Available online at www.sciencedirect.com

SCIENCE DIRECT

Pharmacology, Biochemistry and Behavior 81 (2005) 575 – 584

PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR

www.elsevier.com/locate/pharmbiochembeh

Muscarinic and nicotinic receptor modulation of object and spatial *n*-back working memory in humans

Amity Green[a], Kathryn A. Ellis[b], Julia Ellis[a], Cali F. Bartholomeusz[b], Susan Ilic[b], Rodney J. Croft[b], K. Luan Phan[c], Pradeep J. Nathan[a,*]

[a]*Behavioural Neuroscience Laboratory, Department of Physiology, Monash Centre for Brain and Behaviour, PO Box 13F, Monash University, Melbourne, VIC 3800, Australia*

[b]*Brain Sciences Institute, Swinburne University of Technology, Melbourne, Australia*

[c]*Clinical Neuroscience and Psychopharmacology Research Unit, Department of Psychiatry, Biological Sciences Division and the Pritzker School of Medicine, The University of Chicago, Chicago, IL, USA*

Received 14 January 2005; received in revised form 21 April 2005; accepted 23 April 2005
Available online 3 June 2005

Abstract

Working memory impairments in the *n*-back task in schizophrenia have been linked to sustained deficiency in mesocortical dopamine function. More recently, abnormalities in the cholinergic system have also been documented in schizophrenia, with cortical reductions in both nicotinic and muscarinic receptors. While the cholinergic hypothesis of memory is well established, the role of cholinergic receptors in modulating *n*-back working memory is not known. We investigated the effects of selective and simultaneous muscarinic and nicotinic antagonism on spatial and object *n*-back working memory performance. The study was a double-blind, placebo-controlled repeated-measures design in which 12 healthy subjects were tested under four acute treatment conditions; placebo (P), mecamylamine (M), scopolamine (S) and mecamylamine + scopolamine (MS). Muscarinic antagonism with scopolamine significantly impaired both object and spatial *n*-back working memory, whereas nicotinic antagonism with mecamylamine had little effect. Simultaneous antagonism of both muscarinic and nicotinic receptors produced greater impairments in both object and spatial *n*-back working memory performance than muscarinic or nicotinic antagonism alone. These results suggest that: (1) both muscarinic and nicotinic receptors may functionally interact to synergistically modulate *n*-back working memory, and (2) that *n*-back working memory impairments in schizophrenia may in part be due to reductions in both muscarinic and nicotinic receptors.

Keywords: Cholinergic system; Working memory; Muscarinic receptors; Nicotinic receptors *n*-back; Schizophrenia; Cognitions; Copolamine; Mecamylamine

1. Introduction

Impairments in higher order cognitive processes are one of the most debilitating symptom dimensions of schizophrenia and thought to be a good predictor of poor clinical outcome (Green, 1996; Liddle, 2000). Working memory (i.e. processes involved in maintenance and manipulation of information over a brief period to time to guide task appropriate behaviour) is one construct that has been shown to be impaired in patients with schizophrenia (Park and Holzman, 1992; Goldman-Rakic, 1994; Fleming et al., 1995; Keefe et al., 1997; Conklin et al., 2000). Among the working memory tasks, the *n*-back paradigm has been extensively used to evaluate working memory function in schizophrenia, and studies have consistently found deficits in *n*-back working memory performance in patients with schizophrenia (Carter et al., 1998; Goldberg et al., 2003; Callicott et al., 2000; Abi-Dargham et al., 2002).

Functional neuroimaging studies have demonstrated the engagement of the dorsolateral prefrontal cortex (DLPFC)

* Corresponding author. Tel.: +61 11 613 9214 5216; fax: +61 11 613 9214 5525.
E-mail addresses: pnathan@bsi.swin.edu.au, Pradeep.Nathan@med.monash.edu.au (P.J. Nathan).

0091-3057/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2005.04.010

in the execution of the *n*-back and other working memory tasks (Cohen et al., 1994, 1997; Braver et al., 1997; D'Esposito et al., 1998), and patients with schizophrenia have been shown to have abnormal working memory related activation in the DLPFC (Carter et al., 1998; Barch et al., 2001; Perlstein et al., 2001; Honey et al., 2002). Neurochemical studies in animals and humans have demonstrated a critical role for mesocortical dopamine and D_1 receptors in processes relevant to working memory (Sawaguchi and Goldman-Rakic, 1991, 1994; Arnsten et al., 1994; Arnsten and Goldman-Rakic, 1998; Goldman-Rakic et al., 2000, 2004; Ellis and Nathan, 2001). Consistent with this, alternations in D_1 receptor availability in the DLPFC (i.e. upregulation of D_1 receptors) has been found in patients with schizophrenia (Abi-Dargham et al., 2002) and this increase was shown to be a strong predictor of poorer performance on an *n*-back working memory task (Abi-Dargham et al., 2002). Further, studies investigating functional polymorphisms of the catechol-*O*-methyltransferase (COMT) gene have shown that in both healthy subjects and patients with schizophrenia, those homozygous for the low enzymatic activity *met* allele (greater prefrontal dopamine availability) perform better on *n*-back working memory task than do those subjects with the high enzymatic activity *val* allele (lower prefrontal dopamine availability) (Goldberg et al., 2003).

While a deficiency in mesocortical dopamine has been linked with impairments in *n*-back working memory performance in both normal subjects and patients with schizophrenia, it is likely that other systems including the cholinergic system may also be involved. With neuropathological evidence linking a reduction in cholinergic function to the cognitive decline seen in a number of disorders such as Alzheimer's disease (Perry et al., 1978), as well as pharmacological evidence that anticholinergic drugs consistently produce impairments in learning and memory (Rusted and Warburton, 1988; Broks et al., 1988; Wesnes et al., 1988; Newhouse et al., 1992, 1994; Robbins et al., 1997; Potter et al., 2000; Edginton and Rusted, 2003; Ellis et al., 2005), the cholinergic basis of memory dysfunction has been well established (Bartus et al., 1982). In animals and healthy humans, both muscarinic and nicotinic antagonists have been shown to induce impairments in a number of cognitive domains including working memory (Levin et al., 1993, 1997; Rusted and Warburton, 1988; Wesnes et al., 1988; Rusted et al., 1991; Maviel and Durkin, 2003; Ellis et al., 2005).

Although the link between the cholinergic system and working memory is established, the role of this system in modulating *n*-back working memory is not known. Furthermore, very little is known about the functional interactions between muscarinic and nicotinic receptors, including how they may interact synergistically to modulate selective cognitive processes. 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 upregulation) and at a functional level on various cognitive processes (Vige and Briley, 1988; Levin et al., 1990; Riekkinen et al., 1993; Mirza and Stolerman, 2000; Leblond et al., 2002; Brown and Galligan, 2003). Further, we have recently reported in humans that similar functional synergistic interactions between muscarinic and nicotinic receptors in modulating early information processing (Erskine et al., 2004) sustained attention and working memory (Ellis et al., 2005). It is unknown if *n*-back working memory performance can similarly be synergistically modulated by both receptor systems.

Hence the aim of the present study was to examine the role of the cholinergic muscarinic and nicotinic receptors in modulating spatial and object *n*-back working memory in healthy human subjects. Based on previous animal and human working memory studies, we hypothesised that selective nicotinic and muscarinic receptor antagonism would produce impairments in performance on both object and spatial working memory. Furthermore, we hypothesised that simultaneous antagonism of both nicotinic and muscarinic receptors would impair performance on the *n*-back tasks, over and above the impairments produced by antagonism of either receptor alone.

2. Methods

2.1. Participants

Twelve healthy adult volunteers (4 female, 10 male) aged 19–30 years (M=23.3, S.D.=2.8) with a mean weight of 67.6 kg were recruited through advertisements at local universities. All subjects were university educated and proficient in English. Participants were required to pass a brief semi-structured physical and psychiatric examination and were included in the study if they were non-smokers, not currently on any medication including the oral contraceptive pill, and had no history of psychiatric or medical illness, nor history of abuse of alcohol or psychoactive substances. Participants gave written informed consent prior to taking part in the study, which was approved by the Swinburne University Human Research Ethics Committee.

2.2. Study design

The study employed a double-blind, placebo-controlled, repeated-measures design. Each subject was tested under four acute treatment conditions; placebo (P); mecamylamine 15 mg single oral dose (s.o.d.) (M); scopolamine 0.4 mg intramuscular injection (i.m.) (S); and combined mecamylamine (15 mg)+scopolamine (0.4 mg) (MS). The order of drug treatments was randomised using a Latin square design and treatment conditions were counterbalanced, and were

separated by a 7-day washout period. 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 (Varanda et al., 1985; Brown, 1992; 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 (Wesnes et al., 1988; Ebert et al., 1998), and (2) minimizing the chance of sedation interfering with task performance (especially in the MS condition).

2.3. Procedure

In order to familiarise themselves with the equipment and tasks, and to minimise learning and practice effects, participants were required to attend a practice session prior to the first day of testing in which they completed the 1- and 2-back versions of both the object and spatial *n*-back tasks twice each. All participants attended four morning testing sessions at the Neuropsychopharmacology Laboratory at the Brain Sciences Institute (BSI), Swinburne University. Participants were instructed to have a light breakfast and not consume any alcohol or caffeine for 24 h prior to testing. Female participants were tested only in the follicular phase of the menstrual cycle (days 1–12) in order to minimise the effects of hormonal fluctuations on mood and cognition. On arrival, participants completed a mood questionnaire and baseline (pre-treatment) cognitive testing (working memory tasks), followed immediately by the administration of an oral dose of either mecamylamine or placebo tablets. One hour post-mecamylamine or placebo administration, participants were then given an intramuscular injection of either scopolamine or saline. Two hours post-scopolamine or placebo injection, the mood questionnaire and working memory tasks were re-administered (post-treatment testing). Post-treatment testing was conducted 2 h post-scopolamine and 3 h post-mecamylamine in order to coincide with the drugs' peak pharmacodynamic and pharmacokinetic effects (Safer and Allen, 1971; Young et al., 2001; Ellis et al., 2005). In the period between baseline and post-treatment testing, participants remained in the testing room and carried out non-strenuous activity such as reading or watching videos to keep themselves occupied.

2.4. Working memory n-back tasks

The working memory tasks used in the current study *n*-back tasks were variations of the *n*-back task paradigm, which measures a representative case of working memory and imposes a continuous, parametrically variable load while keeping all other task demands constant (Bartholomeusz et al., 2003; Braver et al., 1997; Cohen et al., 1994, 1997). The *n*-back tasks were developed at the Brain Sciences Institute using Pipscript software, which provides millisecond accuracy in stimulus presentation and response recording (Brain Sciences Institute, Victoria, Australia). Both *n*-back tasks were presented via computer displayed on a high-resolution VGA colour monitor, and all responses were made using an external button box (yes/no). The button box was handheld with thumbs resting upon the respective button. Participants were instructed to respond "as quickly as possible but with accuracy as their priority" on all tasks. Participants were seated approximately 1 m from the computer monitor in a dimly lit room (consistent between sessions) and were requested to sit upright throughout the task.

The spatial and object *n*-back were matched in all task parameters and differed only in stimulus type, with the object task displaying ambiguous objects (i.e. irregular polygons which minimise verbal strategies in encoding or rehearsal) within the centre of the screen, and the spatial task displaying white dots in one of 60 spatial locations on the screen. Each stimulus was presented for 500 ms, with inter-stimulus intervals of 3000 ms. Both tasks comprised two memory load levels (1-back and 2-back). For each memory load level, 80 responses were elicited; 40% response pairs were "matches" of the relevant *n*-back, 10% were incorrect matches (i.e. 2-back in a 1-back task), and 50% were non-matches. The reference tasks involved an equivalent task presentation for both the spatial and object tasks (50% of dots/objects were an *n*-back match, distributed amongst 1- and 2-back), and involved subjects alternating responses between the left and right response buttons. Order of *n*-back task administration was quasi-random.

For the spatial *n*-back task, subjects were required to fixate on the white cross in the centre of the screen, and indicate whether each dot was in the same location as the dot "*n*-back" (either 1-back or 2-back, depending on task instructions) by pressing the appropriate button (yes/no) on the handheld button box. For the object *n*-back task, subjects were required to indicate whether each object was identical to the object "*n*-back" (either 1-back or 2-back, depending on task instructions) by pressing the appropriate button (yes/no) on the handheld button box.

2.5. Critical flicker fusion (CFF) task

The CFF test was used as a measure of sedation and drug-induced drowsiness (Hindmarch and Parrott, 1977). During this task, subjects assumed a seated position, viewing the critical flicker frequency apparatus and holding the response button box with their hands. This task had two sub-components. The first subtest determined

the point at which the lights were changing from a flicker to a steady light source, with flickering frequencies ascending from 25 Hz to 65 Hz. The second subtest assessed the point at which the steady light became a flicker, and in contrast to the first subtest, flicker frequencies were in descending order (descending from 65 Hz to 25 Hz). Higher thresholds (measured in Hz/number of flashes per second) were indicative of better performance discrimination.

2.6. Statistical analyses

2.6.1. Task validity analysis

In order to examine load effects of the spatial and object tasks repeated-measures analyses of variance (ANOVAs) were conducted on data from the placebo condition, with load level (1-back, 2-back) as the independent variable and accuracy and reaction time as the dependent variables.

2.6.2. Spatial and object working memory

Spatial and object *n*-back data were analysed using a drug condition (P, M, MS, S) by time (baseline, post-drug) repeated-measures ANOVA. The 1-back and 2-back tasks were analysed separately with accuracy and reaction time scores as the dependent variables. Planned comparisons were conducted on all significant interactions to investigate the effects of each drug condition compared to placebo and to investigate significant differences between each of the drug conditions. Planned comparisons were determined a priori and α-adjustments were not employed (Tabachnick and Fidell, 1989).

2.6.3. Drug-induced sedation (CFF scores)

Effects of each drug on sedation were analysed using repeated-measures ANOVA for drug (P, M, MS, S) by time (baseline, post-drug) with critical flicker fusion performance discrimination scores as the dependent variable.

3. Results

3.1. Task validity

Participants performed more poorly on the 2-back compared to 1-back versions of the task for both object working memory [accuracy: $F(1,11)=12.47$, $p=0.005$; reaction time: $F(1,11)=3.76$, $p=0.079$], and spatial working memory [accuracy: $F(1,11)=18.84$, $p=0.001$; reaction time: $F(1,11)=7.68$, $p=0.018$], suggesting that for both of the *n*-back tasks used in the present study, 2-back load was more difficult than 1-back load (Table 1).

3.2. Object working memory

3.2.1. 1-Back

A significant drug by time interaction for both accuracy [$F(3,33)=14.72$, $p<001$] and reaction time [$F(3,33)=3.40$, $p<0.05$] was found for object 1-back working memory. Planned contrasts revealed that in the MS condition, subjects made significantly more errors [$F(1,11)=17.23$, $p<0.01$], and reaction times were significantly longer [$F(1,11)=5.36$, $p<0.05$] compared to P. No significant difference was found for the M condition [$F(1,11)=60$, $p=0.46$] or S condition [$F(1,11)=0.28$, $p=0.61$] compared to placebo. Furthermore, in the MS condition, subjects made significantly more errors compared to the M condition [$F(1,11)=19.65$, $p<0.01$], and made more errors [$F(1,11)=19.04$, $p<0.01$] and showed longer response latencies [$F(1,11)=11.15$, $p<0.01$] compared to the S condition (Fig. 1).

Table 1
Means and standard errors (mean±S.E.M.) for object and spatial tasks at baseline and post-drug administration

Measure	P		M		MS		S	
	Baseline	Post-drug	Baseline	Post-drug	Baseline	Post-drug	Baseline	Post-drug
Object 1-back								
Accuracy (%)	89.6 (1.4)	88.6 (1.1)	90.4 (1.5)	88.7 (1.5)	91.1 (1.1)	73.0 (5.4)	88.5 (1.8)	87.6 (1.9)
Reaction time (ms)	715.2 (56.4)	666.3 (41.9)	727.6 (52.4)	722.4 (44.4)	698.3 (48.2)	793.0 (48.9)	743.7 (37.6)	727.1 (35.1)
Object 2-back								
Accuracy (%)	82.9 (2.1)	80.0 (2.6)	85 (2.3)	77.1 (3.7)	84.7 (2.2)	62.5 (4.6)	81.5 (1.9)	67.4 (4.4)
Reaction time (ms)	809.0 (49.4)	792.1 (57.1)	851.1 (61.8)	802.4 (58.8)	832.3 (53.3)	923.8 (54.0)	826.9 (46.5)	810.3 (51.1)
Spatial 1-back								
Accuracy (%)	94.8 (1.1)	93.8 (1.5)	94.2 (1.2)	92.8 (1.7)	92.2 (2.0)	74.9 (5.0)	96.1 (.8)	87.5 (2.9)
Reaction time (ms)	40.8	610.2 (45.4)	619.6 (41.8)	597.9 (34.7)	621.4 (47.0)	763.4 (42.7)	589.6 (32.4)	638.3 (35.1)
Spatial 2-back								
Accuracy (%)	87.6 (1.7)	87 (1.7)	88.9 (1.7)	87.2 (2.0)	87.4 (1.6)	65.1 (5.4)	81.1 (2.0)	76.7 (3.3)
Reaction time (ms)	698.7 (48.3)	667.8 (40.9)	677.3 (46.1)	665.4 (52.2)	703.8 (57.3)	790.9 (47.9)	660.6 (36.8)	662.1 (42.7)

P=placebo, M=mecamylamine, MS=mecamylamine+scopolamine, S=scopolamine.

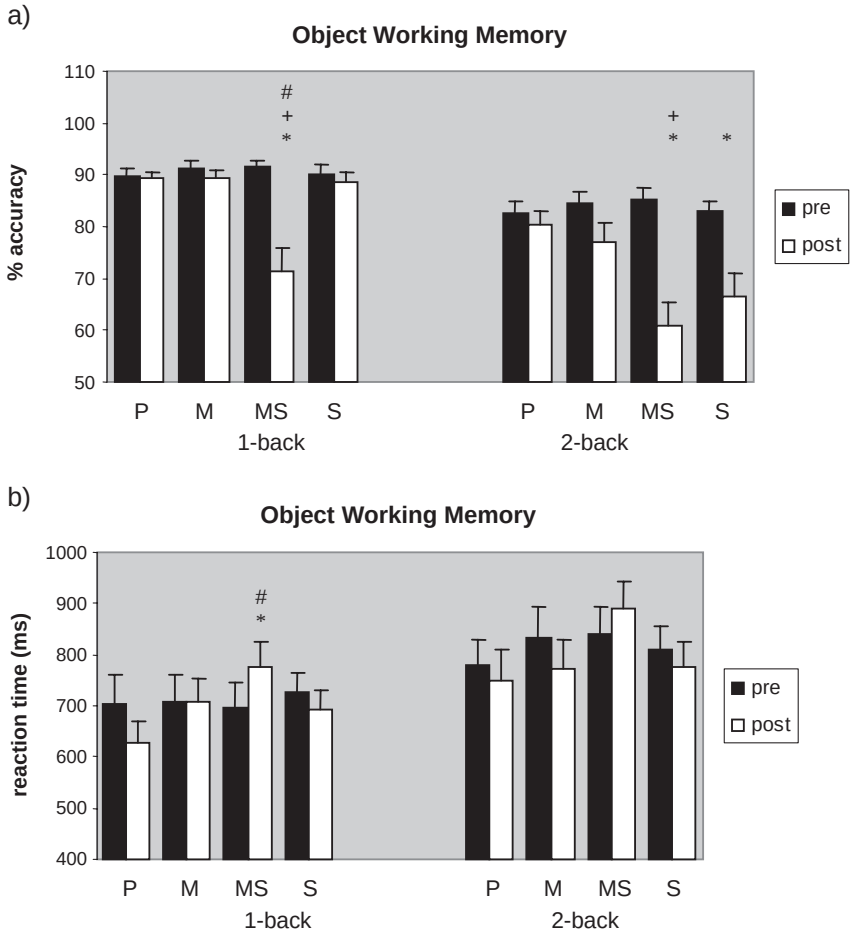

Fig. 1. (a) Accuracy scores for object working memory at baseline and post-treatment (mean ± S.E.M.); (b) reaction time scores for object working memory at baseline and post-treatment (mean ± S.E.M.). P = placebo, M = mecamylamine, MS = mecamylamine + scopolamine, S = scopolamine. * Indicates significant difference between the MS condition and P condition, and a significant difference between the S condition and P condition ($p < 0.05$). # Indicates significant difference between the MS and S conditions ($p < 0.05$). + Indicates significant difference between the MS and M conditions ($p < 0.05$).

3.2.2. 2-Back

A significant drug by time interaction for accuracy [$F(3,33) = 9.27$, $p < 0.001$], but not reaction time [$F(3,33) = 1.76$, $p = 0.17$], was found for object 2-back working memory. Planned comparisons revealed that participants made significantly more errors in both the MS condition [$F(1,11) = 18.64$, $p < 0.01$] and S condition [$F(1,11) = 15.62$ $p < 0.01$] compared to P. There was no significant difference between accuracy scores in the M condition compared to the P condition [$F(1,11) = 1.76$, $p = 0.21$). Furthermore, in the MS condition, subjects made significantly more errors compared to the M condition [$F(1,11) = 8.54$, $p < 0.05$]. There was no significant difference between the MS condition and S conditions [$F(1,11) = 2.73$, $p = 0.13$] (Fig. 1).

3.2.3. Effects of working memory load

A significant interaction between drug, time and load was found for accuracy in the object task [$F(3,33) = 3.04$, $p < 0.05$] and planned comparisons showed that the S condition differentially affected 1- and 2-back object working memory performance compared to P [$F(1,11) = 18.99$, $p < 0.01$], with greater impairments in the 2-back condition.

3.3. Spatial working memory

3.3.1. 1-Back

Significant drug by time interactions for both accuracy [$F(3,33) = 6.36$, $p = 0.01$] and reaction time [$F(3,33) = 13.01$, $p < 0.001$] were found for spatial 1-back working memory. Planned comparisons revealed that subjects made significantly more errors in both the S condition [$F(1,11) = 8.03$, $p < 0.05$] and MS condition [$F(1,11) = 30.52$, $p < 0.001$] compared to P. Similarly, subjects showed longer response times in both the S condition [$F(1,11) = 8.01$, $p < 0.05$] and MS condition [$F(1,11) = 9.71$, $p = 0.01$] compared to the P condition. There was no significant difference between performance on the spatial 1-back task in the M condition compared to P [$F(1,11) = 0.04$, $p = 0.84$]. Furthermore, in the MS condition, subjects made significantly more errors [$F(1,11) = 19.65$, $p = 0.001$], and showed significantly longer response latencies [($F(1,11) = 47.91$, $p < 0.001$] compared to the M condition. Response latencies were

also significantly longer in the MS condition compared to the S condition [$F(1,11)=10.38$, $p<0.01$] (Fig. 2).

3.3.2. 2-Back

A significant drug by time interaction for accuracy [$F(3,33)=12.64$, $p<0.001$], but not reaction time [$F(3,33)=0.89$, $p=0.42$] was found for spatial 2-back working memory. Planned comparisons revealed that subjects made significantly more errors in both the S condition [$F(1,11)=15.62$, $p<0.01$] and MS condition [$F(1,11)=18.64$, $p=0.001$] compared to P. There was no significant difference in spatial 2-back performance in the M condition compared to P [$F(1,11)=1.12$, $p=0.31$]. Furthermore in the MS condition subjects made significantly more errors compared to both the M condition [$F(1,11)=7.27$, $p<0.05$] and S condition [$F(1,11)=4.95$, $p<0.05$] (Fig. 2).

3.3.3. Effects of working memory load

No significant interaction between drug, time and load was found for spatial *n*-back accuracy [$F(3,33)=0.65$, $p=0.55$] or reaction time [$F(3,33)=1.11$, $p=0.36$].

3.4. Critical flicker fusion

There was no significant drug condition by time interaction for the CFF task [$F(3,33)=0.53$, $p>0.5$].

4. Discussion

The current study is the first to examine the effects of muscarinic and nicotinic antagonism on spatial and object *n*-back working memory performance. Nicotinic antagonism with mecamylamine did not significantly impair *n*-back performance for both spatial and object working memory. As hypothesised, selective muscarinic antagonism with scopolamine significantly impaired performance on spatial (1- and 2-back) and object (2-back) working memory. Interestingly, simultaneous antagonism of both muscarinic and nicotinic receptors impaired both spatial and object working memory, more than the impairments induced by muscarinic or nicotinic antagonism alone.

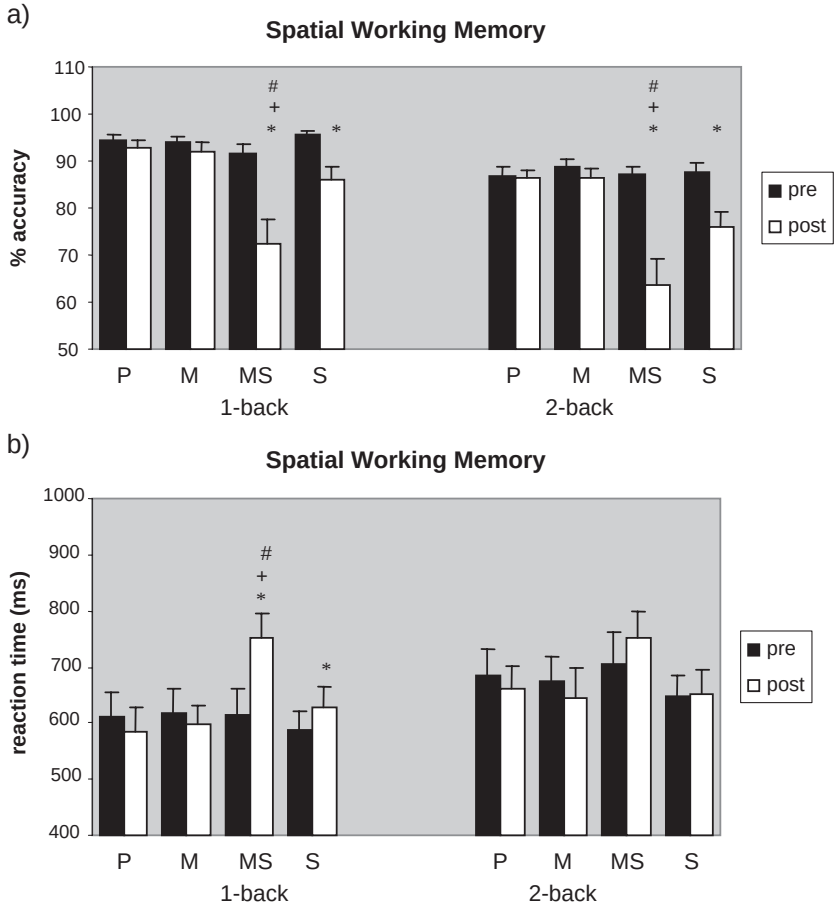

Fig. 2. (a) Accuracy scores for spatial working memory at baseline and post-treatment (mean ± S.E.M.); (b) reaction time scores for spatial working memory at baseline and post-treatment (mean ± S.E.M.). P = placebo, M = mecamylamine, MS = mecamylamine + scopolamine, S = scopolamine. * Indicates significant difference between the MS conditions and P conditions, and the S condition and P condition ($p<0.05$). # Indicates significant difference between the MS and S conditions ($p<0.05$). + Indicates significant difference between the MS and M conditions ($p<0.05$).

The mecamylamine findings were consistent with a number of previous studies that have similarly observed no detrimental effects of low doses of mecamylamine on cognitive function. Previously, mecamylamine (15 mg) has been shown to have no significant effects on performance on a number of cognitive tasks including spatial and non-spatial working memory, declarative memory, and sustained attention (Ellis et al., 2005), as well as early information processing (Erskine et al., 2004). While Little et al. (1998) found that the same dose of mecamylamine produced impairments in some aspects of explicit (list learning) and semantic memory, as well as a trend towards increased preservations in a task of lexical search and retrieval, other studies have only found a significant effect of mecamylamine on cognitive performance at higher doses (i.e. 20 mg) (Newhouse et al., 1992, 1994; Thompson et al., 2000). These findings suggest that there is likely to be a dose–response relationship between nicotinic antagonism and cognitive impairment, and that the dose used in the current study may not have been sufficient to produce detrimental effects specifically on *n*-back working memory performance.

Consistent with the established role of muscarinic receptors as an important modulator of both spatial and non-spatial human working memory processes (Mewaldt and Ghoneim, 1979; Rasmusson and Dudar, 1979; Rusted and Warburton, 1988; Wesnes et al., 1988; Robbins et al., 1997; Ellis et al., 2005), in the current study, muscarinic antagonism with scopolamine induced impairments in both spatial and object *n*-back working memory performance. While our findings indicated that both the 1- and 2-back conditions were impaired in the spatial working memory task, while only the 2-back condition was impaired in the object working memory task, overall there is no significant modality specific effects of cholinergic modulation of working memory. These results are comparable to those of Rusted and Warburton (1988) who similarly found that scopolamine induced decrements in performance on tests of visuospatial working memory and non-spatial memory (recognition memory for abstract shapes). These findings further suggest that the cholinergic modulation of working memory is at the level of the central executive mechanisms rather than the subsystems which it controls. Interestingly, these results can be contrasted to findings of dopaminergic modulation of working memory, in which modality specific effects have been noted in humans. Dopamine agonists such as the selective D_2 agonist bromocriptine, and the D_1/D_2 agonist pergolide, appear to facilitate spatial, but not object working memory (Luciana et al., 1998; Muller et al., 1998; Bartholomeusz et al., 2003). Fewer studies have investigated the effects of dopamine antagonism on working memory in healthy humans; however, findings again suggest a role for dopamine in spatial working memory but not memory for non-spatial (i.e. object) cues (Luciana et al., 1998; Mehta et al., 1999).

Despite the finding that the selective antagonism of nicotinic receptors by mecamylamine at the dose used in this study had no detrimental effects on *n*-back working memory performance, simultaneous nicotinic and muscarinic antagonism induced greater deficits in both spatial and object working memory than the effects of either receptor antagonism alone. These findings support our previous studies that have similarly demonstrated synergistic effects of muscarinic and nicotinic antagonism on range of cognitive processes, including early information processing, sustained attention and working memory (Erskine et al., 2004; Ellis et al., 2005). We previously suggested that the synergistic effects of muscarinic and nicotinic antagonism may be specific to certain cognitive domains such as attention and working memory (Ellis et al., 2005) and it is of interest that in this study, using a different (and more challenging) working memory task, we similarly observed impairments in working memory. However, it is possible that this effect may be driven by modulation of one or related processes such as early information processing or attention. Indeed we have previously shown that simultaneous antagonism of both muscarinic and nicotinic receptors induced larger impairments in early information processing (Erskine et al., 2004) than antagonism of either receptor alone, and impairments in early information processing have been suggested to contribute to impairments in other cognitive domains including memory (Sarter and Bruno, 1999). The importance of both the nicotinic and muscarinic cholinergic receptors in working memory modulation is also supported from findings in animals, where similar impairments in tasks of working memory have been observed with simultaneous antagonism of both receptors (Levin et al., 1990, 1997). Such functional interactions are supported by further evidence for synergistic interactions between muscarinic and nicotinic receptor systems at the cellular level (i.e. receptor sensitization and regulation) (Vige and Briley, 1988; Brown and Galligan, 2003).

Kopelman and Corn (1988) have previously demonstrated an interaction between muscarinic antagonism and processing load whereby simple tasks of working memory such as span tests were not significantly impaired, while short-term memory tasks with heavier processing loads demonstrated larger significant impairments. Similarly, studies investigating nicotinic antagonism and working memory suggest that the more demanding tasks may be more sensitive to nicotinic modulation (Granon et al., 1995). Such effects may be related to an important modulatory influence of cortical function by the cholinergic system, particularly during increased attentional demand (Dalley et al., 2004). In our current study, muscarinic antagonism produced a greater impairment in the more difficult (2-back) compared to 1-back working memory load but only in the object working memory task. Interestingly antagonism of both muscarinic and nicotinic receptors equally impaired both 2-back and 1-back work-

ing memory loads. It is possible that simultaneous antagonism (through synergistic interactions) may have induced maximum possible impairments in the 1-back condition (i.e. a floor effect with regard to cholinergic influence on working memory performance), such that working memory load (i.e. 2-back vs. 1-back) in this condition was not influence by attentional demand.

One important factor that may have influenced our findings is drug-induced sedation. Both scopolamine and mecamylamine have been shown to produce sedative effects, and it is therefore possible that the observed impairments in working memory performance may have been secondary to changes in drowsiness or drug-induced sedation rather than a direct effect on working memory, particularly in the combined treatment condition. However, CFF, a well-established measure of drug-induced sedation (Hindmarch and Parrott, 1977), was found not to be affected by any of the drug conditions. These results indicate that the impairments in *n*-back performance in the scopolamine and combined scopolamine/mecamylamine conditions are unlikely to be the result of sedation following drug administration. One possible limitation of the study is the relatively small sample size. However, the study was adequately powered and the findings were consistent with our previous study (with an identical sample size) on the cholinergic modulation of working memory (using a working memory recognition task) (Ellis et al., 2005). Further the selective effects of muscarinic and nicotinic antagonism on working memory observed in this study are consistent with findings reported in the literature (discussed earlier).

The findings of the current study have relevance to the *n*-back working memory impairments observed in schizophrenia. For example postmortem and brain imaging findings in patients with schizophrenia have demonstrated decreased muscarinic (Dean et al., 1996; Crook et al., 2000, 2001; Collette and Van der Linden, 2002; Raedler et al., 2003; Katerina et al., 2004) and nicotinic receptors (Freedman et al., 1995; Guan et al., 1999; Breese et al., 2000) in a number of brain areas including the cortex. Interestingly, cortical regions where reductions in muscarinic and nicotinic receptors have been noted (i.e. prefrontal cortex) are also regions activated during *n*-back working memory performance (Jansma et al., 2000; Zurowski et al., 2002; Cohen et al., 1997; Jonides et al., 1997). Our findings suggest that the working memory impairments (particularly *n*-back working memory) observed in patients with schizophrenia may in part be related to underlying impairments in nicotinic and muscarinic receptor function. This is supported by previous studies showing improvements in *n*-back working memory following enhancement of cholinergic function with nicotine and the cholinesterase inhibitor, physostigmine (George et al., 2002; Kirrane et al., 2001; Kumari et al., 2003; Jacobsen et al., 2004), and evidence that antipsychotic drugs that have a more profound effect in increasing cortical acetylcholine (Ichikawa et al., 2002; Shirazi-Southall et al., 2002) may have more positive effects on cognitive function, including working memory (Meltzer and McGurk, 1999).

In summary, our findings suggest that spatial and non-spatial (object) *n*-back working memory performance is dependent upon the integrity of both the nicotinic and muscarinic cholinergic receptors, with evidence that both muscarinic and nicotinic receptors may functionally interact to have synergistic effects on performance. These findings indicate that future therapeutic strategies targeting both muscarinic and nicotinic receptors may be another approach to improving cognitive function including working memory in disorders such as schizophrenia.

Acknowledgements

The study was supported by the National Health and Medical Research Council (NHMRC) of Australia (Grant 345709) and Alzheimer's Australia. The authors would like to thank Layton Bioscience for providing the mecamylamine tablets.

References

Abi-Dargham A, Mawlawi O, Lombardo I, Gil R, Martinez D, Huang Y, et al. Prefrontal dopamine D-1 receptors and working memory in schizophrenia. J Neurosci 2002;22:3708–19.

Arnsten AF, Goldman-Rakic PS. Noise stress impairs prefrontal cortical cognitive function in monkeys: evidence for a hyperdopaminergic mechanism. Arch Gen Psychiatry 1998;55:362–8.

Arnsten AF, Cai JX, Murphy BL, Goldman-Rakic PS. Dopamine D1 receptor mechanisms in the cognitive performance of young adult and aged monkeys. Psychopharmacology 1994;116:143–51.

Barch DM, Carter CS, Braver TS, Sabb FW, MacDonald A, Noll DC, et al. Selective deficits in prefrontal cortex function in medication-naive patients with schizophrenia. Arch Gen Psychiatry 2001;58:280–8.

Bartholomeusz CF, Box G, Van Rooy C, Nathan PJ. The modulatory effects of dopamine D-1 and D-2 receptor function on object working memory in humans. J Psychopharmacol 2003;17:9–15.

Bartus RT, Dean RL, Beer B, Lippa AS. The cholinergic hypothesis of geriatric memory dysfunction. Science 1982;217:408–14.

Braver TS, Cohen JD, Nystrom LE, Jonides J, Smith EE, Noll DC. A parametric study of prefrontal cortex involvement in human working memory. Neuroimage 1997;5:49–62.

Breese CR, Lee MJ, Adams CE, Sullivan B, Logel J, Gillen KM, et al. Abnormal regulation of high affinity nicotinic receptors in subjects with schizophrenia. Neuropsychopharmacology 2000;23:351–64.

Broks P, Preston GC, Traub M, Poppleton P, Ward C, Stahl M. Modelling dementia: effects of scopolamine on memory and attention. Neuropsychologia 1988;26:685–700.

Brown JH. Atropine, scopolamine, and related antimuscarinic drugs. In: Goodman Gilman A., Rall TW, Nies AS, Taylor P, editors. The pharmacological basis of therapeutics. Singapore: McGraw-Hill International Editions; 1992. p. 150–66.

Brown EN, Galligan JJ. Muscarinic receptors couple to modulation of nicotinic ACh receptor desensitization in myenteric neurons. Am J Physiol Gastrointest Liver Physiol 2003;285:G37–44.

Callicott JH, Bertolino A, Mattay VS, Langheim FJ, Duyn J, Coppola R, et al. Physiological dysfunction of the dorsolateral prefrontal cortex in schizophrenia revisited. Cereb Cortex 2000;10:1078–92.

Carter CS, Perlstein W, Ganguli R, Brar J, Mintun M, Cohen JD. Functional hypofrontality and working memory dysfunction in schizophrenia. Am J Psychiatry 1998;155:1285–7.

Cohen JD, Forman S, Braver T, Casey B, Servan-Schreiber D, Noll D. Activation of the prefrontal cortex in a non-spatial working memory task with functional MRI. Hum Brain Map 1994;1:293–304.

Cohen JD, Perlstein WM, Braver TS, Nystrom LE, Noll DC, Jonides J, et al. Temporal dynamics of brain activation during a working memory task. Nature 1997;386:604–8.

Collette F, Van der Linden M. Brain imaging of the central executive component of working memory. Neurosci Biobehav Rev 2002;26: 105–25.

Conklin HM, Curtis CE, Katsanis J, Iocono WG. Verbal working memory impairment in schizophrenia patients and their first-degree relatives: evidence from the digit span task. Am J Psychiatry 2000;157(2):275–7.

Crook JM, Tomaskovic-Crook E, Copolov DL, Dean B. Decreased muscarinic receptor binding in subjects with schizophrenia: a study of the human hippocampal formation. Biol Psychiatry 2000;48:381–8.

Crook JM, Tomaskovic-Crook E, Copolov DL, Dean B. Low muscarinic receptor binding in prefrontal cortex from subjects with schizophrenia: a study of Brodmann's areas 8, 9, 10, and 46 and the effects of neuroleptic drug treatment. Am J Psychiatry 2001;158:918–25.

Dalley JW, Theobald DE, Bouger P, Chudasama Y, Cardinal RN, Robbins TW. Cortical cholinergic function and deficits in visual attentional performance in rats following 192 IgG-saporin-induced lesions of the medial prefrontal cortex. Cereb Cortex 2004;14:922–32.

Dean B, Crook JM, Opeskin K, Hill C, Keks N, Copolov DL. The density of muscarinic M1 receptors is decreased in the caudate–putamen of subjects with schizophrenia. Mol Psychiatry 1996;1:54–8.

D'Esposito M, Ballard D, Aguirre GK, Zarahn E. Human prefrontal cortex is not specific for working memory: a functional MRI study. Neuroimage 1998;8(3):274–82.

Ebert U, Oertel R, Wesnes KA, Kirch W. Effects of physostigmine on scopolamine-induced changes in quantitative electroencephalogram and cognitive performance. Hum Psychopharmacol 1998;13:199–210.

Edginton T, Rusted JM. Separate and combined effects of scopolamine and nicotine on retrieval-induced forgetting. Psychopharmacology 2003;170:351–7.

Ellis KA, Nathan PJ. The pharmacology of human working memory. Int J Neuropsychopharmacol 2001;4:299–313.

Ellis JR, Ellis KA, Bartholomeusz CF, Harrison BJ, Wesnes KA, Erskine FF, et al. Muscarinic and nicotinic receptors synergistically modulate working memory and attention in humans. Int J Neuropsychopharmacol 2005;9(May):1–15 [Epub ahead of print].

Erskine FF, Ellis JR, Ellis KA, Stuber E, Hogan K, Miller V, et al. Evidence for synergistic modulation of early information processing by nicotinic and muscarinic receptors in humans. Hum Psychopharmacol 2004; 19:503–9.

Fleming K, Goldberg TE, Gold JM, Weinberger DR. Verbal working memory dysfunction in schizophrenia: use of a Brown–Peterson paradigm. Psychiatry Res 1995;56(2):155–61.

Freedman R, Hall M, Adler LE, Leonard S. Evidence in postmortem brain tissue for decreased numbers of hippocampal nicotinic receptors in schizophrenia. Biol Psychiatry 1995;38:22–33.

George TP, Vessicchio JC, Termine A, Sahady DM, Head CA, Pepper WT, et al. Effects of smoking abstinence on visuospatial working memory function in schizophrenia. Neuropsychopharmacology 2002;26:75–85.

Goldberg TE, Egan MF, Gscheidle T, Coppola R, Weickert T, Kolachana BS, et al. Executive subprocesses in working memory: relationship to catechol-*O*-methyltransferase Val158Met genotype and schizophrenia. Arch Gen Psychiatry 2003;60:889–96.

Goldman-Rakic PS. Working memory dysfunction in schizophrenia. J Neuropsychiatry Clin Neurosci 1994;6:348–57.

Goldman-Rakic PS, Muly EC, Williams GV. D-1 receptors in prefrontal cells and circuits. Brain Res Rev 2000;31:295–301.

Goldman-Rakic PS, Castner SA, Svensson TH, Siever LJ, Williams GV. Targeting the dopamine D1 receptor in schizophrenia: insights for cognitive dysfunction. Psychopharmacology 2004;174: 3–16.

Granon S, Poucet B, Thinus-Blanc C, Changeux JP, Vidal C. Nicotinic and muscarinic receptors in the rat prefrontal cortex: differential roles in working memory, response selection and effortful processing. Psychopharmacology 1995;119(2):139–44.

Green MF. What are the functional consequences of neurocognitive deficits in schizophrenia? Am J Psychiatry 1996;153:321–30.

Guan ZZ, Zhang X, Blennow K, Nordberg A. Decreased protein level of nicotinic receptor alpha7 subunit in the frontal cortex from schizophrenic brain. NeuroReport 1999;10:1779–82.

Hindmarch I, Parrott AC. Repeated dose comparison of nomifensine, imipramine and placebo on subjective assessments of sleep and objective measures of psychomotor performance. Br J Clin Pharmacol 1977;4(Suppl 2):167S–73S.

Honey GD, Bullmore ET, Sharma T. De-coupling of cognitive performance and cerebral functional response during working memory in schizophrenia. Schizophr Res 2002;53:45–56.

Ichikawa J, Dai J, O'Laughlin IA, Fowler WL, Meltzer HY. Atypical, but not typical, antipsychotic drugs increase cortical acetylcholine release without an effect in the nucleus accumbens or striatum. Neuropsychopharmacology 2002;26:325–39.

Jacobsen LK, D'Souza CD, Einar Mencl W, Pugh KR, Skudlarski P, Krystal JH. Nicotine effects on brain function and functional connectivity in schizophrenia. Biol Psychiatry 2004;55:850–8.

Jansma JM, Ramsey NF, Coppola R, Kahn RS. Specific versus nonspecific brain activity in a parametric *N*-back task. Neuroimage 2000;12:688–97.

Jonides J, Schumacher EH, Smith EE, Lauber EJ, Awh E, Minoshima S, et al. Verbal working memory load affects regional brain activation as measured by PET. J Cogn Neurosci 1997;9:462–75.

Katerina Z, Andrew K, Filomena M, Xu-Feng H. Investigation of m1/m4 muscarinic receptors in the anterior cingulate cortex in schizophrenia, bipolar disorder, and major depression disorder. Neuropsychopharmacology 2004;29:619–25.

Keefe RS, Lees-Roitman SE, Dupre RL. Performance of patients with schizophrenia on a pen and paper visuospatial working memory task with short delay. Schizophr Res 1997;26(1):9–14.

Kirrane RM, Mitropoulou V, Nunn M, Silverman J, Siever LJ. Physostigmine and cognition in schizotypal personality disorder. Schizophr Res 2001;48:1–5.

Kopelman MD, Corn TH. Cholinergic 'blockade' as a model for cholinergic depletion A comparison of the memory deficits with those of Alzheimer-type dementia and the alcoholic Korsakoff syndrome. Brain 1988;111:1079–110.

Kumari V, Gray J, Ffytche DH, Mitterschiithaler MT, Das M, Zachariah E, et al. Cognitive effects of nicotine in humans: an fMRI study. Neuroimage 2003;19:1002–13.

Leblond L, Beaufort C, Delerue F, Durkin TP. Differential roles for nicotinic and muscarinic cholinergic receptors in sustained visuo-spatial attention? A study using a 5-arm maze protocol in mice. Behav Brain Res 2002;128(1):91–102.

Levin ED, Rose JE, McGurk SR, Butcher LL. Characterization of the cognitive effects of combined muscarinic and nicotinic blockade. Behav Neural Biol 1990;53:103–12.

Levin ED, Briggs SJ, Christopher NC, Rose JE. Chronic nicotinic stimulation and blockade effects on working memory. Behav Pharmacol 1993;4:179–82.

Levin ED, Kaplan S, Boardman A. Acute nicotine interactions with nicotinic and muscarinic antagonists: working and reference memory effects in the 16-arm radial maze. Behav Pharmacol 1997;8:236–42.

Liddle PF. Cognitive impairment in schizophrenia: its impact on social functioning. Acta Psychiatr Scand; (Suppl 400):11–6.

Little JT, Johnson DN, Minichiello M, Weingartner H, Sunderland T. Combined nicotinic and muscarinic blockade in elderly normal volunteers: cognitive, behavioral, and physiologic responses. Neuropsychopharmacology 1998;19:60–9.

Luciana M, Collins PF, Depue RA. Opposing roles for dopamine and serotonin in the modulation of human spatial working memory functions. Cereb Cortex 1998;8:218–26.

Maviel T, Durkin TP. Role of central cholinergic receptor sub-types in spatial working memory: a five-arm maze task in mice provides evidence for a functional role of nicotinic receptors in mediating trace access processes. Neuroscience 2003;120:1049–59.

Mehta MA, Sahakian BJ, McKenna PJ, Robbins TW. Systemic sulpiride in young adult volunteers simulates the profile of cognitive deficits in Parkinson's disease. Psychopharmacology 1999;146:162–74.

Meltzer HY, McGurk SR. The effects of clozapine, risperidone, and olanzapine on cognitive function in schizophrenia. Schizophr Bull 1999;25:233–55.

Mewaldt SP, Ghoneim MM. The effects and interactions of scopolamine, physostigmine and methamphetamine on human memory. Pharmacol Biochem Behav 1979;10:205–10.

Mirza NR, Stolerman IP. The role of nicotinic and muscarinic acetylcholine receptors in attention. Psychopharmacology 2000;148:243–50.

Muller U, von Cramon DY, Pollmann S. D1- versus D2-receptor modulation of visuospatial working memory in humans. J Neurosci 1998;18:2720–8.

Newhouse PA, Potter A, Corwin J, Lenox R. Acute nicotinic blockade produces cognitive impairment in normal humans. Psychopharmacology 1992;108:480–4.

Newhouse PA, Potter A, Corwin J, Lennox R. Age-related effects of the nicotinic antagonist mecamylamine on cognition and behaviour. Neuropsychopharmacology 1994;10:93–107.

Park S, Holzman PS. Schizophrenics show spatial working memory deficits. Arch Gen Psychiatry 1992;49(12):975–82.

Perlstein WM, Carter CS, Noll DC, Cohen JD. Relation of prefrontal cortex dysfunction to working memory and symptoms in schizophrenia. Am J Psychiatry 2001;158:1105–13.

Perry EK, Tomlinson BE, Blessed G, Bergmann K, Gibson PH, Perry RH. Correlation of cholinergic abnormalities with senile plaques and mental test scores in senile dementia. Br Med J 1978;2:1457–9.

Pickworth WB, Fant RV, Butschky MF, Henningfield JE. Effects of mecamylamine on spontaneous EEG and performance in smokers and non-smokers. Pharmacol Biochem Behav 1997;56:181–7.

Potter DD, Pickles CD, Roberts RC, Rugg MD. The effect of cholinergic receptor blockade by scopolamine on memory performance and the auditory P3. Psychophysiology 2000;14:11–23.

Raedler TJ, Knable MB, Jones DW, Urbina RA, Gorey JG, Lee KS, et al. In vivo determination of muscarinic acetylcholine receptor availability in schizophrenia. Am J Psychiatry 2003;160:118–27.

Rasmusson DD, Dudar JD. Effect of scopolamine on maze learning performance in humans. Experientia 1979;35:1069–70.

Riekkinen Jr P, Riekkinen M, Sirvio J. Cholinergic drugs regulate passive avoidance performance via the amygdala. J Pharmacol Exp Ther 1993;267(3):1484–92.

Robbins TW, Semple J, Kumar R, Truman MI, Shorter J, Ferraro A, et al. Effects of scopolamine on delayed-matching-to-sample and paired associates tests of visual memory and learning in human subjects: comparison with diazepam and implications for dementia. Psychopharmacology 1997;134:95–106.

Rusted JM, Warburton DM. The effects of scopolamine on working memory in healthy young volunteers. Psychopharmacology 1988; 96:145–52.

Rusted JM, Eaton-Williams P, Warburton DM. A comparison of the effects of scopolamine and diazepam on working memory. Psychopharmacology 1991;105:442–5.

Safer DJ, Allen RP. The central effects of scopolamine in man. Biol Psychiatry 1971;3:347–55.

Sarter M, Bruno JP. Abnormal regulation of corticopetal cholinergic neurons and impaired information processing in neuropsychiatric disorders. Trends Neurosci 1999;22:67–74.

Sawaguchi T, Goldman-Rakic PS. D1 dopamine receptors in prefrontal cortex: involvement in working memory. Science 1991;251:947–50.

Sawaguchi T, Goldman-Rakic PS. The role of D1-dopamine receptor in working memory: local injections of dopamine antagonists into the prefrontal cortex of rhesus monkeys performing an oculomotor delayed-response task. J Neurophysiol 1994;71:515–28.

Shirazi-Southall S, Rodriguez DE, Nomikos GG. Effects of typical and atypical antipsychotics and receptor selective compounds on acetylcholine efflux in the hippocampus of the rat. Neuropsychopharmacology 2002;26:583–94.

Tabachnick BG, Fidell LS. Using multivariate statistics, 2nd ed. Northridge, CA: Harper Collins; 1989.

Thompson JC, Stough C, Ames D, Ritchie C, Nathan PJ. Effects of the nicotinic antagonist mecamylamine on inspection time. Psychopharmacology 2000;150:117–9.

Varanda W, Aracava Y, Sherby SM, Vanmeter WG, Eldefrawi ME, Albuquerquem EX. The acetylcholine receptor of the neuromuscular junction recognizes mecamylamine as a non-competitive antagonist. Mol Pharmacol 1985;28:128–37.

Vige X, Briley M. Scopolamine induces up-regulation of nicotinic receptors in intact brain but not in nucleus basalis lesioned rats. Neurosci Lett 1988;88:319–24.

Wesnes KA, Simpson PM, Kidd AG. An investigation of the range of cognitive impairments induced by scopolamine 06 mg sc. Hum Psychopharmacol 1988;3:27–41.

Young JM, Shytle RD, Sanberg PR, George TP. Mecamylamine: new therapeutic uses and toxicity/risk profile. Clin Ther 2001;23:532–65.

Zurowski B, Gostomzyk J, Gron G, Weller R, Schirrmeister H, Neumeier B, et al. Dissociating a common working memory network from different neural substrates of phonological and spatial stimulus processing. Neuroimage 2002;15:45–57.