Muscarinic and nicotinic receptors synergistically modulate working memory and attention in humans

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Abstract

Functional abnormalities in muscarinic and nicotinic receptors are associated with a number of disorders including Alzheimer’s disease and schizophrenia. While the contribution of muscarinic receptors in modulating cognition is well established in humans, the effects of nicotinic receptors and the interactions and possible synergistic effects between muscarinic and nicotinic receptors have not been well characterized in humans. The current study examined the effects of selective and simultaneous muscarinic and nicotinic receptor antagonism on a range of cognitive processes. The study was a double-blind, placebo-controlled, repeated measures design in which 12 healthy, young volunteers completed cognitive testing under four acute treatment conditions: placebo (P); mecamylamine (15 mg) (M); scopolamine (0.4 mg i.m.) (S); mecamylamine (15 mg)/scopolamine (0.4 mg i.m.) (MS). Muscarinic receptor antagonism with scopolamine resulted in deficits in working memory, declarative memory, sustained visual attention and psychomotor speed. Nicotinic antagonism with mecamylamine had no effect on any of the cognitive processes examined. Simultaneous antagonism of both muscarinic and nicotinic receptors with mecamylamine and scopolamine impaired all cognitive processes impaired by scopolamine and produced greater deficits than either muscarinic or nicotinic blockade alone, particularly on working memory, visual attention and psychomotor speed. These findings suggest that muscarinic and nicotinic receptors may interact functionally to have synergistic effects particularly on working memory and attention and suggests that therapeutic strategies targeting both receptor systems may be useful in improving selective cognitive processes in a number of disorders.

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Introduction

The basal forebrain and rostral forebrain cholinergic systems provide a key source of modulatory input to fronto-limbic and fronto-striatal brain regions where they have been implicated in cognitive processes including visual attention, declarative memory and working memory (Everitt and Robbins, 1997; Perry et al., 1999). The neural substrates for the attention and memory modulating effects of the cholinergic system has been linked to the importance of afferent cholinergic pathways in sharpening stimulus representations (i.e. increasing signal to noise) (Sahakian, 1987; Sato et al., 1987), enhancing spatial orientation and sustained attention in the cortex (Davidson and Marrocco, 2000) and enhancing long-term potentiation in the hippocampus (Auerbach and Segal, 1996).

Corroborative clinical and post-mortem studies has shown that disruptions in central cholinergic function, including reductions in muscarinic and nicotinic
receptors in the frontal cortex and hippocampus (Nordberg et al., 1992; Perry et al., 1995; Whitehouse et al., 1988) may underlie or at least contribute to the cognitive deterioration observed in a number of disorders including Alzheimer’s disease, dementia with Lewy bodies, age-related dementias (Auld et al., 2002; Bartus et al., 1982; Graham et al., 2002; Fimlott et al., 2004) and schizophrenia (Breese et al., 2000; Crook et al., 2000, 2001; Freedman et al., 1995; Guan et al., 1999; Katerina et al., 2004). This has been supported by the fact that pharmacological strategies that increase muscarinic and nicotinic receptor function directly or indirectly are current treatments for cognitive decline in Alzheimer’s disease and have been suggested as potential future treatments for improving cognitive function in schizophrenia (Friedman, 2004).

Since the early discovery by Drachman and Leavitt (1974), that muscarinic receptor antagonism could induce amnesic-like deficits in healthy subjects, a number of subsequent studies have shown that antagonism of the muscarinic receptors (with scopolamine) and nicotinic receptors (with mecamylamine) can induce deficits in a range of cognitive domains including attention and memory. For example, muscarinic antagonism has been shown to induce dose-related impairments on tasks of working memory (i.e. visual recognition memory) and declarative memory (i.e. acquisition and retrieval of new information and memory consolidation) (Broks et al., 1988; Edginton and Rusted, 2003; Potter et al., 2000; Robbins et al., 1997; Rusted and Warburton, 1988, 1989; Wesnes et al., 1988), visual sustained attention (Broks et al., 1988; Ebert et al., 1998; Wesnes and Revell, 1984; Wesnes and Warburton, 1983; Wesnes et al., 1988) and psychomotor function (i.e. reaction time) (Broks et al., 1988; Ebert et al., 1998; Flicker et al., 1990; Little et al., 1998; Mintzer and Griffiths, 2003; Nuotto, 1983; Wesnes et al., 1988). While less established, nicotinic antagonism has been shown to impair declarative memory (Newhouse et al., 1992, 1994), attention (Newhouse et al., 1994), psychomotor function (Newhouse et al., 1994) and early information processing (Thompson et al., 2000).

A related question regarding muscarinic and nicotinic modulation of cognitive function, relates to the role of each receptor system in modulating specific cognitive processes. Early findings in animals have suggested that nicotinic receptor antagonism does not impair cognitive function in the same manner as muscarinic receptor antagonism (Everitt and Robbins, 1997). This has been further supported by observations that nicotinic vs. muscarinic receptor modulation of cognitive processes such as working memory may depend on task demand (i.e. the more demanding tasks being sensitive to nicotinic modulation) (Granon et al., 1995), suggesting that muscarinic and nicotinic receptors may exert different effects on cognitive processes. While studies in animals do suggest that nicotinic receptor antagonism can impair attention (Ruotsalainen et al., 2000), working memory (Elrod et al., 1988) and learning (Riekkinen et al., 1993) similar to that observed with muscarinic receptor antagonism, this has not been examined extensively, particularly in humans.

Furthermore, very little is known about the functional interactions between muscarinic and nicotinic receptors, including how they may interact synergistically to modulate selective cognitive process. Animal studies have shown some evidence for synergistic interactions between muscarinic and nicotinic receptor systems at the level of receptor regulation (i.e. sensitization and up-regulation) and at a functional level on various cognitive processes (Brown and Galligan, 2003; Leblond et al., 2002; Levin et al., 1990; Mirza and Stolerman, 2000; Riekkinen et al., 1990; Vige and Briley, 1988). This is supported by a preliminary study in humans, in which simultaneous antagonism of both muscarinic and nicotinic receptors showed nonsignificant trends towards greater impairments in declarative memory (in an explicit word learning task) (Little et al., 1998). Similarly, we recently showed a greater impairment in early information processing (as measured using an inspection time task) with simultaneous antagonism of both receptors in comparison to antagonism of either receptor alone (Erskine et al., 2004). Despite evidence from these studies, questions regarding possible synergistic cognitive effects in humans and the type of cognitive processes specifically modulated by such an interaction remain unanswered. It has been suggested that abnormalities in early information processing, including the ability to detect, select and discriminate sensory stimuli, contribute to the severity of cognitive deficits observed in disorders such as Alzheimer’s disease, including impaired attention, learning and memory (Sarter and Bruno, 1999). Thus, it is possible that, given early information processing was synergistically modulated by both muscarinic and nicotinic receptors (Erskine et al., 2004), that other cognitive processes (including attention and memory) could similarly be modulated by both receptors. Hence the aim of the study was to examine the selective and simultaneous effects of muscarinic and nicotinic receptor antagonism on a range of cognitive processes including attention, working memory, declarative memory and psychomotor function in healthy human subjects.
Methods

Participants

Twelve healthy subjects (10 males, 2 females) aged 19–27 yr (mean 22.4 yr, S.D. = 2.4 yr) were recruited for the study. All subjects (10 Caucasian, 2 Asian) were university educated and proficient in English. Subjects were screened via a semi-structured clinical interview conducted by a medical physician and were excluded based on the following criteria: smokers, substance abuse or medication use (including contraceptives), family or personal history of neurological/psychiatric disorders. All subjects gave written informed consent to participate in this study, which was approved by the Swinburne University Human Research Ethics Committee.

Study design

The study used a double blind, placebo-controlled, repeated measures design in which all subjects were tested under four acute treatment conditions; placebo (P); mecamylamine (15 mg) (M); scopolamine (0.4 mg i.m.) (S); mecamylamine (15 mg)/scopolamine (0.4 mg i.m.) (MS). Each session was separated by a 7-d washout period and the order of drug administration was randomly assigned to subjects over the four testing sessions. Mecamylamine/placebo was administered via tablet form and scopolamine/saline was administered via intramuscular injection. Mecamylamine and scopolamine were chosen for this study, as they are the most selective antagonists available for human use, with high affinity for nicotinic and muscarinic receptors respectively (Brown, 1992; Varanda et al., 1985; Young et al., 2001). The doses of mecamylamine and scopolamine chosen for this study were based on (1) previous findings reporting cognitive impairments following mecamylamine doses ranging from 5 mg to 20 mg (Newhouse et al., 1992, 1994, Pickworth et al., 1997) and scopolamine doses ranging from 0.3 mg to 0.6 mg (Ebert et al., 1998; Wesnes et al., 1988) and (2) minimizing the chance of sedation interfering with task performance (especially in the MS condition).

Procedure

Subjects were requested not to consume alcohol or caffeine for 24 h prior to testing and to consume a light breakfast before each testing session. All subjects attended four half-day testing sessions conducted at the same time of day. All testing in female subjects was conducted during the follicular phase of the menstrual cycle (days 1–12). On each testing day subjects were given a baseline mood and cognitive assessment (pre-treatment testing). This was followed by the administration of either mecamylamine or placebo tablets. One hour post-mecamylamine or placebo administration subjects received either scopolamine or saline intramuscular injection. Post-treatment testing was conducted 3 h post-mecamylamine (or placebo) and 2 h post-scopolamine (or saline injection). The time of post-drug testing was chosen to maximize peak pharmacokinetic and pharmacodynamic effects of both mecamylamine and scopolamine (Kikuchi et al., Little et al., 1998; 1999; Young et al., 2001).

Cognitive assessment

The Cognitive Drug Research (CDR Ltd, Reading, UK) computerized assessment system was used for its validity as a measure of memory, attention and psychomotor function and its proven sensitivity in studies of acute drug manipulation (Ebert et al., 1998; Harrison et al., 2004; Matrenza et al., 2004). All cognitive tests were presented on a computer with high-resolution colour monitor. With the exception of written word recall tests, all responses were made using an external button box (yes/no), and a handheld critical flicker-fusion (CFF) unit. Subjects were instructed to respond as quickly as possible on all tasks where appropriate. Subjects were seated ~1 m from the computer monitor, with lighting dimmed at a constant level during each testing session.

The battery was developed to provide sufficient parallel forms across the eight testing sessions. Before commencing the study, subjects completed a familiarization session of the tests, which included four successful completions of the battery (Wesnes and Pincock, 2002). For each task, the primary measures of speed or accuracy were used as the task end-point. For tasks of working memory and delayed word recognition sensitivity indices (SI) were calculated. The SI combines accuracy scores for the original (target) as well as the novel (distractor) stimuli, providing an index of overall task efficiency (Frey and Colliver, 1973). The SI scores range from 0 to 1 (0, no evidence of discrimination; 1, perfect discrimination). The duration of the test battery was ~35 min. During the delay period of the declarative memory tasks subjects were tested on the other remaining cognitive tasks.

Declarative memory

These tasks measure the ability to store and quickly retrieve verbal and non-verbal information, respectively.
**Word-list learning (immediate and delayed word-recall)**

Fifteen words were presented in the centre of a black screen, matched for frequency (stimulus and inter-stimulus interval – ISI was 1 s). Immediately following the presentation of the final word, subjects were given 60 s to write down as many words remembered. Following a delay of 20 min, subjects were prompted to free-recall as many of the list words as possible. Task scores were based on number of words recalled, minus the number of errors and intrusions; the derived score was converted into a percentage.

**Delayed word recognition**

Words from the original list, interspersed with 15 distracter words matched for length and frequency were presented for 1.5 s, with a 0.5-s delay between consecutive words. Subjects responded *yes* to words identified from the original list, and *no* to all other words.

**Delayed picture recognition**

A series of 20 relatively neutral pictures (e.g. flora, fauna, transport) were presented and subjects instructed to remember them. Following a 20-min delay the original pictures plus 20 distracter pictures were presented for 1.5 s each, with a 0.5-s delay between consecutive pictures. Subjects responded *yes* to words identified from the original presentation and *no* to all other pictures.

**Spatial working memory recognition**

This task is a modified version of Sternberg’s test of working memory maintenance (Sternberg, 1966) and measures intact spatial recognition and the ability to manipulate stored information. The image of a house front displaying nine windows was presented, with four of the nine lights turned on (‘original’ stimuli) and subjects required to memorize the position of the four illuminated windows. Following a short-delay, 36 consecutive presentations of the house front are displayed with only a single window lit up. Subjects were required to identify whether the light was in a matching ‘original’ position by pressing *yes*, or a novel location by pressing *no*.

**Non-spatial working memory (numeric)**

This task measures the ability to keep and retrieve verbal information in memory. Five digits were presented sequentially for the subject to hold in memory. Thirty probe digits were then presented, for each digit the subject indicated whether or not the number appeared in the original series by pressing the appropriate response button.

**Sustained attention**

These tasks measure the ability to attend for a sustained period of time, and ignore distraction.

**Digit vigilance**

A randomly selected target digit was presented constantly throughout the duration of the task on the right-hand side of the computer screen. In the middle of the screen a continuous stream of digits (serially unordered) appeared at a rate of 80 digits per minute. Subjects responded using the *yes* button whenever these two digits matched. The duration of the task was 3 min, with 15 stimulus-target matches.

**Rapid visual information processing (RVIP)**

A series of numbers were presented one at a time in quick succession. Subjects responded with the *yes* button whenever they saw three even (i.e. any three of 2, 4, 6, 8) or odd (i.e. any three of 1, 3, 5, 7, 9) numbers in a row with nothing in between and in no particular order. The task ran for 4 min in a continuous manner.

**Psychomotor function**

**Reaction time (Simple) (SRT)**

This task measures alertness and attention, psychomotor speed and early information processing. Subjects responded to 50 presentations of the word *yes* by pressing the appropriate response button as quickly as possible (ISIs between 1 s and 3.5 s).

**Reaction time (Choice) (CRT)**

This task measures alertness and attention, psychomotor speed, information processing, stimulus discrimination and response organization. Subjects responded to the presentation of the word *yes*, or the word *no*, by pressing the appropriate response button as quickly as possible. Parameters (including number of trials) were equivalent to the SRT task. A cognitive reaction time (COGRT) index was calculated by subtracting SRT scores from CRT scores. This measure enabled the psychomotor (i.e. psychomotor speed) component of the tasks to be factored out, giving a measure of the cognitive component of the task (i.e. information processing, discrimination, response organization).
Critical Flicker Fusion (CFF)

This task is a traditional psychophysical threshold measure of alertness and attention and was used as a measure of drug-induced sedation (Hindmarch and Parrott, 1977). Subjects held the CFF unit, with their preferred eye resting on the upper brim of the tube, fixing their gaze on two red lights at the base of the unit. The light flicker ranged from 25 Hz to 65 Hz in alternating ascending and descending mode, three trials ‘up’ and ‘down’. Subjects were required to indicate when they perceived the light started or stopped flickering, by responding on the button box, or to discriminate flickering between the two lights.

Subjective mood assessment

Subjective mood ratings were obtained using a modified version of the Visual Analogue Mood Scale (VAMS; Bond and Lader, 1974). Consisting of eight 100-mm horizontal lines each representing a bipolar dimensional mood state: Happy–Sad, Sociable–Withdrawn, Relaxed–Tense, Quick Witted–Mentally Slow, Amicable–Antagonistic, Alert–Drowsy, Strong–Feeble, Interested–Bored. Subjects were instructed to place a vertical mark in the location on each scale that best described their current mood state.

Statistical analysis

All behavioural data were analysed using repeated-measures ANOVAs for treatment condition (P, M, MS, and S) × time (pre-drug and post-drug). Planned contrasts were carried out on the results of significant interactions, to examine the effects of each treatment condition compared to placebo and compared to each other. Contrasts were determined a priori and α-adjustments were not employed (Tabachnick and Fidell, 1989). All other exploratory correlations are reported at appropriate α-adjusted levels.

Results

Declarative memory

A significant treatment condition × time interaction was found for immediate word recall [F(3, 33) = 6.60, p = 0.001, ηp² = 0.37] and delayed word recall [F(3, 33) = 5.44, p < 0.01, ηp² = 0.33]. Planned contrasts revealed that compared to P, there was a significant decrease in words recalled in the MS and S conditions only. There was also a significant difference in words recalled between the MS and M conditions, with subjects recalling fewer words in the MS condition (see Figure 1 for F and p values).

Spatial working memory

There was a significant treatment condition × time interaction for spatial working-memory original stimulus accuracy [F(1.66, 17.9) = 4.7, p < 0.05, ηp² = 0.30] (Greenhouse–Geisser adjusted), SI [F(1.38, 15.26) = 6.02, p < 0.05, ηp² = 0.30] (Greenhouse–Geisser adjusted), reaction time (RT) [F(3, 33) = 4.96, p < 0.01, ηp² = 0.31]. Planned contrasts revealed that compared to P, there was a significant decrease in accuracy for the original stimulus in the MS condition.

Figure 1. Immediate (■) and Delayed (□) word recall performance after 3 h (mean ± S.E.M. % correct). P, placebo; M, mecamylamine; MS, mecamylamine + scopolamine; S, scopolamine. * Indicates significant difference between P and MS conditions [Immediate F(1, 11) = 10.42; Delayed F(1, 11) = 6.50] at p < 0.05 level; ** significant difference between P and S conditions [Immediate F(1, 11) = 11.18; Delayed F(1, 11) = 8.67] at p < 0.01 level; # significant difference between M and MS conditions [Immediate F(1, 11) = 5.94; Delayed F(1, 11) = 5.54] at p < 0.05 level.

Delayed word recognition

There was a significant treatment condition × time interaction across measures of word recognition original stimulus accuracy [F(3, 30) = 4.43, p < 0.05, ηp² = 0.31], novel stimulus accuracy [F(3, 30) = 6.42, p < 0.01, ηp² = 0.39] and SI [F(3, 30) = 6.88, p = 0.001, ηp² = 0.41]. Planned contrasts revealed that compared to P, there was a significant decrease in the SI in the MS and S conditions (see Figure 2a for F and p values). No significant differences were found between the treatment conditions for original stimulus accuracy. However, compared to P, there was a significant difference in novel stimulus accuracy for the S condition (see Figure 2b for F and p values).

Delayed picture recognition

There was no significant treatment condition × time interaction for delayed picture recognition.
Furthermore, compared to the MS condition there was a significant difference in the accuracy for the original stimulus in the M condition (see Figure 3a for F and p values). Compared to P, there was a significant decrease in SI in the MS condition. There was also a significant difference in SI between the MS and M conditions (see Figure 3b for F and p values). Planned contrasts also revealed that compared to P, there was a significant increase in RT in the MS condition. In addition, compared to MS, there was a significant difference in RT in the S and M conditions, with subjects performing more poorly whilst in the MS condition (see Figure 3c for F and p values).

Non-spatial working memory (numeric)

There was a significant treatment condition × time interaction for numeric working-memory original stimulus accuracy [F(3, 27) = 6.04, p < 0.01, η²p = 0.40], SI [F(3, 33) = 6.81, p = 0.001, η²p = 0.38], RT [F(1,29, 14.18) = 13.30, p < 0.01, η²p = 0.55] (Greenhouse–Geisser adjusted). Planned contrasts revealed that compared to P, there was a significant decrease in SI and an increase in RT in the MS condition. In addition, compared to P, there was a significant increase in RT in the S condition, but not in the M condition. Further planned contrasts showed significant differences in SI and RT measures between the MS and both S and M conditions, with subjects performing more poorly whilst in the MS condition (see Figure 4a, b for F and p values).
There was a significant treatment condition \( \times \) time interaction for digit vigilance accuracy \( F(3, 33) = 11.64, p < 0.001, \eta_p^2 = 0.51 \). False alarms \( F(3, 33) = 5.44, p < 0.01, \eta_p^2 = 0.33 \), and RT \( F(3, 33) = 10.43, p < 0.001, \eta_p^2 = 0.49 \). Planned contrasts revealed that compared to P, there was a significant decrease in accuracy in both the MS and S conditions. Significant differences in accuracy were found between the MS condition and both the S and M conditions, with subjects making more errors in the MS condition (see Figure 5a for \( F \) and \( p \) values). In addition, compared to P, there was a significant increase in false alarms in both MS and S conditions. Significant differences in false alarms were also found between the MS and M conditions (see Figure 5b for \( F \) and \( p \) values). Compared to P, there was a significant increase in RT in both the MS and S conditions. Significant differences in RT were also found between the MS and M conditions (see Figure 5c for \( F \) and \( p \) values).
Rapid visual information processing

There was a significant treatment condition × time interaction for RVIP accuracy \( F(3, 33) = 3.39, p < 0.05, \eta_p^2 = 0.25 \). Planned contrasts revealed no significant differences between the conditions. Interestingly, there was a trend towards a treatment condition × time interaction on RT \( (p = 0.06) \) (see Table 1).

Psychomotor function

Reaction time

There was a significant treatment condition × time interaction for RT on both the SRT \( F(1.41, 15.46) = 28.54, p < 0.001, \eta_p^2 = 0.70 \) (Greenhouse–Geisser adjusted) and CRT tasks \( F(1.58, 17.47) = 9.84, p < 0.01, \eta_p^2 = 0.47 \) (Greenhouse–Geisser adjusted). Planned contrasts revealed that compared to P, there was a significant increase in RT for the SRT and CRT tasks in both the MS and S conditions, but not for M condition. Further planned contrasts revealed significant differences in RT between the MS and S conditions for the CRT task. In addition, significant differences in RT between MS and M conditions were found for both SRT and CRT tasks (see Figure 6 for F and p values). There were no significant treatment condition × time effects for the COGRT measure.

Critical Flicker Fusion

There was no significant treatment condition × time interaction for CFF (see Table 1).

Subjective mood and correlations with cognitive processes

Repeated-measures ANOVAs revealed significant treatment condition × time interactions for the following mood states: Happy–Sad \( F(3, 33) = 7.76, p < 0.001, \eta_p^2 = 0.41 \), Sociable–Withdrawn \( F(3, 33) = 10.19, p < 0.001, \eta_p^2 = 0.48 \), Strong–Feeble \( F(3, 33) = 7.53, p = 0.001, \eta_p^2 = 0.41 \). Planned contrasts revealed that compared to P, subjects reported feeling more sad in the MS condition \( F(1, 11) = 21.5, p = 0.001 \), more withdrawn in the MS \( F(1, 11) = 26.4, p < 0.001 \), and S \( F(1, 11) = 22.0, p = 0.001 \) conditions and more feeble in the S condition \( F(1, 11) = 20.6, p = 0.001 \). The percentage change in mood ratings between pre- and post-treatment was calculated for each of the above three mood states during the MS, and S conditions. The percentage change for each mood state was then correlated with corresponding percentage changes in cognitive function in each treatment condition. The correlations between the three mood states and various cognitive processes varied, \( 0.01 > \gamma < 0.63 \) with no significant correlation found for either the MS, or S conditions (see Table 2).

Correlations with attention and memory measures

To examine the relationship between attention and memory performance, the percentage change in sustained attention performance (Digit Vigilance task) was correlated with working memory and declarative memory scores. No significant correlations were found between attention and working-memory task efficiency (SI) under the MS \( (0.00 > \gamma < 0.38), S (0.03 > \gamma < 0.38) \) or M conditions \( (0.02 > \gamma < 0.42) \). A moderate correlation was found between attention and working-memory reaction times under the MS condition \( (\gamma = 0.6, p < 0.05) \). No significant correlations were found between attention and declarative memory performance under the MS \( (0.01 > \gamma < 0.27), S (0.07 > \gamma < 0.40) \) or M \( (0.04 > \gamma < 0.41) \) conditions.

Discussion

The current study examined the effects of selective and simultaneous antagonism of the muscarinic and nicotinic receptors on various cognitive processes in healthy subjects. The major findings of the study were: (1) blocking muscarinic receptors with scopolamine was associated with impairments in working memory (spatial and non-spatial), declarative memory (immediate and delayed word recall and recognition), sustained visual attention (digit vigilance) and psychomotor function (reaction time); (2) blocking nicotinic receptors with mecamylamine had no significant effects on any of the cognitive processes and (3) that combined blockade of both muscarinic and nicotinic receptors with scopolamine and mecamylamine impaired all cognitive processes impaired by scopolamine and produced greater deficits than either muscarinic or nicotinic blockade alone on working memory, sustained attention and psychomotor function. These findings were observed independent of changes in mood or drug-induced sedation (assessed using the CFF test).

The scopolamine-induced deficits in both spatial and non-spatial working memory are consistent with previous studies implicating an important role for the cholinergic system in modulating general working-memory function in humans (Robbins et al., 1997; Rusted and Warburton, 1988; Wesnes et al., 1988). Scopolamine was also found to impair both immediate and delayed word recall, suggesting that scopolamine impairs not only the acquisition and encoding of
Table 1. Means and standard errors (mean ± S.E.M.) for CDR cognitive assessment at pre- and post-drug administration for non-significant results

<table>
<thead>
<tr>
<th>Measure</th>
<th>P</th>
<th>M</th>
<th>MS</th>
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<td>Memory</td>
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<tr>
<td>Accuracy (original) (%)</td>
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<tr>
<td>Immediate word recall</td>
<td>57.4 (4.2)</td>
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<td>Delayed word recall</td>
<td>47.4 (4.9)</td>
<td>31.3 (5.2)</td>
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<td>Delayed word recognition</td>
<td>74.5 (4.4)</td>
<td>80.6 (5.4)</td>
<td>73.9 (5.6)</td>
<td>69.1 (6.7)</td>
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<td>Delayed picture recognition</td>
<td>87.7 (3.6)</td>
<td>85.4 (4.4)</td>
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<td>Accuracy (novel) (%)</td>
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<td>Spatial working memory</td>
<td>98.6 (0.7)</td>
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<td>98.2 (0.75)</td>
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<td>96.8 (0.8)</td>
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<td>92.3 (1.9)</td>
<td>91.8 (2.3)</td>
<td>90.0 (2.3)</td>
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<td>Reaction time (ms)</td>
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<tr>
<td>Delayed word recognition</td>
<td>638.2 (26.9)</td>
<td>721.3 (37.2)</td>
<td>656.7 (30.5)</td>
<td>708.1 (41.6)</td>
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<td>771.1 (45.7)</td>
<td>792.7 (42.4)</td>
<td>779.3 (39.8)</td>
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<td>147.4 (11.0)</td>
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<td>Sensitivity Indices (%)</td>
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<td>Delayed picture recognition</td>
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<td>77.8 (6.3)</td>
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<td></td>
</tr>
<tr>
<td>Accuracy (original) (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rapid visual info processing</td>
<td>76.1 (4.6)</td>
<td>65.1 (5.3)</td>
<td>67.0 (7.3)</td>
<td>62.8 (6.0)</td>
</tr>
<tr>
<td>Reaction time (ms)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rapid visual info processing</td>
<td>466.8 (16.9)</td>
<td>455.2 (16.8)</td>
<td>474.8 (18.7)</td>
<td>501.5 (15.1)</td>
</tr>
<tr>
<td>Psychomotor Function</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Accuracy (novel) (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Choice reaction time</td>
<td>94.0 (1.1)</td>
<td>92.8 (1.0)</td>
<td>93.5 (1.7)</td>
<td>92.5 (1.1)</td>
</tr>
<tr>
<td>Critical Flicker Fusion</td>
<td>33.1 (1.5)</td>
<td>34.3 (1.6)</td>
<td>34.8 (1.9)</td>
<td>33.5 (1.3)</td>
</tr>
<tr>
<td>Threshold (Hz)</td>
<td></td>
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<td></td>
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</tr>
</tbody>
</table>

P, placebo; M, mecamylamine; MS, mecamylamine + scopolamine; S, scopolamine.
words in short-term memory, but also disrupts consolidation. These findings are comparable to those of previous studies demonstrating impairments in acquisition of new information and memory consolidation (Broks et al., 1988; Edginton and Rusted, 2003; Wesnes et al., 1988) following scopolamine administration. In addition to effects on memory and learning, scopolamine also impaired sustained attention as evidenced by impairments in the Digit Vigilance task and psychomotor function as evidenced by impairments in SRT and CRT tasks. These findings are again consistent with previous studies showing deficits in sustained attention (Broks et al., 1988; Ebert et al., 1998; Wesnes and Revell, 1984; Wesnes and Warburton, 1983; Wesnes et al., 1988) and psychomotor performance (i.e. SRT and CRT) with scopolamine (Broks et al., 1988; Ebert et al., 1998; Flicker et al., 1990; Little et al., 1998; Mintzer and Griffiths, 2003; Nuotto, 1983; Wesnes et al., 1988). In comparison to the effects of scopolamine, antagonism of nicotinic receptors with mecamylamine had no detrimental effects on any of the cognitive measures examined. These findings are comparable to other studies (using equivalent doses) that similarly found no effects of mecamylamine on measures of information processing, attention, psychomotor function (i.e. SRT) and declarative memory (Erskine et al., 2004; Newhouse et al., 1992, 1994). However, studies employing higher doses of mecamylamine (20 mg) have found significant effects on declarative memory, attention and psychomotor function (i.e. CRT) (Newhouse et al., 1992, 1994). These findings support animal studies, which show that nicotinic receptor antagonism can impair attention (Ruotsalainen et al., 2000), working memory (Elrod et al., 1988) and learning (Riekkinen et al., 1993).

Of interest is the finding that combined blockade of both muscarinic and nicotinic receptors impaired working memory (spatial and non-spatial), short-term memory (immediate word recall), declarative memory (immediate and delayed word recall and delayed recognition), sustained visual attention (digit vigilance) and psychomotor function (reaction time) and in addition induced larger impairments in working memory, sustained attention and psychomotor function, which were over and above those produced by scopolamine or mecamylamine alone. These findings suggest that both the muscarinic and nicotinic receptor systems interact functionally to have synergistic effects on specific cognitive processes. Interestingly the effects were selective to only working memory (spatial and numeric working speed), sustained attention (digit vigilance speed and accuracy) and psychomotor function (SRT and CRT). While the findings by Little et al. (1998), demonstrated larger impairments in declarative memory following combined muscarinic and nicotinic blockade, this was contrary to our findings in which synergistic effects were not found on any of the tasks of declarative memory (recognition and recall tasks). The findings of Little et al. (1998), however, should be interpreted with caution as this study was underpowered and the reported effects were only trends. In contrast, the observed power for the declarative memory tasks, were moderate in the present study (average of 0.54), suggesting that the lack of a synergistic effect on declarative memory was unlikely due to inadequate power. Hence, it is possible that the effects observed may be specific to certain cognitive processes and predominantly driven by effects on one
or related processes such as early information processing or attention. This is particularly relevant, as we have previously shown (with the same study sample) that simultaneous antagonism of both muscarinic and nicotinic receptors induced larger impairments in early information processing (in an inspection time task) than antagonism of either receptor alone (Erskine et al., 2004) and impairments in early information processing has been suggested to contribute to impairments in other cognitive domains including attention and memory (Sarter and Bruno, 1999). Our findings are also comparable with animal studies that have shown evidence for synergistic effects of muscarinic and nicotinic receptor antagonism on tests of attention and working memory (Leblond et al., 2002; Levin et al., 1990; Mirza and Stolerman, 2000). These findings are supported by further evidence for synergistic interactions between muscarinic and nicotinic receptor systems at the cellular level (i.e. receptor sensitization and regulation) (Brown and Galligan, 2003; Vige and Briley, 1988).

It is interesting to note that previous research using identical tasks as the current study have shown nicotine administration to improve performance on tasks of attention (Parrott et al., 1996; Wesnes and Parrott, 1992; Wesnes and Revell, 1984; Wesnes and Warburton, 1984), but not declarative memory (delayed word recall and recognition) (Wesnes and Parrott, 1992). It is thus important that in the present study, while attentional impairments were found to be greater in the MS condition than with the S alone condition, this was not the case for the declarative memory tasks. This suggests that while both nicotinic and muscarinic receptors play a role in attention, only muscarinic receptors may play a role in declarative memory. In addition, dementia with Lewy bodies (DLB) has been shown to have more pronounced nicotinic receptor loss than Alzheimer’s disease (Perry et al., 1995). This condition has been shown to have greater attentional and working-memory deficits on the CDR tasks used in this study than with Alzheimer’s patients, but smaller deficits to declarative memory (Ayre et al., 1997). Juxtaposing these findings with those of the present study suggests that the consequence of the extra nicotinic loss in DLB may be seen in the additional attentional disruption in the condition. A further differentiation between Alzheimer’s disease and DLB is that cognitive reaction time is not prolonged in Alzheimer’s disease compared to normal subjects, whereas there is a marked prolongation for cognitive reaction time in DLB (Ballard et al., 2002). One possible explanation for this prolongation in DLB is the extra nicotinic loss in the condition. A further finding from this study was that combined muscarinic and nicotinic blockade did not affect cognitive reaction time, strongly suggesting that its prolongation in DLB may be related to loss of nicotinic receptors.

Previous research in animals and humans have shown that manipulation of the cholinergic system can exert dissociable effects on attention and memory, including working memory (Baxter et al., 1995; Chappell et al., 1998; Park et al., 2000; Robbins, 2002; Wenk et al., 1994), suggesting that acetylcholine may mediate these processes independently (Everitt and Robbins, 1997). More recent studies suggest that while dissociable effects may be observed, these effects depend on task demands (Chudasama et al., 2004), with both working memory and attention modulated by the cholinergic system when attentional demands are high.

### Table 2. Visual Analogue Mood Scale (VAMS) ratings (mean ± S.E.M.)

<table>
<thead>
<tr>
<th>Condition</th>
<th>P Baseline</th>
<th>P Post</th>
<th>M Baseline</th>
<th>M Post</th>
<th>MS Baseline</th>
<th>MS Post</th>
<th>S Baseline</th>
<th>S Post</th>
<th>Condition × time interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sad</td>
<td>22.3 (4.3)</td>
<td>16.4 (3.7)</td>
<td>20.3 (4.3)</td>
<td>17.7 (3.4)</td>
<td>20.6 (3.1)</td>
<td>34.2 (5.0)</td>
<td>24.0 (3.7)</td>
<td>29.3 (3.9)</td>
<td>F(3, 33) = 7.76*</td>
</tr>
<tr>
<td>Withdrawn</td>
<td>24.5 (3.3)</td>
<td>16.1 (2.6)</td>
<td>21.0 (4.2)</td>
<td>21.2 (3.3)</td>
<td>24.8 (4.7)</td>
<td>36.1 (5.9)</td>
<td>21.2 (3.3)</td>
<td>32.4 (4.2)</td>
<td>F(3, 33) = 10.19*</td>
</tr>
<tr>
<td>Antagonistic</td>
<td>20.8 (4.1)</td>
<td>18.9 (3.6)</td>
<td>18.5 (3.6)</td>
<td>18.3 (3.5)</td>
<td>19.7 (3.3)</td>
<td>25.4 (5.4)</td>
<td>19.7 (3.5)</td>
<td>21.4 (3.7)</td>
<td>F(3, 33) = 1.31</td>
</tr>
<tr>
<td>Mentally slow</td>
<td>31.3 (4.1)</td>
<td>28.7 (4.7)</td>
<td>27.9 (3.8)</td>
<td>33.7 (3.5)</td>
<td>39.5 (5.9)</td>
<td>53.4 (6.1)</td>
<td>29.9 (3.1)</td>
<td>45.7 (4.5)</td>
<td>F(3, 33) = 2.78</td>
</tr>
<tr>
<td>Tense</td>
<td>19.3 (3.1)</td>
<td>16.7 (3.5)</td>
<td>16.6 (3.3)</td>
<td>20.4 (2.8)</td>
<td>23.9 (4.9)</td>
<td>31.5 (5.9)</td>
<td>22.4 (3.7)</td>
<td>29.2 (5.7)</td>
<td>F(3, 33) = 1.41</td>
</tr>
<tr>
<td>Drowsy</td>
<td>33.0 (5.3)</td>
<td>30.1 (5.4)</td>
<td>34.3 (5.4)</td>
<td>40.7 (6.1)</td>
<td>40.5 (6.3)</td>
<td>52.7 (7.2)</td>
<td>30.3 (4.1)</td>
<td>55.2 (5.1)</td>
<td>F(3, 33) = 5.01</td>
</tr>
<tr>
<td>Feeble</td>
<td>26.0 (4.3)</td>
<td>26.2 (4.1)</td>
<td>22.5 (3.8)</td>
<td>27.2 (4.4)</td>
<td>28.1 (5.7)</td>
<td>42.3 (6.8)</td>
<td>25.7 (2.7)</td>
<td>45.2 (4.8)</td>
<td>F(3, 33) = 7.53*</td>
</tr>
<tr>
<td>Bored</td>
<td>27.7 (3.8)</td>
<td>30.6 (4.9)</td>
<td>29.3 (4.6)</td>
<td>37.3 (5.2)</td>
<td>28.6 (4.3)</td>
<td>43.0 (4.9)</td>
<td>32.8 (3.9)</td>
<td>40.2 (3.7)</td>
<td>F(3, 33) = 1.10</td>
</tr>
</tbody>
</table>

P, Placebo; M, mecamylamine; MS, mecamylamine + scopolamine; S, scopolamine.

Larger scores indicate greater intensity of the mood state for mood dimensions. *p < 0.01.
(Chudasama et al., 2004). This finding is further supported by evidence showing diffuse release of acetylcholine throughout the cortex during periods of high attentional demand (Phillis and Chong, 1965). Furthermore, functional neuroimaging studies in humans, suggest that the cholinergic system may modulate processing during both working memory and attention (i.e. attentional aspects of the working-memory task) (Furey et al., 2000), particularly when there is anatomical overlap of the neural networks responsible for both processes (Awh et al., 1999; Awh and Jonides, 2001; LaBar et al., 1999). In the present study both working memory and sustained attention were impaired by simultaneous muscarinic and nicotinic antagonism (with evidence for some correlation between both processes). As discussed earlier, it is possible that the effects on attention and memory may be driven by a predominant effect on early information processing and attention and possibly through cholinergic modulation of signal-to-noise ratios in the cortex (Gu, 2002; Sahakian, 1987; Sato et al., 1987). Alternatively, the impairment may be related, in part to overlap between two complex tasks with regard to task requirements and distinct abilities such as sustained attention, working memory and psychomotor speed.

In summary, this study examined the effects of selective and simultaneous antagonism of muscarinic and nicotinic receptors on a wide range of cognitive processes. Supporting previous research, muscarinic receptor antagonism with scopolamine resulted in deficits in working memory, declarative memory, sustained visual attention and psychomotor speed, while nicotinic receptor antagonism with mecamylamine had no detrimental effects. Simultaneous antagonism of both muscarinic and nicotinic receptors induced larger impairments, particularly in working memory, sustained attention and psychomotor function suggesting that these receptors may interact functionally to have synergistic effects on selective cognitive processes. These findings together with previous findings in animals and humans highlight the importance of cholinergic integrity in maintaining memory over time as well as optimizing information processing and attention.

Acknowledgements

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Statement of Interest

None.

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