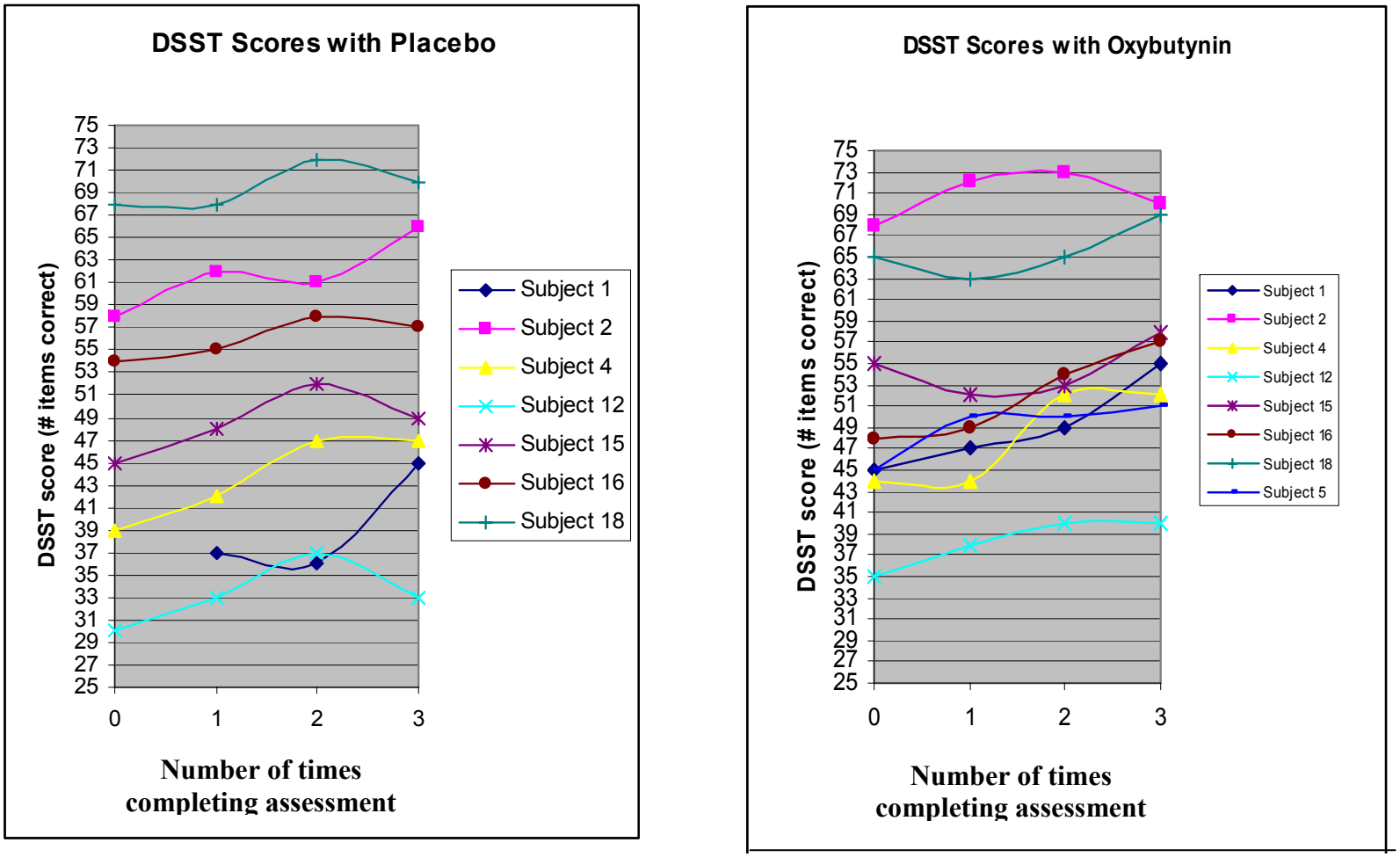
Figure 6.14. Observed versus population predicted DSST scores



The predicted population DSST scores were determined using the best fit model discussed above [F = constant + m*(number of times completing DSST)]. The pink line is a unit line.

The population mean (interindividual variability) for the constant [e.g., F = constant + m*(number of times completing DSST)] was 46.2 (25%) and 49.5 (19%), for the placebo and active groups, respectively. The constant population mean was slightly higher for the oxybutynin session, as compared to the placebo session. One possibility for this is that there may have been a difference in learning across study sessions depending on which study medication was administered on study session 1. For instance, when subjects received placebo on study session 1 (n=4), the average improvement in baseline scores from session 1 (placebo) to session 2 (active) was 8.3 items. The subjects who received oxybutynin on study session 1 (n=3) only demonstrated an average improvement of 1.3 items at baseline study session 2 when placebo was received.

Figure 6.15. Individual DSST scores across time following placebo and oxybutynin.

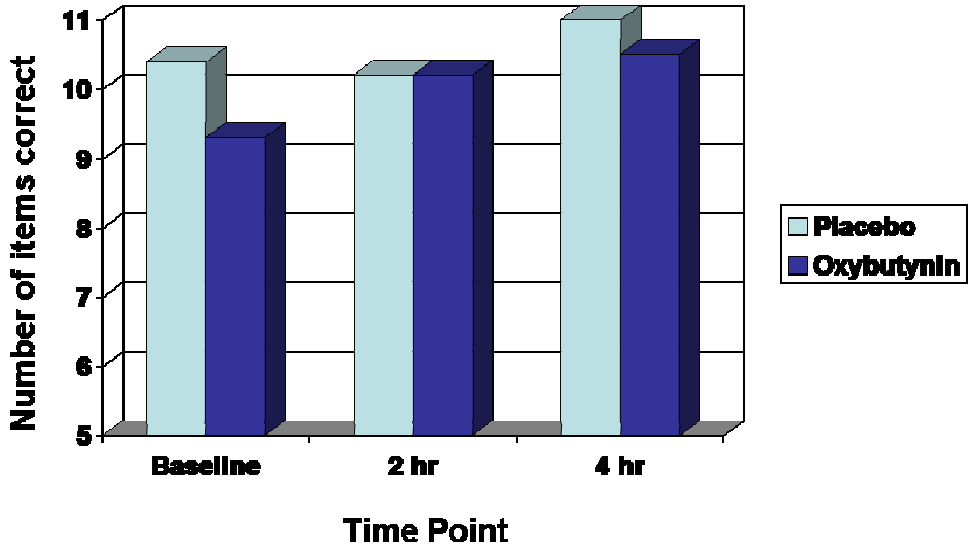


The same version of the DSST was completed four times during each study session (e.g., at baseline and approximately 1, 2, and 4 hours following study medication administration). Zero on the X-axis indicates DSST scores at baseline. Subject 1 did not complete the DSST at baseline during the placebo session. Subject 5 only participated in one study session (active) and therefore is not represented in the placebo figure.

6.5.4 Letter-Number Sequencing Test

Parallel versions of this task were administered at 3 time points during each study session (i.e., at baseline and approximately 2 and 4 hours post study medication administration) in an approximate latin square design. A total of 7 subjects during placebo session and 8 subjects during active session completed this task. One placebo baseline was not performed due to administrative reasons. This subject was removed from placebo analysis. Approximately half way through the study, the letters C, D, L, and S were removed from all versions of this task due to subjects consistently recalling back a similar sounding letter. Two subjects were dropped from analysis since it was apparent that hard of hearing (e.g., difficulty distinguishing between letters) interfered with their performance. This left 5 subjects during the placebo session and 6 subjects during the active session for analysis. The average (SD; range) baseline scores for session 1 and session 2 were 9.6 (1.5; 7 to 11) and 10.2 (2.3; 7 to 13), respectively, indicating that some learning may have occurred across study sessions (e.g., including only the individuals who participated in both baseline assessments). No apparent difference was seen between the two arms (Figure 6.16).

Figure 6.16. Letter-Number Sequencing scores



6.5.5 N-back task

Both the 1N-back and the 2N-back were administered at baseline and approximately 90 minutes post study medication for each study session. A total of 7 subjects during placebo session and 8 subjects during active session completed this task. One placebo baseline was not performed due to administrative reasons. This subject was dropped from the placebo arm for this task. This same subject was dropped from the active arm as well since she failed did properly follow directions during the baseline assessment. Thus, there were 6 subjects, placebo and 7 subjects, active available for analyses. Scores are reported as fraction correct.

The average (SD; range) baseline 1N-back scores for session 1 and session 2 were 97 (3.7; 91 to 100) and 93 (6.4; 83 to 100) percent, respectively (e.g., including only the individuals who participated in both baseline assessments). The average (SD; range) baseline scores for the placebo and active arms were 95 (6.7; 83 to 100) and 96 (4.1; 90 to 100) percent, respectively. A

decrease in scores was evident at the 90 minute time point for both cohorts. However, the active arm had approximately two-fold greater decrease in scores than the placebo arm (e.g., 16 versus 7 percent, respectively). These differences did not reach statistical significance using a two-way repeated measures ANOVA.

The average (SD; range) baseline 2N-back scores for session 1 and session 2 were 45 (23.6; 17 to 82) and 55 (21.3; 30 to 91) percent, respectively indicating that some learning may have occurred across sessions (e.g., including only the individuals who participated in both baseline assessments). The average (SD; range) baseline scores for the placebo and active arms were 45 (28.1; 17 to 91) and 54 (13.9; 41 to 82) percent, respectively (Figure 6.17). A 5 percent decrease in scores was seen following oxybutynin administration, whereas the placebo arm showed an improvement of 11 percent at this time point. These differences did not reach statistical significance using a two-way repeated measures ANOVA.

Figure 6.17. 2N-Back scores for placebo and oxybutynin sessions

Figure 6.17A

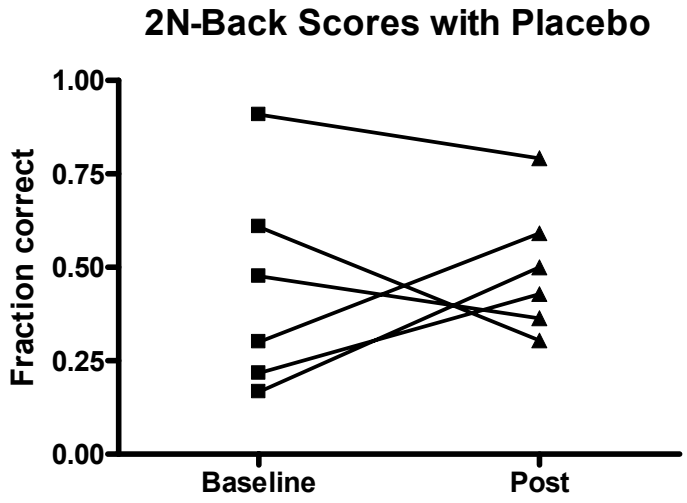
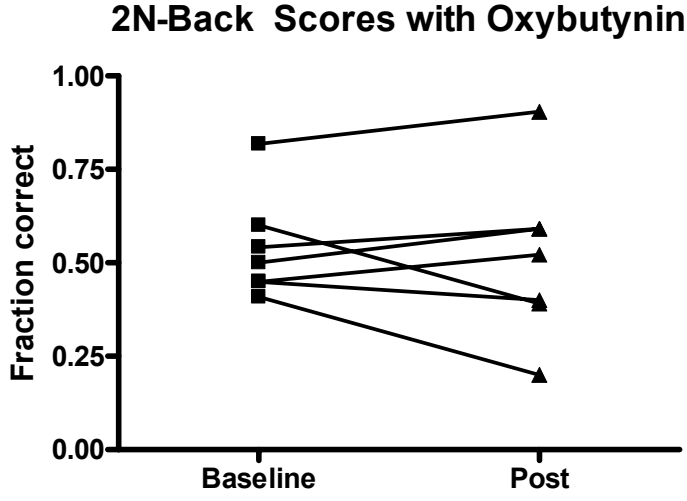


Figure 6.17B



Parallel versions of the 2N-back task were administered at baseline and approximately ninety minutes following study medication administration. On the left side of each graph are the fraction correct scores for each subject at baseline. On the right side of each graph are the scores that the same subjects received approximately ninety minutes following study medication (i.e., lines connecting individual subjects).

6.5.6 Discussion

Some impairment was evident for verbal learning, attention/motor speed (e.g., DSST), long-term memory (e.g., paragraph recall), and working memory (e.g., N-back tasks) when tasks were administered approximately 1 to 2 hours following oral administration of oxybutynin, 5 mg. In contrast, there were no apparent deficits with an additional working memory assessment, the letter-number task.

A decrease in long-term memory (paragraph recall) was evident following oxybutynin administration. In addition, although individuals improved overall with each subsequent DSST administration on the day of oxybutynin, it did not appear that subjects maintained these practice effects inter-occasion (e.g., separated by seven or more days). Very few investigators have previously examined the effects of anticholinergic medication on long-term memory.[217] The majority of reports on anticholinergic-induced impairment have investigated episodic memory using a delayed recall of 20-45 minutes. This memory is classified as short-term memory by neurobiologists based on neuromolecular mechanisms.[218-220] The findings from this current study highlight the importance of including long-term measures when assessing the cognitive effects of medications in older adults.

On average, subjects had an immediate recall decline of approximately 30% following oxybutynin administration at all subsequent time points. This is in agreement with results reported by Katz and colleagues.[63] However, a noticeable decline in verbal learning test scores was also seen following placebo administration at the 2 and 4 hour time points. Multiple factors could be contributing to this decline seen with placebo including 1) the lack of "parallelness"

among test versions, 2) tiredness occurring as the day progresses, and 3) interference from previous word lists. Each study session was approximately 8 hours long with an intensive schedule (e.g., ocular and cognitive measurements and blood draws) the first 5 to 6 hours of the day. The majority of subjects reported often taking naps or inadvertently falling asleep during the day while at home. Moreover, many indicated increased tiredness as the study session progressed. Tiredness or the natural circadian rhythm of "alertness" could have influenced testing performance. Another strong possibility is interference from previous word lists. This phenomenon, although not well studied, has been reported with administration of word lists with only brief testing intervals.[221] Many subjects spontaneously complained of difficulty recalling words from the current word list due to interference from previous (including even screening or words from past study session) word lists.

Oxybutynin appeared to cause a small impairment in one working memory task (N-back), but not another (Letter-Number). There are multiple possible explanations for this inconsistency. In certain aspects, the N-back tasks may be more difficult. For instance, subjects must be able to control the interference from previous letters when deciding whether a stimulus was presented either one back or two back. In addition, the examinee must be able to sustain attention without breaking for 150 seconds. Some subjects from this study reported that their mind began to "wander" the last 60 seconds of the N-back tasks. Another possibility is that the Letter-Number task may have been performed too late following oxybutynin administration in order to capture central anticholinergic effects (e.g., the Letter-Number task was performed approximately one hour following the N-back tasks).

There was an obvious discrepancy between some subjects' cognitive test scores and subjective and objective noting of cognitive impairment and confusion. Some subjects showed little or no decline on cognitive measurements despite 1) subjective reports of feeling "fuzzy-headed" and/or feeling mild confusion, 2) inability to recall study procedures (e.g., forgot how to perform a task despite performing correctly multiple times previously), 3) objective noting of decreased ability to follow directions (e.g., directions for a task that had been performed several times, now needed multiple reviews in order for subject to correctly understand and follow the directions); and 4) objective noting of slowing of cognitive processing. Two subjects had to remain one or more hours following completion of study procedures in order for them to return at or near baseline functioning. Despite these subjective and objective findings, some subjects demonstrated minimal cognitive effects on the measures employed. One possibility for this is that subjects are able to maintain motivation and attention for short testing periods. Perhaps increasing assessment time for specific tasks such as the DSST or the N-back task may improve the ability to detect impairment. In addition, the cognitive tasks utilized may not have been the most optimal. It appeared that subjects had the greatest impairment for long-term memory and in difficulty following/learning directions. Future work should include assessments which can better capture these impairments. For instance, using a lengthy driving simulation task would incorporate multiple domains known to be impaired (e.g., attention, processing speed, motor control, following directions) and has real-life relevance.

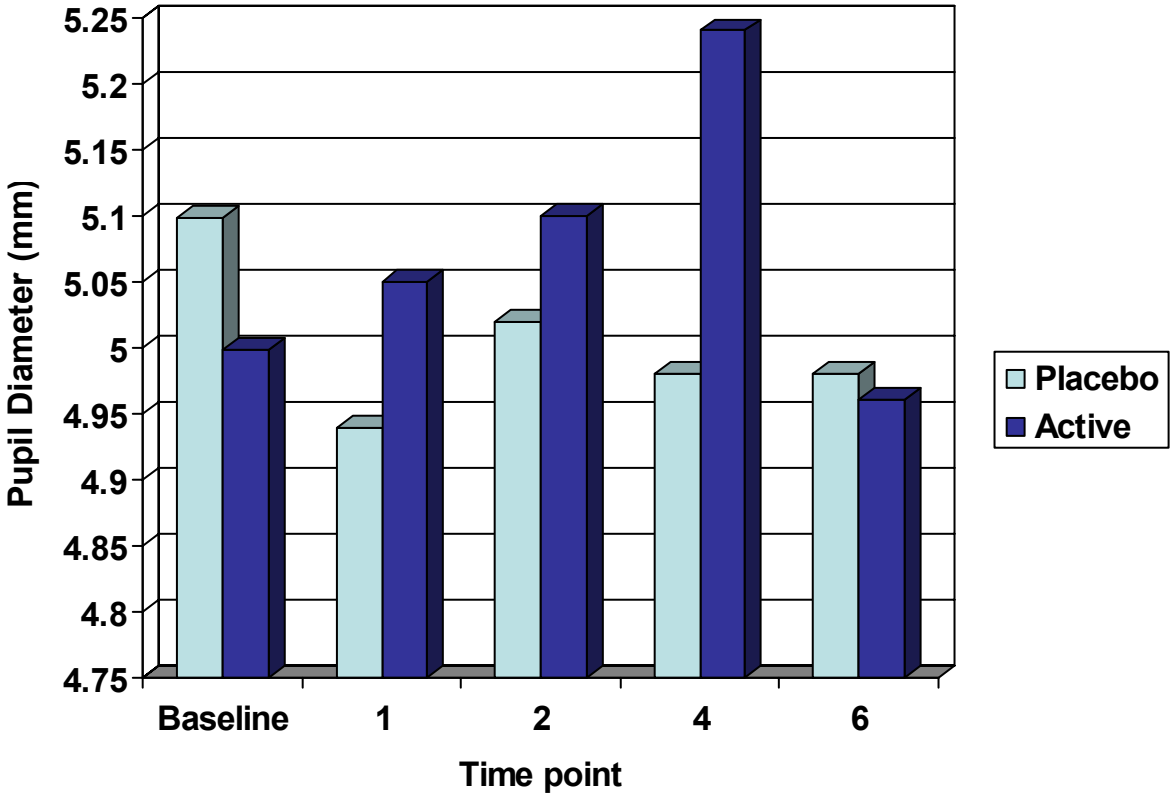
6.6 PUPIL DIAMETER

Pupil diameter measurements using the Neuroptics® handheld device was attempted at least thrice at 8 time points for each study session (i.e., at baseline and approximately 0.33, 0.66, 1, 1.5, 2, 4, and 6 hours post study medication administration). A total of 7 subjects during placebo session and 8 subjects during active session completed this task. Subject 16 had pupil diameter measured for both the right and left eyes. Only data from the left eye for this subject was used for analysis as the right eye was approximately 1 mm smaller and had a diminished response to light. The right eye was used for all other subjects. No pupil diameter values were missing from the placebo arm. Two subjects had 4 missing data values (e.g., 1 time point due to lack of time and for subject 16, the left eye was only measured during select time points) from the active arm. The average (SD; range) baseline pupil diameters for session 1 and session 2 were 5.1 (0.80; 3.7 to 6.1) and 4.9 (0.81; 4.0 to 6.3) mm, respectively (e.g., including only the individuals who participated in both baseline assessments). One possible explanation for this small difference in baseline values is that some subjects were more apprehensive for their first study session and therefore had greater central activation and a subsequent increase in pupil diameter. All subjects displayed what appeared to be sinusoidal wave patterns or linear quadratic trends during the placebo session. This pattern dampened in most subjects following oxybutynin administration.

Figure 6.18 depicts average pupil diameter across time following placebo or oxybutynin administration. The average (SD; range) baseline pupil diameters for placebo and active arms are 5.1 (0.77; 4 to 6.1) and 5.0 (0.79; 3.7 to 6.3) mm, respectively. The average peak increase following study medication was 0.13 (0 to 0.5) and 0.35 (0 to 0.6) for the placebo and active

groups, respectively (p=0.036). Four out of the 7 subjects showed a decrease in pupil diameter across time following placebo, 2 subjects showed a small increase in diameter at 1 or more time points, and 1 subject had a 0.5 mm increase at a single time point. Only 1 out of the 8 subjects following oxybutynin administration did not demonstrate an increase in pupil diameter. The average increase in pupil diameter peaked at time point 4, when R-oxybutynin and R-desethyloxybutynin levels were decreasing (Figure 6.16). Moreover, of the 7 subjects demonstrating an increase in diameter, all but 1 had a larger pupil at time point 6 as compared to baseline, despite low or undetectable levels of oxybutynin and its active metabolite.

Figure 6.18. Average pupil diameter following placebo or oxybutynin administration



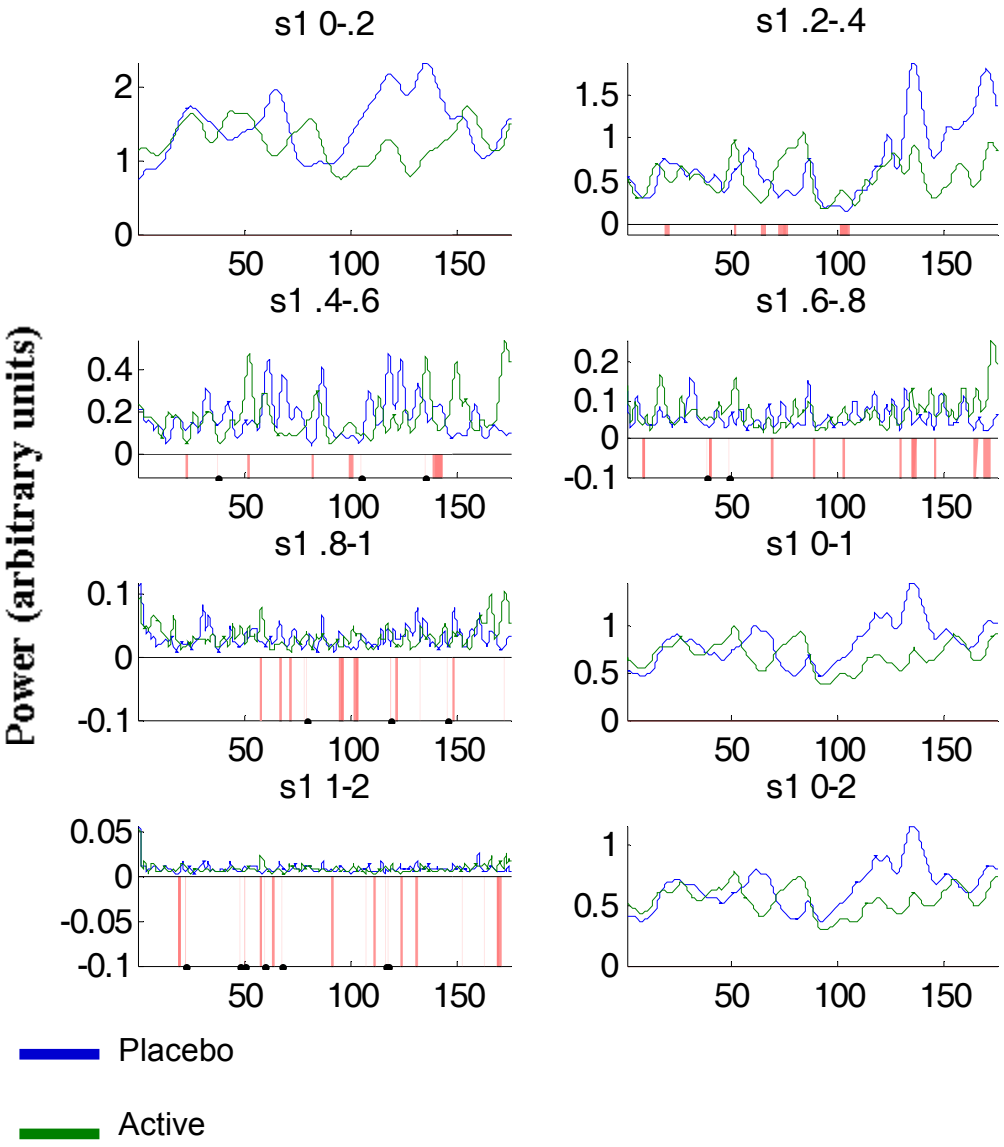
6.7 OSCILLATIONS

Pupillary motility was measured for 3 minutes using the ISCAN pupillometer at four time points during each study session (i.e., at baseline and approximately 1, 2, and 4 hours post study medication administration). Data was available at all 4 time points from 7 subjects during the placebo session and 8 subjects during the active session. Figure 6.19 presents the average power of oscillations (e.g., for the 7 subjects participating in both study sessions) across time for frequencies of less than 2.0 Hz (Hz-cycles per second).

The average power for the first sixty seconds decreased following oxybutynin administration for all frequency groups, with the exception of 1.0 to 2.0 Hz (Figures 6.19 and 6.20). However, power in the 1.0 to 2.0 Hz frequency range increased across time for the placebo arm, yet remained steady following oxybutynin administration. Statistically significant differences between placebo and oxybutynin were evident within one hour following study medication administration. Specifically, changes were prominent for the 0.81 to 1.0, 0.0 to 1.0, and 0.0 to 2.0 Hz approximately one hour following oxybutynin administration. The remaining frequency categories (i.e., with the exception of 0.61 to 0.80) showed obvious differences at the 2 hour time point. Moreover, oscillatory changes were still seen at the four hour time point, despite decreasing serum oxybutynin and desethyloxybutynin concentrations.

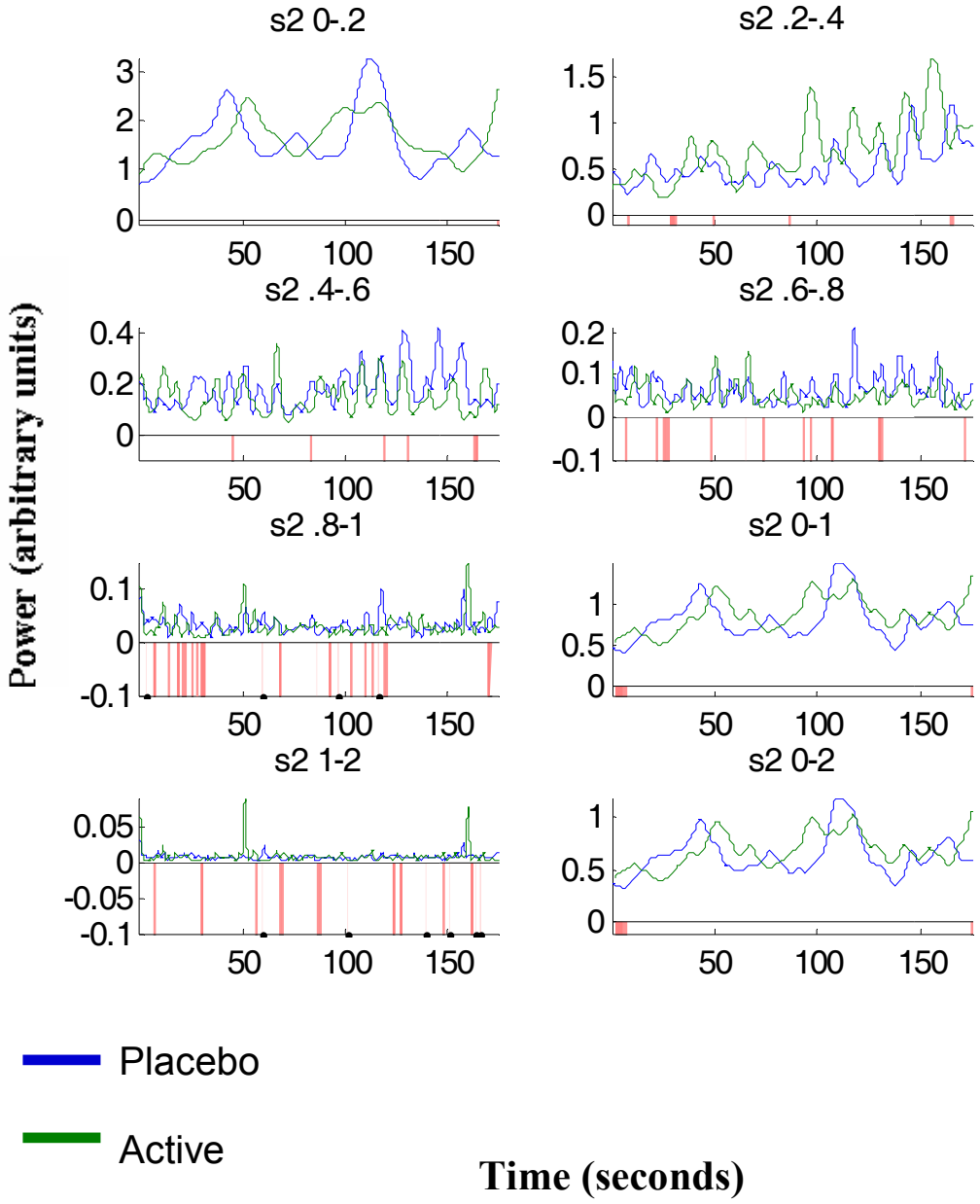
Figure 6.19. Pupillary oscillations at low level frequencies

Figure 6.19a. Pupillary oscillations at low level frequencies: Baseline



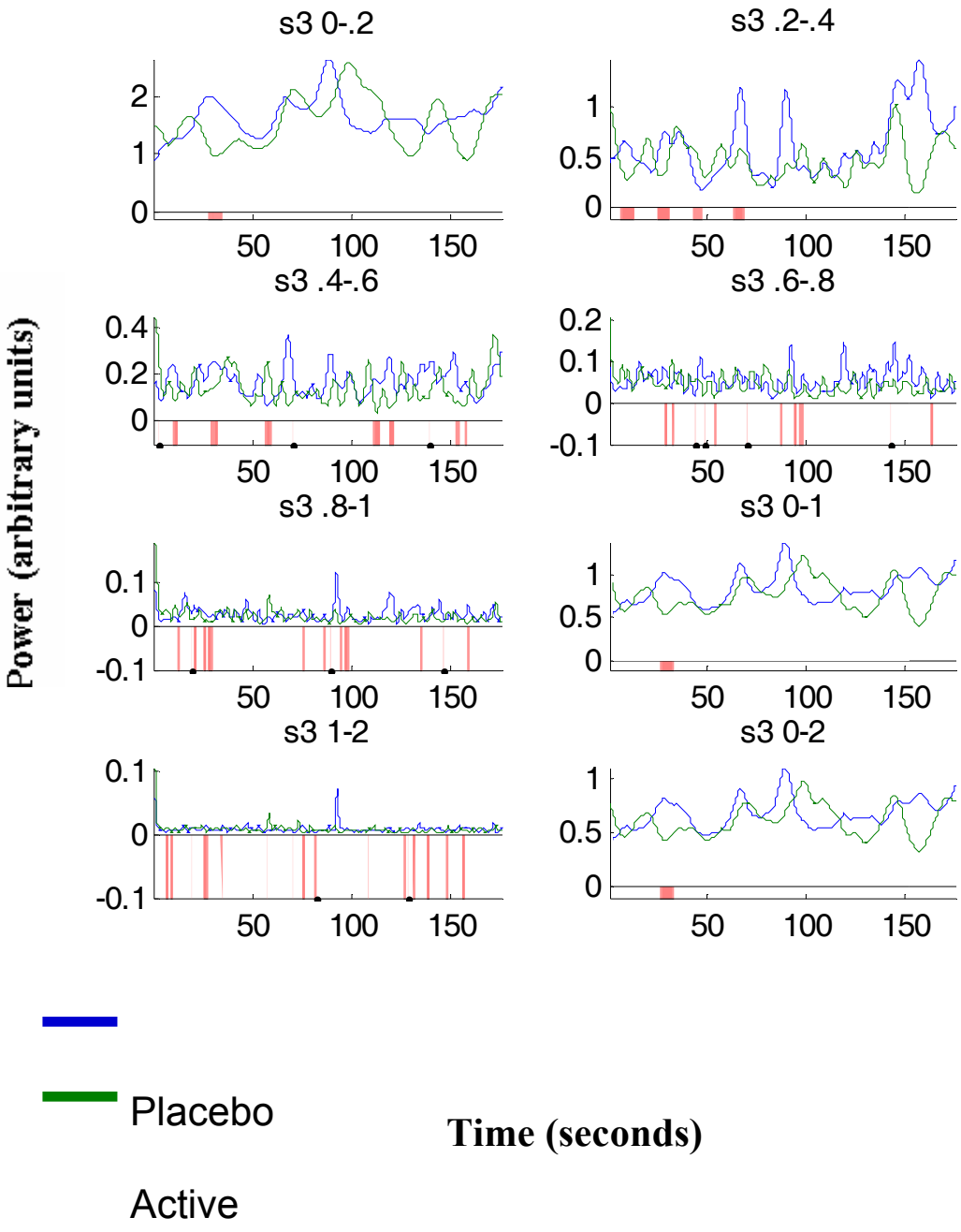
Pupil motility was measured for three minutes at each time point. The data presented here are averages of the subjects who participated in both study sessions. Only oscillatory amplitudes at lower frequencies of less than 2.0 Hz were examined (Hz-cycles per second). Oscillatory frequencies were divided into eight categories and are represented above. The dark pink bands reflect areas of significance lasting longer than one second ($p<0.5$);[222] the light pink bands reflect areas with a p values of <0.10. The higher the power, the greater the oscillations and/or amplitude changes. Note, power at different frequencies cannot be directly compared.

Figure 6.19b. Pupillary oscillations at low level frequencies: 1 hour



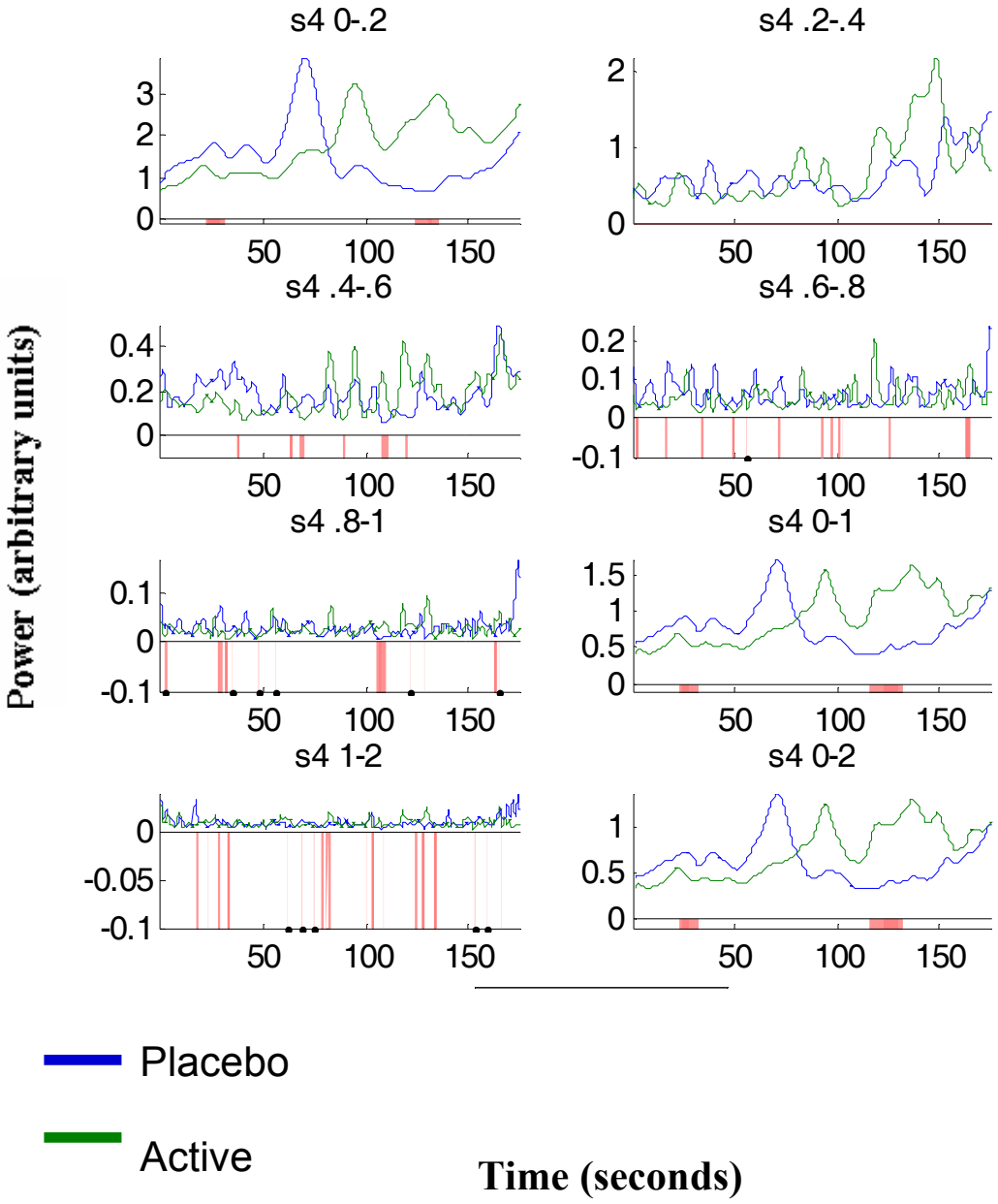
Pupil motility was measured for three minutes at each time point. The data presented here are averages of the subjects who participated in both study sessions. Only oscillatory amplitudes at lower frequencies of less than 2.0 Hz were examined (Hz-cycles per second). Oscillatory frequencies were divided into eight categories and are represented above. The dark pink bands reflect areas of significance lasting longer than one second ($p<0.5$);[222] the light pink bands reflect areas with a p values of <0.10. The higher the power, the greater the oscillations and/or amplitude changes. Note, power at different frequencies cannot be directly compared.

Figure 6.19c. Pupillary oscillations at low level frequencies: 2 hour



Pupil motility was measured for three minutes at each time point. The data presented here are averages of the subjects who participated in both study sessions. Only oscillatory amplitudes at lower frequencies of less than 2.0 Hz were examined (Hz-cycles per second). Oscillatory frequencies were divided into eight categories and are represented above. The dark pink bands reflect areas of significance lasting longer than one second ($p<0.5$);[222] the light pink bands reflect areas with a p values of <0.10. The higher the power, the greater the oscillations and/or amplitude changes. Note, power at different frequencies cannot be directly compared.

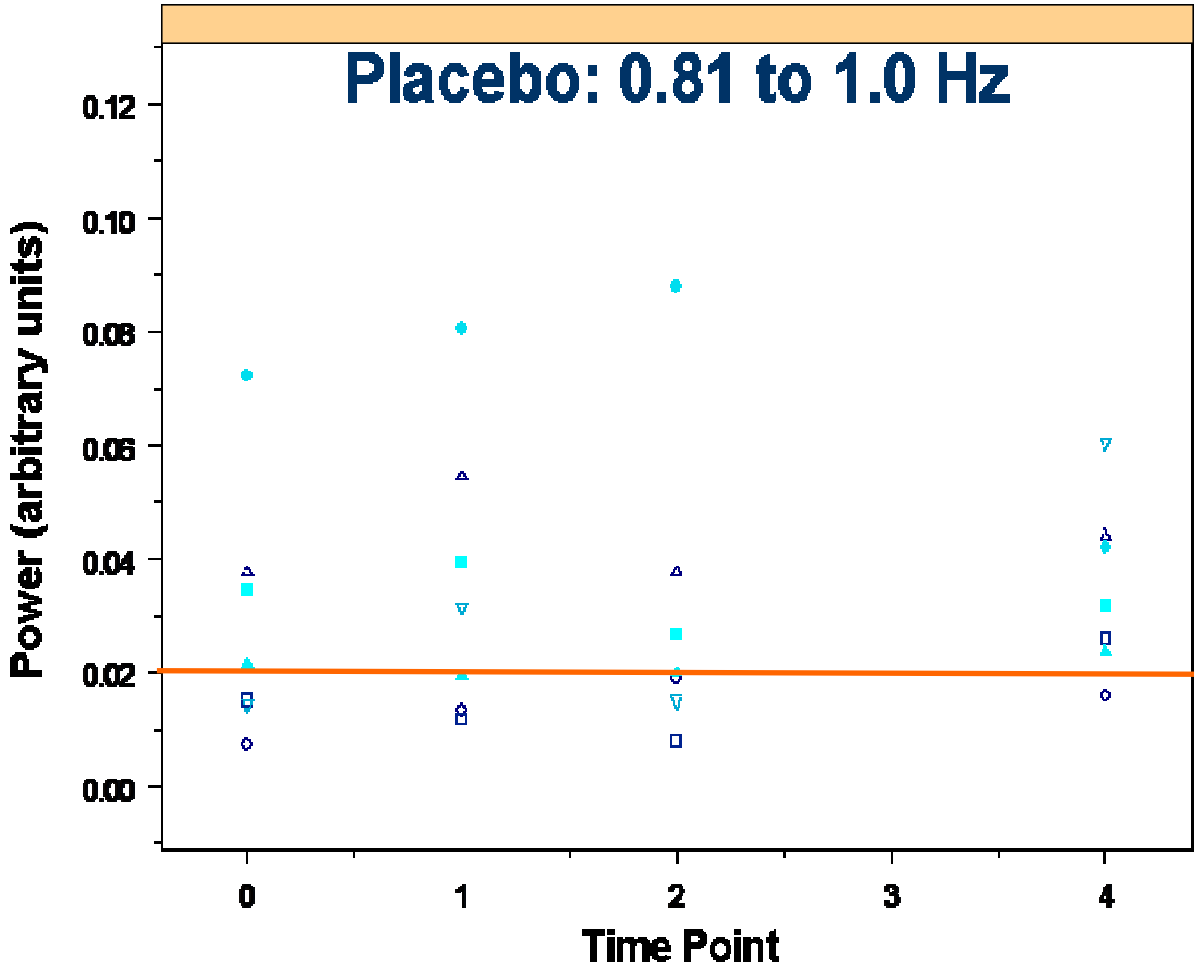
Figure 6.19d. Pupillary oscillations at low level frequencies: 2 hour



Pupil motility was measured for three minutes at each time point. The data presented here are averages of the subjects who participated in both study sessions. Only oscillatory amplitudes at lower frequencies of less than 2.0 Hz were examined (Hz-cycles per second). Oscillatory frequencies were divided into eight categories and are represented above. The dark pink bands reflect areas of significance lasting longer than one second ($p<0.5$);[222] the light pink bands reflect areas with a p values of <0.10. The higher the power, the greater the oscillations and/or amplitude changes. Note, power at different frequencies cannot be directly compared.

Figure 6.20. Average power at 0.81 to 1.0 Hz for the first sixty seconds for each subject

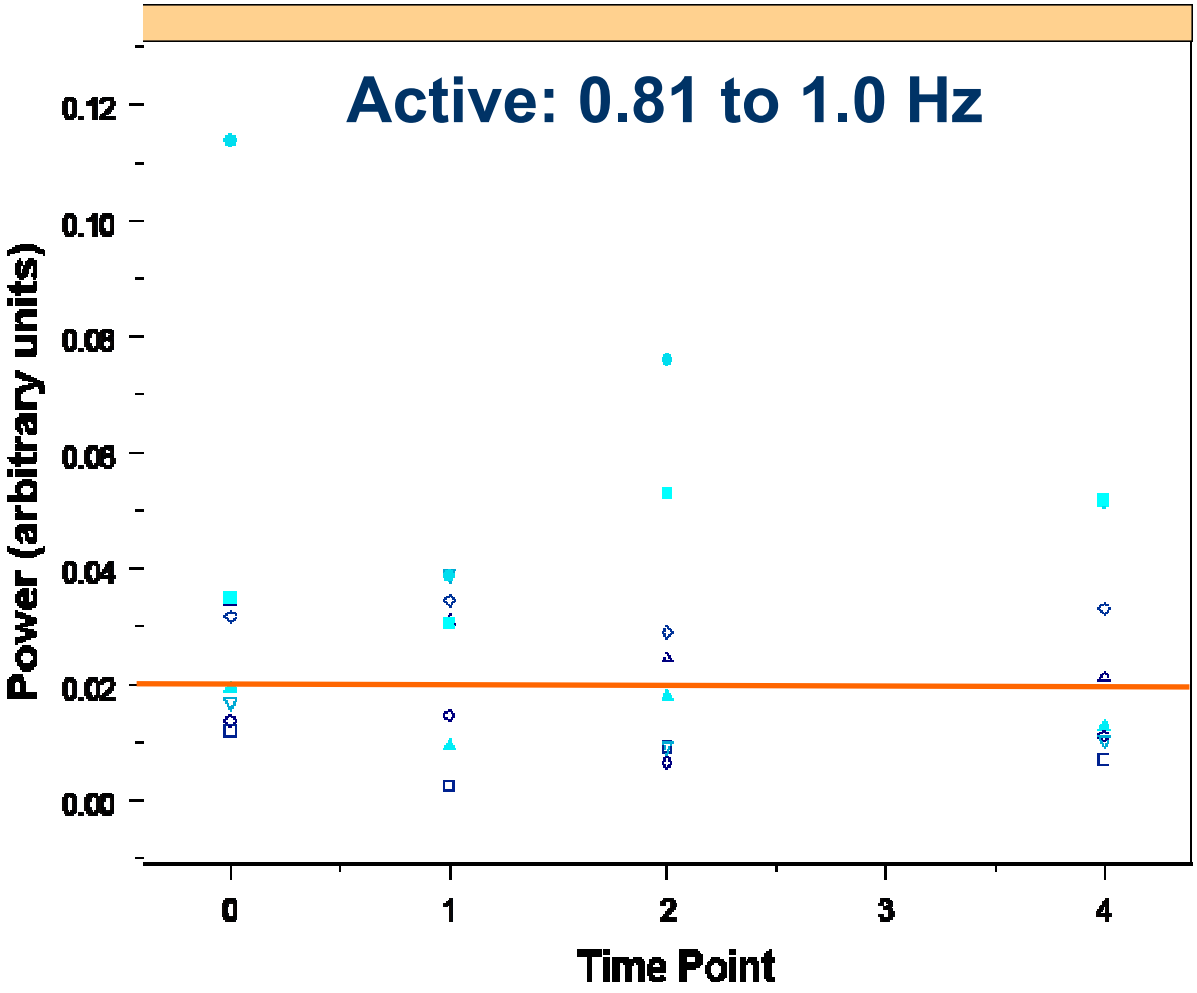
A.



Pupil motility was measured for three minutes at baseline and approximately 1, 2 and 4 hours following study medication administration. The above values represent the average power over the first sixty seconds of measurement for the frequency range 0.81 to 1.0 Hz. Each subject is represented by the same symbol across all four time points. The higher the power, the greater the oscillations and/or amplitude changes. The horizontal orange line was arbitrarily placed to demonstrate the decrease in power over time following oxybutynin, as compared to placebo. Note that by the 4 hour time point following placebo, all but one subject had an average power greater than 0.02. Conversely, five out of eight subjects had power at or below 0.02.

Figure 6.20. Average power at 0.81 to 1.0 Hz for the first sixty seconds for each subject (cont)

B.

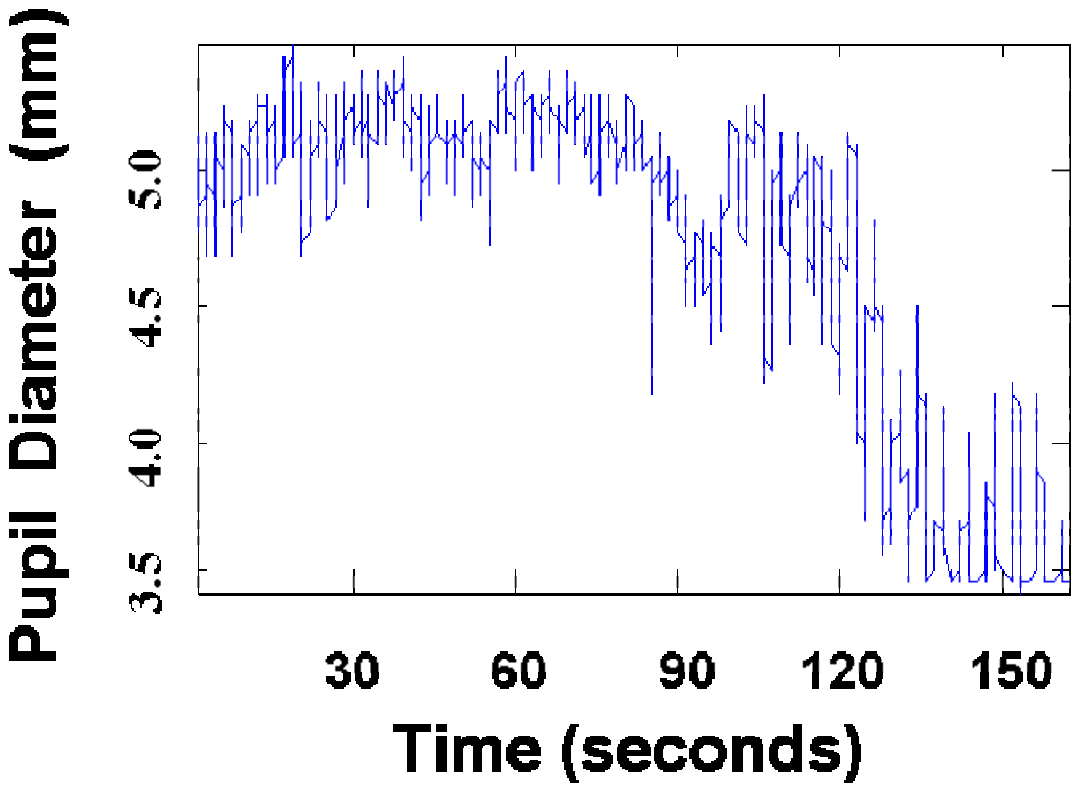


Pupil motility was measured for three minutes at baseline and approximately 1, 2 and 4 hours following study medication administration. The above values represent the average power over the first sixty seconds of measurement for the frequency range 0.81 to 1.0 Hz. Each subject is represented by the same symbol across all four time points. The higher the power, the greater the oscillations and/or amplitude changes. The horizontal orange line was arbitrarily placed to demonstrate the decrease in power over time following oxybutynin, as compared to placebo. Note that by the 4 hour time point following placebo, all but one subject had an average power greater than 0.02. Conversely, five out of eight subjects had power at or below 0.02.

In contrast to the first sixty seconds, oscillatory power following oxybutynin was greater than placebo at specific frequencies after approximately 2 minutes of pupillary measurement. This discrepancy may be due to an increase in tiredness following oxybutynin and a subsequent increase in "fatigue waves". In healthy younger adults, "fatigue waves", or large changes (0.3 to 0.5 mm and upward) in pupil size, occur after sitting quietly (typically in the dark) for approximately 7 minutes. Older adults are particularly likely to display these types of waves, often after only sitting for a minute or so, and may be independent of whether or not the subject subjectively complains of fatigue.[160]

For this study, individual plots of pupil diameter across 3 minutes frequently showed what appeared to be fatigue waves (Figure 6.21). In fact, some subjects displayed rapid, large changes in pupil diameter within the first 30 to 60 seconds of measurement. These fatigue waves increase the power of oscillations at lower frequencies (especially in the range of 0.0 to 0.2 Hz), despite a central deactivation or a decrease in an arousal actually occurring. Hence, these waves may interfere with the ability to detect differences between the oxybutynin and placebo arms. One option to limit the influence of these waves is to focus our analysis (Section 6.8) only on the first thirty to sixty seconds of measurement. In addition, higher frequency ranges (e.g., 0.81 to 1.0 Hz) may ultimately prove to be more reliable, as it is thought that the majority of fatigue waves occur below 0.2 Hz.

Figure 6.21. Pupil oscillations over time in a single subject



The above figure shows data from a single subject at a single time point following oxybutynin administration. Pupil motility was measured for three minutes. Beyond ninety seconds of continuous measurement, this subject displayed increasingly greater amplitude changes in pupil diameter.

6.7.1 Discussion

Pupil motility was measured for three minutes at baseline and approximately 1 hour, 2 hour, and 4 hours following oxybutynin (5 mg oral) and placebo administration. On average, oscillatory power was decreased across frequency ranges of less than 2.0 Hz for the first 60 seconds of measurement following oxybutynin administration. Statistically significant differences between

placebo and oxybutynin were evident within one hour following study medication administration for specific frequency ranges. Oscillatory changes were still seen at the four hour time point, despite decreasing serum oxybutynin and desethyloxybutynin concentrations.

Studying the exact relationship between individual pupillary oscillations and oxybutynin/desethyloxybutynin levels was limited by the few pupillary measurement time points and the small number of subjects investigated. In addition, the intensive nature of the study may have confounded results (e.g., by contributing to an increase in fatigue and possibly testing anxiety). Following oxybutynin, average power decreases in the 0.81 to 1.0 Hz range were evident prior to notable changes in the lower frequency categories. One possibility for this is that fatigue waves (e.g., which contribute to an increase in power and appear with frequencies of less than 0.81 Hz), were prevalent in the majority of subjects, following both study medications (although more prominent following oxybutynin), and occurred even within the first sixty seconds of pupil motility measurement. It is unclear why oscillatory changes were still prominent at the four hour time point, despite decreasing serum oxybutynin and desethyloxybutynin concentrations. One possibility for this is a discrepancy between serum and central pharmacokinetics. Future studies should utilize multiple doses of oxybutynin, limit the intensity of cognitive testing, as well as increase pupil motility sampling times in order to better understand the relationship between anticholinergic medications and pupil oscillations.

6.8 RELATIONSHIP BETWEEN OSCILLATIONS AND COGNITIVE PERFORMANCE

The relationship between centrally mediated pupillary oscillations and cognitive performance was explored using linear and nonlinear models with various statistical forms for interindividual and residual variability using NONMEM-V®. The DSST was chosen for the cognitive measure in this analysis. Although DSST scores did not show a significant change following oxybutynin administration (Section 6.5.3), performance on this assessment has been shown to be impaired with anticholinergic use by previous investigators. Moreover, the DSST was performed four times (e.g., baseline, and approximately 1, 2, and 4 hours post study medication) during each session, making it more feasible to estimate and control for practice effects. Verbal learning immediate and delayed recall tasks were the a priori cognitive measures for this analysis. However, given the possible discrepancy in difficulty among the "parallel" versions, verbal learning scores could not be used. Working memory (e.g., the n-back tasks) did appear to be impaired with a small acute dose of oxybutynin. However, practice effects were also apparent. It would have been difficult to separate out practice effects given the small n and that the N-back was performed at only one time point post study medication administration.

Only data from the active session was used for the following analyses. A total of eight subjects participated in both pupillary motility measurements and the DSST assessment at four time points throughout the active session. The DSST was performed approximately five minutes following completion of pupil measurement. The model discussed in section 6.5.3 was used as the initial base model [F = constant + m*(number of times completing DSST), with interindividual variation on the constant and additive error for residual variability]. The

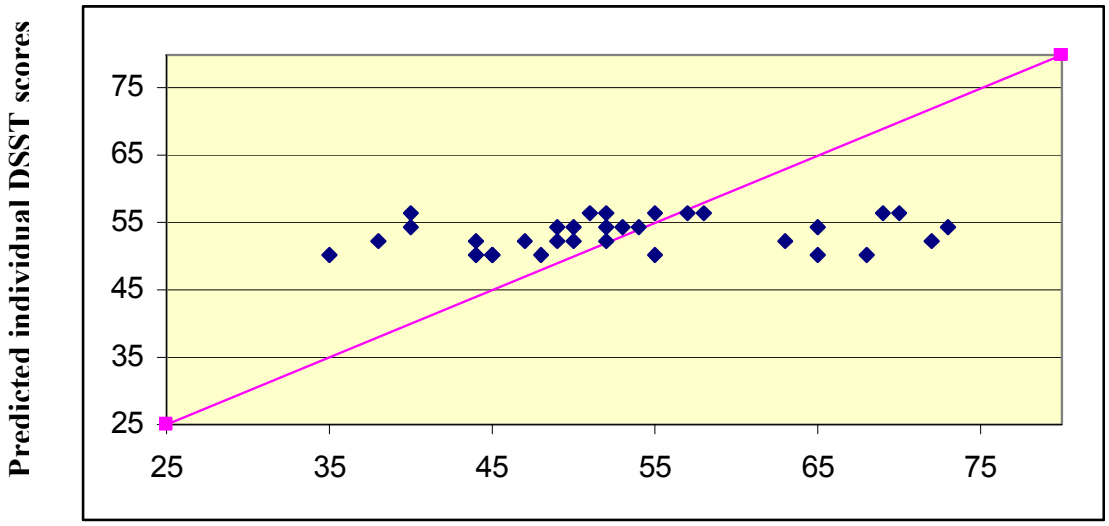
relationship between oscillations and cognition was examined for each of the eight chosen frequency categories. Average power for the first sixty seconds, as well as the average power from 120 to 150 seconds was specifically examined. Average power at 0.0 to 0.20, 0.21 to 0.40, 0.41 to 0.0, 0.81 to 1.0, and 1.0 to 2.0 for the first 60 seconds of measurement were all significantly positively associated with cognitive performance ($p<0.01$) when assessed individually in the base model. No relationship was found using the last 30 seconds of pupil measurements, most likely due to the confounding effects of "fatigue waves" (Section 6.7) which were more prominent during the last minute of pupil measurement. Serum AA as a continuous variable was not associated with DSST performance, either alone or with oscillations also incorporated into the model.

The base model used above with constant (interindividual variation) and practice effects alone accounted for a significant portion of the data spread (Figure 6.13). We were interested in examining how much oscillation power could improve the model fit when only the population means (e.g., no interindividual variation) for constant and practice effects were used. The individual variation on the constant was removed and oscillatory power of 0.81 to 1.0 Hz for the first 60 seconds of measurement was again examined [e.g., F = constant + m*(number of times completing DSST) + b*(power of oscillations)]. We targeted this frequency range because there is putatively no or only minimal fatigue waves occurring at this frequency. Using just the population mean for the constant and for practice effects, average power at 0.81 to 1.0 Hz significantly improved the model ($p<0.00l$; Figures 6.22 and 23). However, an improvement was seen only if interindividual variation on b was allowed. Serum AA as a continuous variable was

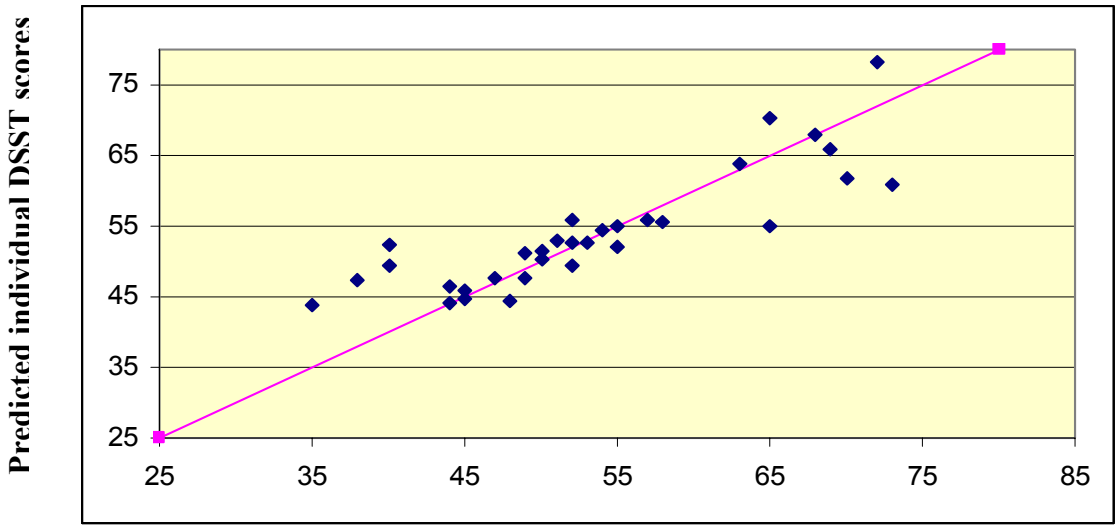
not associated with DSST performance in this model. The geometric mean (SD; range) of the slope for oscillations was 4.4 (5.4; 0.54 to 16.0).

Figure 6.22. Observed versus individual predicted DSST scores

A. Base model alone (no interindividual variation)



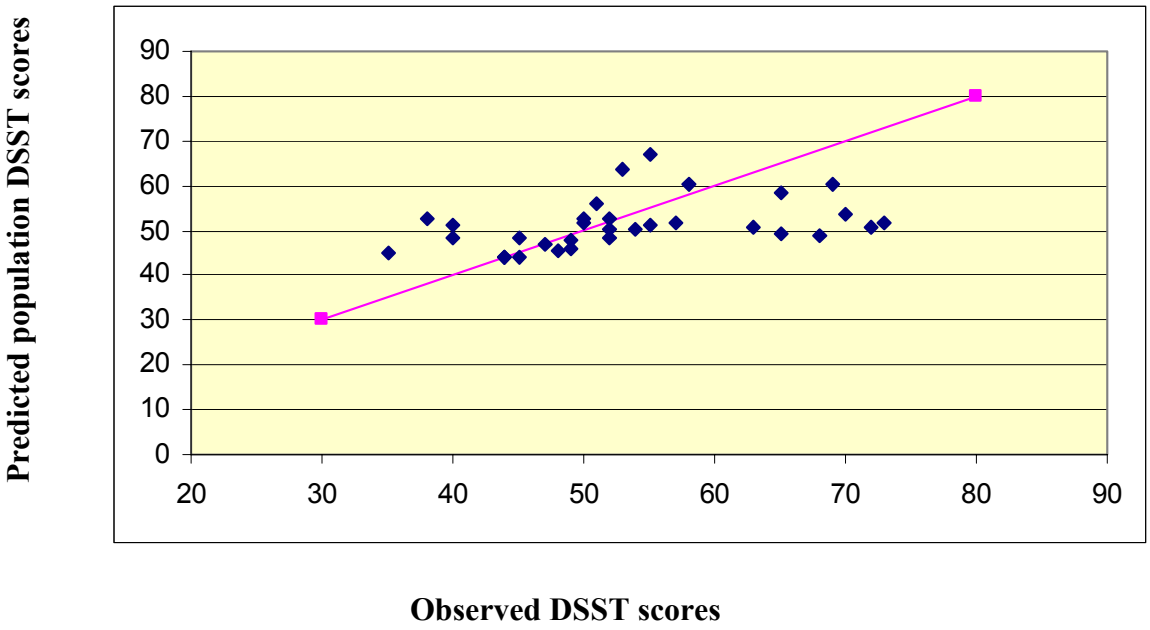
B. Base model with oscillations added



The predicted individual DSST scores were determined using the models discussed above. Figure 6.22A is F = constant + m*(number of times completing DSST), no interindividual variability on either of the parameters; Figure 6.22B is F = constant + m*(number of times completing DSST) + (b*oscillations), interindividual variability on b. The pink line is a unit line.

Figure 6.23 Observed versus population predicted DSST scores

Base model with oscillations added



The predicted population DSST scores were determined using the models discussed above [e.g., F = constant + m*(number of times completing DSST) + (b*oscillations), interindividual variability on b. The pink line is a unit line.

6.8.1 Discussion

The relationship between oscillations and Digit Symbol Substitution Test scores was examined for oscillation frequencies of less than 2.0 Hz. Average power for the first sixty seconds for 0.0 to 0.20, 0.21 to 0.40, 0.41 to 0.60, 0.81 to 1.0, and 1.0 to 2.0 were all significantly positively associated with individual performance ($p<0.01$). No relationship was found between the average power of the last thirty seconds of pupillary measurements and individual DSST scores. In addition, serum AA as a continuous variable was not associated with DSST scores when incorporated into the model by itself or with oscillatory power.

It is unclear why serum AA was not associated with DSST scores. One possibility is that the number of subjects involved in this study was too small in order to adequately capture this relationship. Basic linear regression investigating the association between serum AA and DSST scores had a p value of approximately 0.10. Another possibility is that there could be a delay between what is seen peripherally (e.g., serum AA) and what is actually occurring centrally (e.g., due to delay in medication distribution into the CNS or due to downstream events). Finally, serum AA may not be a sensitive enough measure for small changes in serum AA to be associated with cognitive changes. In a larger cohort, the predictive value of categorical versus continuous serum AA values needs to be explored.

This study represents only initial efforts of the examination of the relationship between serum AA, ocular response and cognition. We have shown that anticholinergic administration is associated with a decrease in power of oscillations at lower frequencies. We have also shown that pupillary oscillations are associated with DSST performance. However, from this, we cannot directly draw the conclusion that pupil oscillations would be a good tool to predict who is at risk for anticholinergic-induced cognitive impairment. For instance, it could be the natural circadian rhythm of the "alertness" or central activation which is driving the relationship between oscillations and cognitive performance. Moreover, when specifically investigating the power of 0.81 to 1.0 Hz to improve the model fit, a very large range of individual slopes was estimated (0.54 to 16.0), making the predictive value of oscillations uncertain. Finally, we have shown that oscillatory power is correlated with DSST performance, an assessment which is a measure of attention, perceptual speed, motor speed, visual scanning and memory. Thus we

cannot say if oscillatory power is associated with other cognitive domains such as working memory, episodic memory, the ability to follow directions, etc.

In order to better understand the relationship between pupillary oscillations and cognitive performance, future studies (e.g., including a larger number of subjects) need to examine the relationship using a variety of cognitive assessments in the absence of any specific anticholinergic medication and following different acute doses of oxybutynin (e.g., 2.5, 5.0, and 10.0). In addition, possible covariates (e.g., age, education, IQ) need to be investigated.

7.0 CONCLUSIONS

This doctoral dissertation addresses serum AA and pupil oscillations as possible peripheral markers for the assessment of anticholinergic-induced cognitive impairment. Serum AA holds promise as a useful clinical tool to determine which individuals may be at risk for anticholinergic toxicity. Several studies have shown that serum AA is associated with global cognitive performance, verbal memory, self-care capacity, and presence of delirium in older nondemented or mildly demented elderly. Moreover, in one study serum AA was found to be a stronger predictor of cognitive impairment than age or total number of medications. However, prior to its incorporation in a clinical setting, there are multiple items which need to be examined to enhance the reliability and clinical applicability of the assay. This doctoral dissertation reports on the investigation of the *in vitro* AA of 106 anticholinergic medications and the possible use of pupillary unrest as means to assess central AA.

The muscarinic receptor binding profiles of most medications and their metabolites have never been examined. Thus, even if a clinician decides that a patient is suffering from anticholinergic-induced toxicity, he/she has little guidance on which medication(s) to adjust. To address this issue, we investigated the *in vitro* AA of 106 commonly used prescription and over-the-counter medications at various therapeutic concentrations representative of dose ranges commonly used in the elderly. To make these data more useful to clinicians, we then utilized published pharmacokinetic data from elderly patients to translate the concentration/AA relationship into an estimated dose/AA relationship.[92] Each of the therapeutic classes investigated had at least one medication which demonstrated AA at therapeutic levels. In total, 36 percent of the medications examined demonstrated detectable AA. Of these, 21 medications had dose-dependent AA and 17 demonstrated AA only at the highest (e.g., supratherapeutic) concentrations examined.

Some of the findings that we reported were expected (e.g., amitriptyline, nortriptyline, paroxetine, oxybutynin, tolterodine). However, other results will come as a surprise to most. For example, previous affinity studies have inferred that citalopram, fluoxetine, and quetiapine would have negligible binding at muscarinic receptors with clinical doses. However, these studies typically based their conclusions on results from nonclinical concentrations, extending over a wide range. Conversely, at clinically relevant concentrations, we found modest, yet significant AA for citalopram, fluoxetine, and quetiapine, which alone or in combination with other agents may cause some cognitive impairment in some individuals.

Although valuable, our results have their limitations. Foremost, AA is not to be used as the deciding factor in whether or not a medication should be administered. It is meant only as a tool to assist clinicians in assessing the risk-benefit profile of a medication in conjuction with the total clinical picture of the patient (e.g., disease status, additional medications, age). The exact relationship between serum AA and cognitive performance is still not known. In fact, some individuals demonstrate a relatively high *in vivo* AA without any subjective or objective noting of memory deficits. In addition, only a small number of medications have been assessed thus far. Moreover, of the medications assessed, metabolites have yet to be examined. Thus, we may have classified a drug with an antimuscarinic metabolite as exhibiting no AA at therapeutic doses. One possible example of this is loratadine. Our lab as well as others, have reported loratadine to have no anticholinergic activity. However, its metabolite, desloratadine has been shown to have *in vitro* muscarinic receptor binding. Future work needs to assess an increased number of prescription and over-the-counter medications, as well as their metabolites. We also

need to better understand the relationship between cognitive performance and serum AA. The second part of this doctoral dissertation addresses in part this relationship.

A pilot study (n=8) was conducted to assess the feasibility of using serum AA and pupillary oscillations together to predict cognitive performance. A secondary aim of this study was to assess serum AA over time in the absence of any specific medication administration. Serum AA following placebo exhibited profiles one would expect to see if indeed medication present in serum is driving AA levels. Oxybutynin did produce apparent, but nonsignificant deficits in verbal learning, working memory, and long-term recall approximately 1 to 2 hours following oral administration of oxybutynin. A decrease in pupillary unrest in lower amplitude frequencies (e.g., < 2.0 Hz) was evident following oxybutynin administration through the last testing point at approximately 4 hours post medication. Pupillary unrest following oxybutynin administration was positively associated with the DSST (e.g., an assessment which measures attention, perceptual speed, motor speed, and visual scanning). However, continuous serum AA was not associated with this specific assessment.

This study provides the groundwork for future investigations of possible peripheral markers for anticholinergic-induced cognitive impairment. We have shown oxybutynin to be feasible as a anticholinergic probe--as an acute dose of this medication was well tolerated by the subjects, produced significant changes in pupil diameter and pupil oscillations, and did show some, nonsignficant objective and subjective cognitive deficits. However, future studies need to address the utility of the cognitive assessments chosen for this study. Many subjects subjectively complained of feeling confused and the clinician noted obvious impairments in the ability to

follow directions, as well as slowing of cognitive processing. Yet overall, this was not reflected well in cognitive testing. Additional measures need to be examined with a focus on long-term memory, ability to follow directions, and on those measures which have greater day to day relevance for the subject. Another future focus is better understanding of pupillary oscillations in older adults. Specifically, what is the inter and intra individual variation across time; how does different doses of oxybutynin affect oscillations (e.g., 2.5, 5.0, 10.0); what is the relationship between serum AA, oscillations, and a variety of cognitive assessments; and how might covariates affect this relationship.

In conclusion, the results of this work provide the impetus for future investigations of anticholinergic induced memory impairment. The long-term goal of our lab is to 1) extend the *in vitro* AA investigation to include additional medications and metabolites, 2) examine baseline serum AA in a large community-dwelling population so that we may better understand the AA-cognition relationship and possible covariates which may affect this, and 3) examine pupillary oscillations, serum AA, and cognitive performance in a larger cohort.

APPENDIX A

BRIEF DESCRIPTIONS OF SPECIFIC COGNITIVE DOMAINS

Autobiographical memory: Memory of past personal life. This specific memory is commonly impaired in patients with Alzheimer's Disease. An example measure assessing autobiographic memory is a questionnaire that elicits the recall of 10 personal episodes per decade of life through 7 decades and measure 4 kinds of information: facts ("what happened"), people ("who was there"), places ("where did it occur"), and dates ("when did it occur").[50]

Declarative memory: This type of memory refers to the learning, retention, and retrieval of information stored for periods longer than 30 seconds. It is the conscious recollection of past events relating to a specific time and place. Declarative memory is often broken down into episodic and semantic memories.

Episodic memory: A type of declarative memory, this is memory for past and personal experiences and conception-based knowledge. Autobiographical and verbal learning memories are both examples of episodic memory.

Explicit memory: See declarative memory

Immediate Memory: See working memory

Implicit memory: See procedural memory

Long-term memory (also see short-term memory): Memory which is consolidated and held in storage approximately 3 to 6 hours following an event and which is stored indefinitely. Some investigators also use long-term memory to refer to any memory which occurs approximately 30 seconds or longer following an event. Distinct physiological mechanisms support the use of the former definition.

Praxis: Memory for the planning, executing, and sequencing of motor movements.

Primary memory: See working memory

Procedural memory: This type of memory is the unconscious, no intentional form of memory (e.g., learning how to ride a bike).

Secondary memory: (See explicit, implicit, and semantic memory)

Semantic memory: A type of declarative memory, this memory is concept-based knowledge not related to specific experiences. Knowledge or words and how to apply them (e.g., is a lion an

insect?), reading, writing, and mathematics are all examples of semantic memory. Common measures of semantic memory include 1) asking an individual to recall as many words that they can beginning with a specified letter in a 90 second time frame, 2) object naming, and 3) spelling.

Short-term memory (also see long-term memory): Memory approximately 30 seconds up to 6 hours following a stimulus. Short-term memory is a separate process from long-term memory. Experimental manipulations can produce deficits in short or long-term memory independent of the other. (Some investigators also use short-term memory interchangeably with immediate or working memory. In these instances, short-term memory refers to memory which is held for approximately 15-30 seconds following an event.

Working memory: This type of memory refers to tasks or processes in which small quantities of currently relevant information are recalled over short-retention periods of approximately 15 to 30 seconds. Examples of working memory include the comprehension of a sentence, recalling back a number of digits such as a telephone number, and completing a simple calculation without paper.

APPENDIX B

TELEPHONE SCREENING

Hello, my name is Marci Chew and I am a researcher at the University of Pittsburgh, School of Pharmacy. I was calling in reference to the letter that Dr. Nebes, from Western Psychiatric Institute and Clinic, sent you about participating in a research study. Is it a convenient time for me to speak with you?

{If No}: May I call you back at another time?

{If Yes}: The purpose of our research study is to look at the relationship between medications, memory, and eye responses such as pupil size. Your participation in this study is completely voluntary and you have no obligation to participate. This study involves a short screening visit (approximately 2 ½ hours) and two study visits (approximately 8 hours each). The study visits include taking a single dose of either oxybutynin (a medication commonly used for bladder control) or placebo (a pill with no medication in it), testing memory, measuring eye responses such as pupil size and measuring certain chemicals in the blood. The information from this study will be used to see if we can develop a tool to help

predict which individuals are at risk for memory impairment caused by medications. Do you think that you might be interested in participating in this study?

{If No}: Thank you very much for your time.

{If Yes}: Before enrolling people in this study, we need to determine if they are eligible. We are looking for male or female non-brown eyed Caucasians 65 years or older to participate. The reason that at this time we are only looking for non-brown eyed Caucasians is that both eye color and ethnicity may affect eye response to different medications. The reason that we are looking for only individuals greater than 65 years of age is that we are specifically interested in the relationship between medications, memory and eye responses in older adults. What I would like to do now is to ask you a series of questions. There is a possibility that some of these questions may make you feel uncomfortable; if so, please let me know. You do not have to answer those questions if you do not want to. The purpose of these questions is only to determine whether you are eligible for our study. Remember your participation is voluntary and you do not have to complete these questions. All of the information that you tell me today will be kept confidential. This information will be shredded if we determine that you are ineligible to participate in this study or if you decide that you are not interested in participating. However, if you are eligible to participate, we will keep all of the information that I receive from you today, including your name, under lock and key

Do I have your permission to ask you these questions?

{If No}: Thank you very much for your time.

{If Yes}:

1. How old are you?
2. What is the color of your eyes?
3. Are you Caucasian?
4. Are you a current or past smoker? When was your last cigarette?
5. Do you use any other tobacco products such as chewing tobacco? If so, how often do you use these products?
6. For this study, we request that subject's do not consume any nicotine for 2 days before and the day of the study? Do you think that you would have a problem doing this?
7. How much caffeine do you consume in one day (e.g., coffee, tea, soda, chocolate)?
8. For this study, we request that subject's do not consume any caffeine for 2 days before and the day of the study? Do you think that you would have a problem doing this? Would you get a headache?
9. How much alcohol (e.g., beer, wine, liquor) do you consume in one week? In one day?
10. For this study, we request that subject's do not consume any alcohol for 2 days before and the day of the study? Do you think that you would have a problem doing this?
11. What are your current medical conditions?
12. Do you have any problems with digestion or your stomach? Please list and describe.
13. Have you ever been diagnosed with major depression within the past year, anxiety disorder, bipolar disorder, panic attacks, obsessive compulsive disorder, schizophrenia, or glaucoma.
14. Do you have any eye problems? Please list and describe.
15. Do you ever use any eye drops? For what?
16. Do you have any problems urinating?
17. What medications are you currently taking?
18. What over-the-counter or store bought medications do you take? Vitamins? Herbal products?
19. Do you ever take any medications to help you sleep?
20. Do you take any medications for anxiety or depression?
21. Are you allergic to any medications?
22. Have you ever had a bad reaction to a medication?
23. Has your doctor ever told you not to take a specific medication or class of medications?

APPENDIX C

MEDICAL SCREENING DOCUMENT

**Have individual bring all medications (including OTC, herbal, vitamins) to the screening session.*

Date of Birth: **Age:**

Race: **Eye color:**

Handedness: **Visual Acuity:**

Weight: **Height:**

Sitting Blood Pressure: **Pulse:**

Native language:

1. **How old are you?** *(if not 65 years or greater, end the interview)*

2. **What is the color of your eyes?** *(if not non-brown, end the interview)*

3. **Are you Caucasian?** *(if no, end the interview)*

4. **What is your dominant hand?** *(if left, end the interview)*

5. **Are you a current smoker?** *(if yes, end the interview)*

6. **Were you a smoker in the past? If so, when was your last cigarette?**

7. **Do you use any nicotine products (e.g., chewing tobacco, cigars, nicotine gum or patches)?**

8. **How much caffeine do you consume in one day?**

    a. **How many cups of coffee in one day?**

    b. **How many cups of caffeinated soda such as Pepsi, Coke, Mountain Dew?**

    c. **How may cups of iced tea or hot tea?**

    d. **How much chocolate do you consume?**

    e. **How often do you take store bought products that contain caffeine such as Excedrin, midol, vivarin, nodoz,).**

    f. **Are you using any over the counter weight loss products such as dexatrim?**

    g. **Do you take any medications for migraines?**

h. Are there any other sources of caffeine that you use that I have not listed yet?

_____

_____

9. For this study, we request that subject's do not consume any caffeine for 2 days before and the day of the study? This includes all liquids such as coffee or soda, food products such as chocolate, and medications such as excedrin. Do you think that you would have a problem doing this (e.g., would you get a headache)?

_____

_____

10. For this study, you will have to swallow a small pill. Do you think that you will have a problem with this?

11. How much alcohol (e.g., beer, wine, liquor) do you consume in one day; in one week?

_____

_____

12. For this study, we request that subject's do not consume any alcohol for 2 days before and the day of the study? Do you think that you would have a problem doing this?

13. What is the last year of education completed?

_____

_____

14. Do you wear corrective eye-wear? Be specific (e.g., glasses or contact; for near or far vision).

_____

_____

15. Do you consider yourself a morning person, an afternoon person, or a night person? When do you feel that you think best?

_____

_____

16. Do you have any hearing problems?

_____

_____

17. When was the last time that you visited your primary care physician? {If it has been since the telephone screening, ask subject reason for visit and if any new medications were prescribed}

_____

_____

18. What are your medical conditions?

a._____ **f.**_____

b._____ **g.**_____

c._____ **h.**_____

d._____ **i.**_____

e._____ **j.**_____

19. When was the last time you visited an eye specialist (e.g., optometrist or ophthalmologist)?

_____

_____

20. What medications are you currently taking?

a._____ **h.**_____

b._____ **i.**_____

c._____ **j.**_____

d._____ **k.**_____

e._____ **l.**_____

f._____ **m.**_____

g._____ **n.**_____

******Anticholinergic agents may potentially alter absorption of some concomitantly administered drugs due to effects on GI motility. May be a concern for drugs with narrow therapeutic index.***

21. Are there medications that you are prescribed, but you do not take? List and describe?

_____

_____

22. Do you ever take any medications which are prescribed for somebody else besides you?

_____

_____

23. What over-the-counter (OTC) medications do you take? Vitamins? Herbal products?

a._____ **h.**_____

b._____ **i.**_____

c._____ **j.**_____

d._____ **k.**_____

e._____ **l.**_____

f._____ **m.**_____

g._____ **n.**_____

24. Are there any medications or OTC products that you take occasionally or rarely?

_____

_____

25. Do you ever have problems sleeping? Do you taken any over the counter or prescription medications for this problem (e.g., Ativan, Benedryl, Tylenol PM, cold medications)?

_____

_____

26. Do you ever take prescription or OTC products for constipation or diarrhea?

_____

_____

27. Are you allergic to anything (e.g., dust, perfume, etc). If yes, what do you take when your allergies are bothering you?

_____

_____

28. Do you take any medications when you have a headache or your sinuses are bothering you? What do you take and how often?

_____

_____

29. Do you take aspirin, Tylenol, ibuprofen, aleve or other pain reliever medications? If so, how often and for what?

_____

_____

30. Do you take any weight loss products such as dexatrim?

_____

_____

31. What medications do you take for an upset stomach? How often do you take them?

_____

_____

32. Have you ever taken prescription pain relievers such as vicodin or oxycodon

_____

_____

33. Do you ever take Benadryl or other sinus, cough, or cold medications? How often?

_____

_____

34. Do you take any medication for osteoporosis?

_____

_____

35. Are there prescription or over-the counter medications that you were taking within the last month, that you are no longer taking?

_____

_____

36. Are you allergic to any medications?

_____

_____

37. Have you ever had a bad reaction to a medication?

_____

_____

38. Has your doctor ever told you not to take a specific medication or class of medications?

_____

_____

39. Have you ever gotten confused or delirious after taking a medication?

_____

_____

40. Have you ever been hospitalized? Please list and describe?

_____

_____

41. Do you have any dietary restrictions?

_____

_____

42. Have you participated in other studies? Please list and describe?

_____

_____

43. Have you ever had you memory tested before? Please list and describe?

_____

_____

44. Have you ever passed out, lost consciousness, or fainted while giving blood?

_____

_____

Gastrointestinal:

Do you have any stomach or digestive disorders? Please list and describe them.

1. **Do you have any of the following:**
    a. **ulcers**
    b. **painful swallowing**
    c. **reflux disease or GERD**
    d. **ulcerative colitis**
    e. **paralytic ileus**
    f. **intestinal atony**
    g. **gastric obstruction**
    h. **gastrointestinal obstruction**
    i. **hiatal hernia**
    j. **diverticulitis**
    k. **gastritis**
    l. **constipation (how often do you take medication to treat constipation; have you ever had problems as a result of your constipation such as bowel obstruction)**
    m. **h/o small bowel obstruction**
    n. **h/o large bowel obstruction**

*****oxybutynin is contraindicated in patients with gastric retention and other severe decreased GI motility conditions. Should be used in caution with GI obstructive disorders**

_____

Urinary System:

Do you have any bladder or urinating difficulties? If so, please name and describe them.

1. **Have you ever had a urinary tract infection or a UTI? When was the most recent infection? How many UTIs have you had in the past year?**
2. **Have you recently had a bladder or kidney stones?**
3. **Have you ever been diagnosed with any other disorder which may cause your bladder to be blocked or obtstructed.**
4. **Were you ever diagnosed with interstitial cystitis?**
5. **Do you have difficulty urinating?**
6. **Do you ever feel like you do not completely empty your bladder?**
7. **Have you ever been diagnosed with enlarged prostate?**
8. **Have you ever taken a medication for enlarged prostate (be specific)?**

******Ditropan may aggravate the sxs of prostatic hypertrophy***

Eye Disorder:

Do you have eye disorders? If so, please list and describe them.

1. **Do you have any of the following:**
   a. **Glaucoma**
   b. **Cataracts**
   c. **Retinopathy (or visual changes related to diabetes)**
   d. **Macular degeneration**
2. **Do you ever use eye drops?**
3. **Do you have a history of ocular surgery?**
4. **Do you wear glasses or contacts?**

Cardiovascular/Cerebral:

Do you have any cardiovascular disorders? If so, please list and describe them?

1. **Do you have any of the following:**
    a. **Arrhythmias (were you ever treated for irregular heartbeat)**
    b. **low or fast heart rate**
    c. **uncontrolled hypertension**
    d. **angina**
    e. **congestive heart failure**
    f. **h/o myocardial infarction**
    g. **h/o stroke**

******Ditropan may aggravate the sxs of coronary heart disease and congestive heart failure***

Cardiovascular/Cerebral:

_____

_____

Endocrine/Neurologic

1. **Have you ever been diagnosed with myasthenia gravis?**
2. **Have you ever had a seizure or a convulsion? Do you have a seizure d/o?**
3. **Have you ever been diagnosed with Parkinson's disease**
4. **Have you ever been diagnosed with another neurological disorder?**

_____

_____

Other

1. Have you ever been diagnosed with renal (kidney) or liver disease? Have you ever been diagnosed with kidney or liver impairment? Please list and describe?

2. Have you ever had a head injury? Did you lose consciousness or require hospitalization? Please describe?

3. Have you ever lost consciousness? If so, please describe?

4. Do you any type of inhaler?

5. Have you ever been diagnosed with cancer?

6. When was the last time that you have donated blood (e.g., to the central blood bank).

_____

_____

Psychiatric:

1. **Have you ever been diagnosed with a psychiatric disorder? If so what?**
2. **Have you ever been diagnosed:**
    a. **Depression**
    b. **Anxiety disorder**
    c. **Panic disorder/panic attacks**
    d. **Schizophrenia or schizoaffective disorder**
    e. **Bipolar disorder**
    f. **Eating disorder**
3. **Have you ever taken an antianxiety agent such as Valium or Ativan? If so for what?**
4. **Have you ever received treatment for drug or alcohol abuse? Have you ever thought or have somebody tell you that you may have a drinking disorder?**
5. **Are you currently using cocaine, heroin, opiates, amphetamines, marijuana or other illegal medications? If you have in the past used any of these agents, when was the most recent time.**

_____

_____

APPENDIX D

Assessments at Screening and Study Visits 1 and 2

Assessments	*Screening (Pre-study session)*	*Study Session 1 & 2 Baseline*	*20 min*	*40 min*	*60 min*	*1.5 hr*	*2 hr*	*3 hr*	*4 hr*	*6 hr*	*~24 hr*
Medical, medication, psychiatric review	X	X									
Vital signs	X	X	X	X	X		X	X	X	X	
Temperature		X	X	X	X		X	X	X	X	
Electrocardiogram	X										
Visual acuity test	X										
Biochemical Tests	X										
Geriatric Depression Scale	X										
North American Reading Test	X										
Mini-Mental State Exam	X										
Digit symbol substitution test	X	X			X		X		X		
Letter-number sequencing test (concurrently measuring pupillary response)	X	X			X		X		X		
Verbal learning test (concurrently measuring pupillary response)	X	X			X		X		X		
N-back test (concurrently measuring pupillary response)	X	X			X						
Valence Identification (concurrently measuring pupillary response)		X				X					
Paragraph Test						X					X
Pupil diameter/light reflex		X	X	X	X	X	X		X	X	
Continual measurement of pupillary response		X			X	X	X		X		
Near-point of accommodation		X	X	X	X	X	X		X	X	
SAA/oxybutynin blood draw		X	X	X	X	X	X		X	X	
Timed walking test		X			X		X		X		

ADVERSE EVENTS FORM

Adverse Event Questionnaire

Please circle the severity of any of the following problems that you have at THIS MOMENT.

1) Tiredness or fatigue	None	Mild	Moderate	Severe
2) Dry mouth	None	Mild	Moderate	Severe
3) Increased Salivation	None	Mild	Moderate	Severe
4) Dizziness/ Lightheadedness	None	Mild	Moderate	Severe
5) Nausea	None	Mild	Moderate	Severe
6) Stomach Pain/ Indigestion	None	Mild	Moderate	Severe
7) Headache	None	Mild	Moderate	Severe
8) Diarrhea	None	Mild	Moderate	Severe

9) Difficulty Urinating	None	Mild	Moderate	Severe
10) Dry eyes	None	Mild	Moderate	Severe

Please circle the severity of any of the following problems that you have at THIS MOMENT.

11) Weakness/ Lack of Strength	None	Mild	Moderate	Severe
12) Tension/ Nervous Restlessness	None	Mild	Moderate	Severe
13) Tremor (shaking)	None	Mild	Moderate	Severe
14) Confusion	None	Mild	Moderate	Severe
15) Heart Palpitations (rapid beating)	None	Mild	Moderate	Severe
16) Blurred Vision	None	Mild	Moderate	Severe
17) Increase in Thirst	None	Mild	Moderate	Severe
18) Concentration Difficulties	None	Mild	Moderate	Severe

Time administered: _____

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