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ARTICLE

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Muscarinic antagonist effects on executive control of attention

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Abstract

Acetylcholine plays a major role in mediating attention processes. We investigated the muscarinic antagonist effect of scopolamine on functional neuro-anatomy of attention and cognition. We assessed 12 healthy volunteers while performing the Attention Network Task on 0.4 mg scopolamine and placebo in a single-blind randomized trial in a 1.5 T magnetic resonance scanner. Neurocognitive measures included verbal learning, verbal memory, verbal fluency, trail making, digit span, a continuous performance task and a planning task (Tower of London). When compared to placebo, scopolamine increased reaction times for conflicting stimulus processing, together with decreasing brain activation in the anterior cingulate cortex (a brain region involved in conflict processing) suggestive of a muscarinic antagonist effect on executive control of attention. Contrary to the notion of a predominantly right-hemispheric lateralization of cognitive processes associated with orienting attention, scopolamine reduced brain activity in left superior and left middle frontal brain areas. Our neuropsychological test data revealed a selective effect of scopolamine on verbal learning and memory while other cognitive domains, such as planning and working memory, were unaffected. These findings are consistent with muscarinic modulation of dopaminergic neurotransmission in frontal attention networks when processing conflicting information.

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Key words: Anterior cingulate cortex, Attention Network Task, functional magnetic resonance imaging, scopolamine, verbal memory.

Introduction

Acetylcholine (ACh) plays a major role in mediating attention (Callejas *et al*. 2004; Fan *et al*. 2002; Freedman *et al*. 1988, 1999, 2000; Ochoa & Lasalde-Dominicci, 2007; Sabri *et al*. 2008). ACh exerts its diverse physiological actions by binding to and activating two structurally and functionally distinct families of cell-surface receptors: (1) the nicotinic ACh receptors, which form ligand-gated ion channels, and (2) the G protein-coupled muscarinic ACh receptors. Both muscarinic and nicotinic receptors have been implicated in cognition, particularly in attention and memory function (Friedman, 2004; Green *et al*. 2005; Sarter *et al*. 2005) and have also been linked to conditions like Alzheimer's disease and schizophrenia (Buchanan *et al*. 2007; Langmead *et al*. 2008; Sarter *et al*. 2005). There is also some evidence suggesting potentially beneficial effects of muscarine agonists when targeting cognitive

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impairment and psychotic symptoms (Shannon *et al*. 1994; Shekhar *et al*. 2008).

Most experimental studies have investigated the effects of nicotinic and muscarinic antagonists on cognition. For instance, the muscarinic antagonist scopolamine has detrimental effects on working and declarative memory, sustained visual attention (Furey *et al*. 2008), and psychomotor speed learning when tested in healthy subjects (Ellis *et al*. 2006; Sitaram *et al*. 1978). Sherman *et al*. (2003) reported impaired recollection and familiarity of memory performance in a set of complex visual images in response to a single dose of 0.4 mg scopolamine. Koller *et al*. (2003) reported impaired memory and vigilance performance irrespective of dose (i.e. 0.3 mg and 0.6 mg). Green *et al*. (2005) further demonstrated that scopolamine significantly impaired both object and spatial N-back working-memory performance. These effects were not present with the nicotinic antagonist mecamylamine, thus suggesting some specificity of the muscarinergic ACh receptors in mediating the reported effects on cognition.

Other reports also suggest that scopolamine interferes with sensory information processing. Pekkonen *et al*. (2001) reported decreased mismatch negativity amplitudes with scopolamine when assessing pre-attentive auditory sensory memory function with frequency deviants. In contrast, middle-latency magnetic fields in response to auditory stimuli increase with scopolamine (Jaaskelainen *et al*. 1999).

These findings in humans are largely supported by animal research in non-human primates. For instance, scopolamine disrupts auditory short-term memory in a delayed matching to sample task (Plakke *et al*. 2008) while intracranial infusion of scopolamine into the intraparietal cortex slowed covert orienting performance (Davidson & Marrocco, 2000). Scopolamine also impairs visual short-term memory (Bartus & Johnson, 1976) and reduces attention modulation in primary visual cortex (Herrero *et al*. 2008). The latter effect was not present with mecamylamine, thus supporting the notion of some specificity of the muscarinic ACh receptors in modulating attention and working-memory processes.

Functional magnetic resonance imaging (fMRI) studies on healthy volunteers indicate reduction of extrastriate, middle frontal, and inferior frontal brain activation associated with repetition suppression in left neocortex in response to scopolamine (Thiel *et al*. 2001). Scopolamine also disrupts experience-dependent plasticity in auditory cortex (Thiel *et al*. 2002) and de-activates hippocampal, fusiform, and inferior prefrontal regions when performing a face-name association task (Sperling *et al*. 2002). However, functional brain-imaging studies specifically investigating scopolamine effects on attention processes are lacking.

The present study investigated the effects of scopolamine on cognition and, specifically, assessed the functional neuro-anatomy of attention processes in healthy subjects when performing the Attention Network Task (ANT; Fan *et al*. 2002; Thienel *et al*. 2009 for mecamylamine effects). This task allows for the discrimination of *alerting*, *orienting* and *executive control* processes of attention.

Each of these processes has been linked to different brain regions and neuromodulators. *Alerting* is predominantly associated with thalamic, anterior and posterior cortical activation (Fan *et al*. 2005) and involves noradrenergic up-modulation of frontal and parietal regions in the right hemisphere (Witte & Marrocco, 1997). *Executive control* appears to be mainly modulated by dopamine and engages the anterior cingulate cortex (ACC) and the lateral prefrontal cortex (Bush *et al*. 2000; MacDonald *et al*. 2000; Marrocco & Davidson, 1998).

Most relevant to this study, ACh plays a major role in *orienting* (see Ochoa & Lasalde-Dominicci, 2007 for review; Posner & Petersen, 1990) which engages the temporo-parietal junction and the inferior frontal gyrus in the right hemisphere (Corbetta *et al*. 2000).

Based on these findings, we predicted reduced frontal and parietal activation for *orienting* when challenged by scopolamine. However, effects of muscarinic signalling on mid-brain dopamine release via M_4 receptors (Langmead *et al*. 2008) suggest changes in mesocortical neurotransmission further downstream, thus also affecting *executive control* performance. Hence we predicted decreased activation in the ACC and the lateral prefrontal cortex with scopolamine when performing *executive control* along with increased response times when processing conflicting information. Muscarinic antagonist effects on *alerting* should not be present.

Methods

Subjects

Following approval by the RWTH Aachen Medical Research Ethics Committee, 12 male right-handed (Edinburgh Handedness Inventory Score 9 ± 0.9) subjects with a mean age of 26 ± 2.1 yr (range 23–29 yr), were recruited into the study which also investigated nicotinic effects on ANT performance in a parallel functional brain-imaging study as reported in a companion paper (Thienel *et al*. 2009). Subjects achieved

a mean verbal IQ of 118±13.8 [Mehrfachwahl-Wortschatz-Intelligenztest (Multiple choice vocabulary test); Lehrl, 2005] and did not meet criteria for a current or lifetime psychiatric diagnosis [including current nicotine or other substance abuse or lifetime diagnosis of substance addiction (SCID-I)], or any other relevant past or present medical or neurological condition, including standard MRI exclusion criteria. Subjects gave informed, written consent and received a small honorarium for participation.

Study design

Subjects took part in three successive MRI sessions at least 1 wk apart and were randomized into a single-blind, crossover design. Active drugs and placebo were administered in a double-dummy procedure as described by Thienel *et al*. (2009) for mecamylamine effects on ANT performance: (1) A single dose of 0.4 mg scopolamine was injected intravenously and placebo given orally; (2) a single dose of 15 mg mecamylamine was given orally with a saline injection intravenously; or (3) placebo was administered orally together with an intravenous saline injection. Mecamylamine findings are reported elsewhere (Thienel *et al*. 2009).

Stimuli and tasks

A modified version (Thienel *et al*. 2009) of the ANT (Fan *et al*. 2002, 2005; Konrad *et al*. 2005) served as cognitive challenge in the MRI scanner. Three cueing conditions were used: (1) no cue (central fixation cross presented), (2) a central cue, or (3) a spatial cue which predicted the location of a target arrow 4° to the right or 4° to left of the central fixation cross. Left- or right-pointing target arrows were presented with four flanker arrows (two above and two below) either pointing in the same direction (congruent condition) or in the opposite direction (incongruent condition) to the target arrow.

Ten repetitions of the 24 possible stimulus combinations (i.e. three cue conditions, two flanker conditions, two target presentation sides, and two target-pointing directions) were presented in a randomized and counterbalanced sequence of 240 trials. Study participants were asked to respond by pressing a button with their right index or middle finger depending on the direction the target arrow was pointing (see Thienel *et al*. 2009 for more detail). Reaction times were analysed for congruency, cueing and drug conditions using a full-factorial linear mixed-effects model (Pinheiro & Bates, 2000).

Following each MRI session, participants underwent neuropsychological testing consisting of (1) the Verbal Learning Memory Test [VLMT; Helmstaedter *et al*. 1990, the German adaptation of the Rey Auditory Verbal learning Test (RAVLT; Schmidt, 1996)]; (2) the Tower of London task (ToL; Shallice, 1982; adapted by Tucha & Lange, 2004); (3) Trail Making Test (TMT A/B; Reitan, 1958); (4) Verbal Fluency [Regensburger Wortfluessigkeitstest (RWT); Aschenbrenner *et al*. 2000]; (5) Digit Span (Haerting *et al*. 1999); and (6) d2 test (d2; Brickenkamp, 1994). Analyses of variance (ANOVA) for repeated measures were employed to compare scopolamine *vs*. placebo test performance at $p<0.0016$ (Bonferroni-corrected for multiple comparisons).

MRI data acquisition

Echo-planar imaging (EPI) took place at the Jülich Research Centre (Germany) using a 1.5 T Siemens Symphony MRI scanner (90° flip angle, 3 s repetition time, and 60 ms echo time). Images consisted of 30 slices of 4-mm thickness (gap between slices was 0.4 mm) resulting in an in-plane resolution of 64×64. The field of view was 200×200 mm^2 with voxel dimensions of $3.125\times3.125\times4$ mm^3. A series of 286 images was acquired for each subject discarding the initial three images to account for T1 stabilization effects.

MRI data analyses

MRI data were analysed using SPM2 (http://www.fil.ion.ucl.ac.uk/spm/). Images were aligned to the first volume using rigid-body transformations. Acquisition delays of individual slices were corrected by the 15th slice of each volume as reference in time before transforming into standard MNI space by normalizing to an EPI template. Data were spatially interpolated to a voxel size of $2\times2\times2$ mm^3 and spatially smoothed using an isotropic Gaussian kernel of 10 mm full width at half maximum.

Target stimulus onset determined event onset for haemodynamic modelling. Non-sphericity correction was performed to control for heteroscedasticity and covarying conditions. *T* contrasts were calculated at $p<0.01$ (voxel level) and clusters of >54 continuous voxels (equivalent to Monte Carlo-corrected threshold of $p<0.05$, 1000 iterations; Slotnick *et al*. 2003) for correctly performed trials for scopolamine *vs*. placebo for (1) central *vs*. no cue (*alerting*), (2) spatial *vs*. central cue (*orienting*) and (3) incongruent *vs*. congruent (*executive control*), respectively, by repeated-measures ANOVA with reaction time as covariate of no interest (see

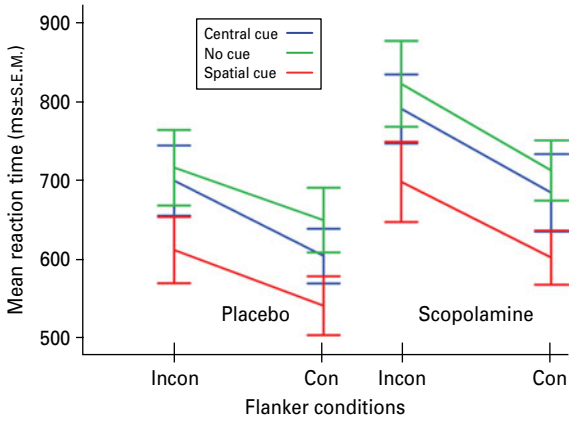


Fig. 1. Mean reaction times (S.E.M.) for correctly performed trials recorded in non-cued, central cued or spatially cued and congruent (Con) or incongruent (Incon) flanker conditions for placebo (left) and scopolamine (right).

Thienel *et al*. 2009 for more details). Inclusive masking with main placebo effects at $p<0.05$ were conducted to reveal which areas with drug interaction were also activated with placebo alone. Automatic anatomic labelling was performed in SPM (automatic anatomic labelling, http://www.fil.ion.ucl.ac.uk/spm/ext/#AAL; Tzourio-Mazoyer *et al*. 2002).

Results

Adverse drug effects and behavioural data

None of the participants reported subjective drug effects when tested with placebo. Scopolamine induced modest side-effects in all participants (i.e. hyposalivation and mild sedation). However, one participant was excluded from our study due to excessive sedation.

The results for the placebo condition are reported in detail in Thienel *et al*. (2009). Briefly, with placebo the overall correct response rate was 98%. Across all ANT conditions, mean reaction time (±S.E.M.) was 638±11 ms. Alerting (central cue *vs*. no cue) and orienting (spatial cue *vs*. central cue) significantly facilitated response time by 31±7 ms and 75±11 ms [$F(2,55)>40.8$, $p<0.0001$] and incongruent *vs*. congruent flanker conditions, assessing for executive control, slowed response times by 77±8 ms. [$F(1,55)=245.2$, $p<0.0001$]. Cue interacted with flanker type [$F(2,55)=5.4$, $p<0.008$; Fig. 1].

Rate of correct responses was not affected by scopolamine when compared to placebo ($F<1.0$). In contrast, scopolamine slowed mean reaction times (±S.E.M.) across all task conditions by 81±22 ms *vs*. placebo [$F(1,121)=39.8$, $p<0.0001$] with a significant reaction time increase of 91±9 ms for the incongruent condition [congruency × drug interaction: $F(1,121)=5.7$, $p=0.02$] when assessing for executive control and comparing to placebo. Scopolamine also abolished the cue × flanker type interaction seen in the placebo condition. However, no specific scopolamine effects were found for alerting and orienting conditions with comparable facilitations of response times [$F(2,55)>511.0$, $p<0.0001$] by 30±7 ms and 87±9 ms, respectively; when comparing with placebo performance.

Functional imaging data

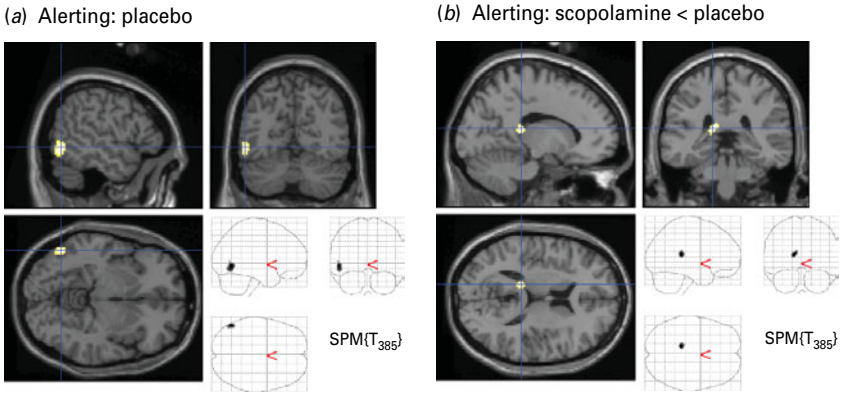
Alerting was predominantly associated with increased blood oxygenation level dependent (BOLD) effect in the left interior temporal gyrus with placebo. When contrasted with the placebo condition, scopolamine increased the alerting effect in the right middle temporal gyrus and the left middle occipital gyrus but reduced the alerting effect in the left hippocampus (Table 1, Fig. 2). Covarying reaction times did not change the results except for hippocampus BOLD activation becoming non-significant.

Bilateral prefrontal cortex, right precuneus, and left caudate activation was present with orienting with placebo while the orienting effect was significantly reduced with scopolamine in the left prefrontal and right precentral gyrus when compared to placebo. The prefrontal area was confirmed as a region activated with scopolamine *vs*. placebo and placebo only as well (Table 2, Fig. 3). Covarying reaction times did not change the results except for precentral BOLD activation becoming non-significant.

Executive control was associated with increased BOLD bilaterally in the ACC, right superior frontal and parietal gyri, left gyrus rectus, right angular and left inferior occipital gyrus and bilateral precuneus in the placebo condition. The executive control effect was significantly reduced for scopolamine *vs*. placebo in regions also activated by placebo alone, e.g. the right superior and middle orbito-frontal gyrus, precuneus and the left gyrus rectus. Other areas affected by scopolamine included the right ACC, right middle orbicular frontal gyrus, lingual, inferior temporal, and precuneus as well as left lingual and inferior parietal gyrus with reduced executive control effect, while left inferior parietal gyrus showed increased executive control effect (Table 3, Fig. 4). Covarying reaction times did not change the results for clusters with increased BOLD (i.e. left inferior parietal lobe) or reduced BOLD (i.e. left gyrus rectus and right lingual

Table 1. Local maxima for the clusters associated with alerting (central cue *vs.* no cue) and scopolamine *vs.* placebo contrast (cluster threshold >54 continuous voxels)

	MNI coordinates	Z score	*p* value (uncorr.)	Cluster size (voxels)
Scopolamine < placebo				
L hippocampus	−14, −34, 14	3.01	0.001	61
Scopolamine > placebo				
R middle temporal gyrus	48, −20, −16	3.80	<0.001	163
L middle occipital gyrus	−16, −94, −2	3.40	<0.001	149



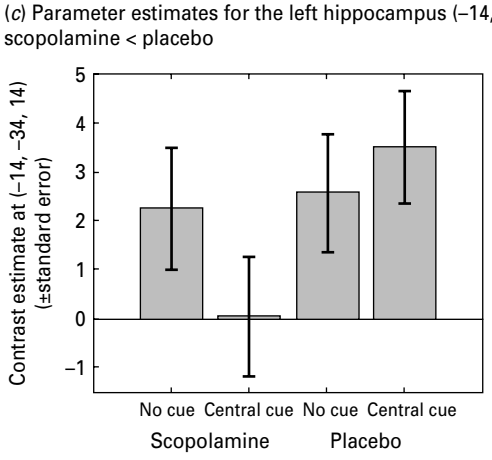


Fig. 2. For the alerting contrast (central cue > no cue) the glass brain and intersecting sagittal, coronal, and transaxial slices (SPM-2 T1 template) show (*a*) left inferior temporal gyrus activation with placebo and (*b*) a reduction in brain activation with scopolamine < placebo in left hippocampus (Monte Carlo-corrected threshold of $p < 0.05$ for clusters >54 continuous voxels). (*c*) The parameter plot shows a BOLD increase with placebo after central cues whereas the inverse effect is evident with scopolamine.

gyrus) in response to scopolamine. However, peak activity of some clusters slightly shifted within the same anatomical areas in right superior frontal, precuneus, middle orbicular frontal, ACC, and inferior temporal gyrus.

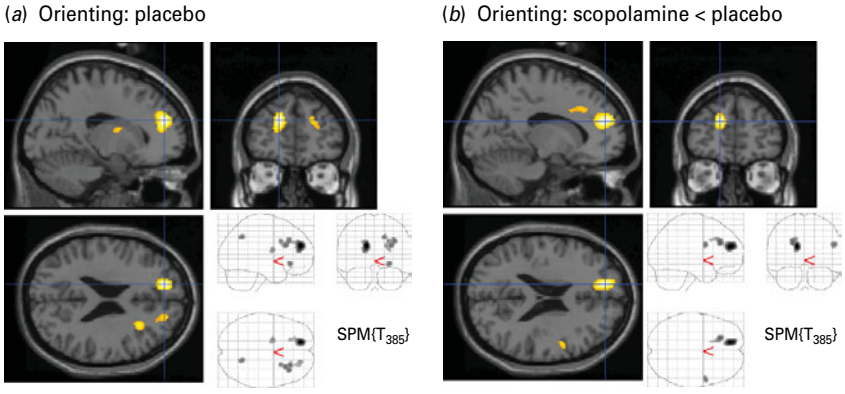
Neuropsychological data

Scopolamine significantly impaired verbal learning memory performance (VLMT) by reducing the number of words reproduced in the free recall sessions 1–5

Table 2. Local maxima for the clusters associated with orienting (spatial cue *vs.* centre cue) scopolamine *vs.* placebo contrast (cluster threshold >54 continuous voxels)

	MNI coordinates	Z score	*p* value (uncorr.)	Cluster size (voxels)
Scopolamine < placebo				
L superior frontal gyrus[a]	−14, 50, 22	4.49	<0.001	387
L middle frontal gyrus[a]	−22, 30, 30	3.40	<0.001	203
R precentral gyrus	52, 4, 26	3.20	0.001	77

[a] Brain areas co-activated with placebo and drug challenge.



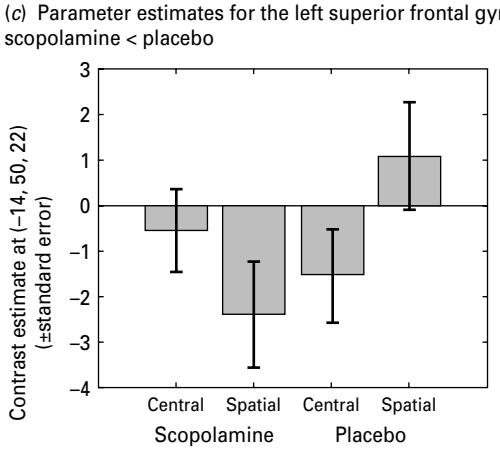


Fig. 3. For the orienting contrast (spatial cue > central cue) the glass brain and intersecting sagittal, coronal, and transaxial slices (SPM-2 T1 template) show (*a*) bilateral superior and right inferior frontal gyrus, right precuneus (only shown in glass brain) and left caudate activation with placebo and (*b*) scopolamine reduced BOLD in the left superior and middle frontal gyrus compared to placebo (Monte Carlo-corrected threshold of $p<0.05$ for clusters >54 continuous voxels). (*c*) The parameter plot shows with placebo a BOLD increase in the left superior frontal gyrus after spatial cues and a reduction after central cues, while with scopolamine central and to a larger extent spatial cues lead to a decrease in activation in this brain area.

from 56±6 (values are ±S.D.) to 49±8 compared to 56±6 for placebo [$F(1,11)=12.9$, $p=0.004$]. Delayed recall of word after 30 min was also affected by scopolamine from 14±2 to 12±4 [$F(1,11)=10.7$, $p=0.007$]. No further effects of scopolamine on neuropsychological test performance was confirmed ($F<1.0$).

Discussion

Our functional imaging findings support our predictions that muscarinic antagonism significantly reduced brain activation in parts of the orienting and executive control network. The latter finding is

Table 3. Local maxima for the clusters associated with executive control (incongruent *vs.* congruent flanker) and scopolamine *vs.* placebo contrast (cluster threshold >54 continuous voxels)

	MNI coordinates	Z score	*p* value (uncorr.)	Cluster size (voxels)
Scopolamine < placebo				
L gyrus rectus[a]	−8, 54, −20	3.46	<0.001	803
R lingual gyrus	16, −52, 2	3.51	<0.001	506
R superior frontal gyrus[a]	28, 30, 54	3.49	<0.001	467
R precuneus[a]	8, −50, 26	2.92	0.002	357
R middle orbicular frontal gyrus[a]	36, 46, −14	3.00	0.001	273
R superior frontal gyrus[a]	14, 42, 38	2.91	0.002	208
L lingual gyrus	−24, −46, −6	3.08	0.001	172
R inferior temporal gyrus	62, −42, −14	3.13	0.001	82
R anterior cingulate cortex	14, 28, 18	2.76	0.003	68
R inferior temporal gyrus	64, −22, −22	2.79	0.003	66
Scopolamine > placebo				
L inferior parietal	−58, −30, 52	3.39	<0.001	279
L inferior parietal	−26, −42, 44	3.20	0.001	269

[a] Brain areas co-activated with placebo and drug challenge.

supported by our behavioural data showing a selective drug effect on processing incongruency. This association is further supported by parameter plots showing that muscarinic antagonism reverses brain activation when compared to placebo.

The most pronounced scopolamine effect was found with executive control when increasing reaction times were assessed after incongruent flanker conditions. This finding is consistent with our functional imaging data which demonstrate that brain activation associated with executive control in the ACC – a brain region subserving conflict processing as shown in previous studies (Fan *et al*. 2005) – is abolished by scopolamine. In particular, the ACC is engaged in conflict resolution (Botvinick *et al*. 2001; Bush *et al*. 2000) as induced by incongruent conditions, while executive function tasks generally activate frontoparietal cortical areas (Perfetti *et al*. 2007; Specht *et al*. 2008). Since executive control is hypothesized to be associated with dopaminergic neurotransmission, this finding can also be interpreted on the basis of the modulatory role of muscarinic receptors in mesocortical dopamine pathways (Langmead *et al*. 2008).

When controlling for reaction time, the main results were largely confirmed with a few exceptions (i.e. hippocampus activation in alerting contrast; precentral activation in orienting contrast). While some peak activation slightly shifted when controlling for reaction time, they largely centred within the same areas as those identified for the placebo main effects. However, ACC differed in this respect. Scopolamine challenge activated a more dorsal portion of ACC compared to placebo. Nicotinic challenge, on the other hand, resulted in a general slowing of reaction times, irrespective of attention components along with no confounding effects on BOLD activation contrasts (Thienel *et al*. 2009).

Our neuropsychological data further demonstrate that muscarinic antagonism selectively impairs verbal learning memory performance. Largely consistent with our results, Sherman *et al*. (2003) reported that scopolamine impaired the recollection and familiarity of items that had been learned previously. Ellis *et al*. (2006) also found detrimental effects of scopolamine on immediate and delayed recall. Surprisingly little or no effect was recorded for any other cognitive domain in our study which may be explained by a ceiling effect in our well-performing cohort of healthy volunteers or lack of test specificity in relation to executive control of attention (Riedel *et al*. 1997).

On the other hand, potentially relevant to our neurocognitive findings are reports of beneficial effects of the selective M_1/M_4 mAChR agonist xanomeline on verbal learning and short-term memory functions in patients with schizophrenia (Shekhar *et al*. 2008). Verbal memory deficits are also one of the few cognitive markers known to be sensitive to prodromal states and transition to psychosis, including schizophrenia (Lencz *et al*. 2006), thus potentially providing a

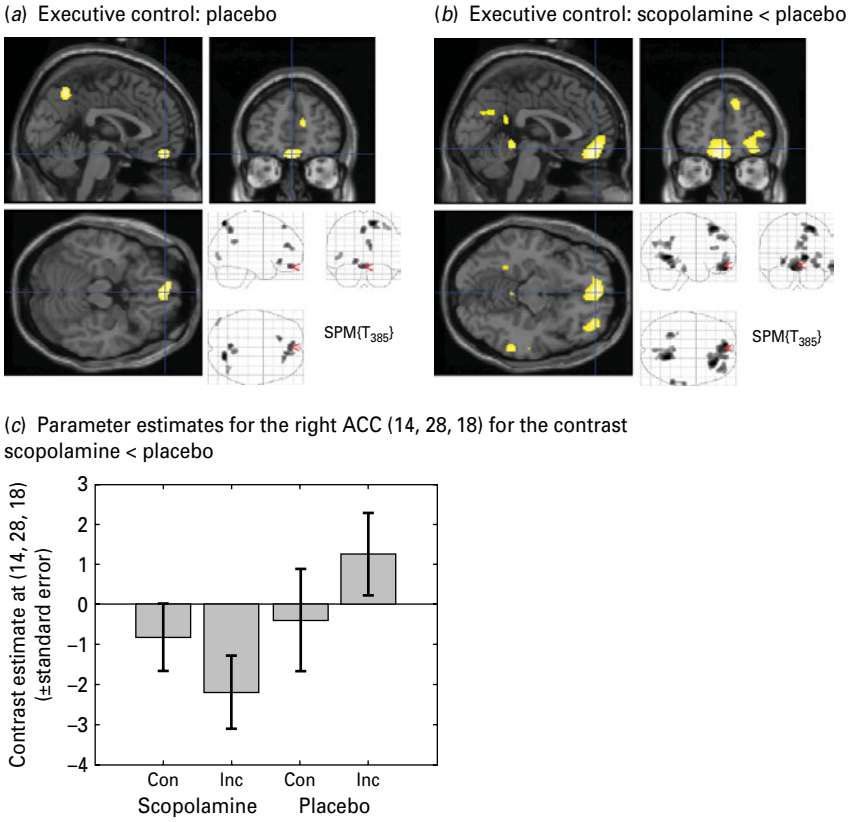


Fig. 4. For the executive control contrast (incongruent flanker > congruent flanker) the glass brain and intersecting sagittal, coronal, and transaxial slices (SPM-2 T1 template) show (*a*) bilateral precuneus, angular gyrus and anterior cingulate cortex (ACC) (both shown only in the glass brain), left gyrus rectus, inferior occipital gyrus and right superior parietal gyrus (both shown only in the glass brain) activation with placebo and (*b*) decreased brain activation in the bilateral lingual, the right superior frontal and middle orbicular frontal gyrus, the left gyrus rectus, as well as only shown in the glass brain the right precuneus and right ACC, right inferior temporal, left inferior parietal, under scopolamine < placebo (Monte Carlo-corrected threshold of $p < 0.05$ for clusters > 54 continuous voxels). (*c*) The parameter plots show that scopolamine abolishes BOLD increase induced by incongruent flankers as evident with placebo.

therapeutic target for selective M_1/M_4 mAChR agonists in 'ultra high-risk' populations by increasing ACh and dopamine release in the medial prefrontal cortex and hippocampus (Li *et al*. 2007; Weiner *et al*. 2004). Other preclinical studies provide supportive data for M_1 agonists when treating cognitive deficits (Harries *et al*. 1998; Loudon *et al*. 1997; Schwarz *et al*. 1999; Shannon *et al*. 1994), while M_4 agonists are more likely to act as an antipsychotic by inhibiting dopamine release (Langmead *et al*. 2008).

Following placebo administration the orienting contrast (spatial *vs*. central cue) activated, as predicted, the predominantly right-lateralized neural network comprising inferior and superior frontal cortical areas, precuneus and caudate. This largely confirms previous reports (Corbetta *et al*. 2000; Fan *et al*. 2005); however, our findings differ in parietal activation by involvement of the right precuneus rather than the intraparietal sulcus (Corbetta *et al*. 2000). Scopolamine disrupted the orienting effect in the left superior and left middle frontal brain areas, contrary to the notion of a predominantly right-hemispheric orienting network (see also Thienel *et al*. 2009).

Our alerting (central *vs*. no cue) findings also have some inconsistencies with previous reports. In contrast to our data, Corbetta *et al*. (2000) and Fan *et al*. (2005) described right-hemispheric activation in the temporo-parietal junction and the inferior frontal gyrus. Our results showed increased alerting effects in the left inferior temporal gyrus with placebo and reduced brain activation in response to scopolamine *vs*. placebo in the left hippocampus. Consistent with previous studies (Fan *et al*. 2005), this region did not show a placebo response for the alerting contrast.

Furthermore, drug effects on hippocampus activation were not confirmed when controlling for reaction times.

Inconsistencies with other studies may also reflect differences in our study design when presenting the central cue as a fixation aid rather than a fixation cross, thus diminishing the 'alerting salience' of our cueing stimulus. This may also explain inconsistent findings for the alerting contrast with mecamylamine challenge, showing increased BOLD response in the left superior orbito-frontal and the right precentral, middle temporal, and middle occipital gyri (Thienel *et al*. 2009) that is not supported by the literature (Witte & Marrocco, 1997). Nevertheless, scopolamine did increase brain activation in the right middle temporal gyrus, although this brain region was silent in the placebo condition, thus potentially indicating compensatory mechanisms in order to execute the task successfully. Given that the alerting network is proposed to be predominantly modulated by noradrenaline, our findings may also reflect a non-specific effect of scopolamine.

Cerebrovascular coupling is defined as the global effect of a drug on local blood flow independent of changes in neuronal metabolic activity. However, this mechanism is unlikely to explain the specificity of our other findings in relation to neural networks and associated attention processes as operationalized by specific contrasts (e.g. spatial *vs*. central cue, etc.). This approach also controls for generalized drug effects, such as sedation, but may still play a role in non-specific compensatory mechanisms in response to the drug challenge in areas otherwise silent with placebo.

In summary, our study is a first attempt to uncover muscarinic antagonist effects on functional and behavioural correlates of attention. Consistent with the notion of muscarinic modulation of dopaminergic neurotransmission in frontal attention networks (Langmead *et al*. 2008; Levin *et al*. 1990) our findings provide evidence for disruptive effects of scopolamine on executive control processes. Our findings are only partly consistent with the notion of muscarinic modulation of orienting. While muscarinic blockade did not affect the critical brain areas subserving orienting processes in the right hemisphere, our data on nicotinic antagonism illustrate a down-regulation of this network (Thienel *et al*. 2009). The findings from both studies together suggest a larger degree of nicotinic modulation of *orienting* and a stronger muscarinic effect on executive control. Our neuropsychological data further demonstrated a selective scopolamine effect on verbal learning and memory while other cognitive domains, such as planning and working memory, were not affected by scopolamine.

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Statement of Interest

None.

References

Aschenbrenner S, Tucha O, Lange KW (2000). In: *Regensburger Wortflüssigkeitstest* (*RWT*). Hogrefe, Göttingen.

Bartus RT, Johnson HR (1976). Short-term memory in the rhesus monkey: disruption from the anti-cholinergic scopolamine. *Pharmacology Biochemistry and Behavior* **5**, 39–46.

Botvinick MM, Braver TS, Barch DM, Carter CS, Cohen JD (2001). Conflict monitoring and cognitive control. *Psychological Review* **108**, 624–652.

Brickenkamp R (1994). *Test d2 Aufmerksamkeits-Belastungs-Test. 8. ueberarbeitete und neu normierte Auflage*. Hogrefe Verlag, Goettingen.

Buchanan RW, Freedman R, Javitt DC, Abi-Dargham A, Lieberman JA (2007). Recent advances in the development of novel pharmacological agents for the treatment of cognitive impairments in schizophrenia. *Schizophrenia Bulletin* **33**, 1120–1130.

Bush G, Luu P, Posner MI (2000). Cognitive and emotional influences in anterior cingulate cortex. *Trends in Cognitive Sciences* **4**, 215–222.

Callejas A, Lupianez J, Tudela P (2004). The three attentional networks: on their independence and interactions. *Brain and Cognition* **54**, 225–227.

Corbetta M, Kincade JM, Ollinger JM, McAvoy MP, Shulman GL (2000). Voluntary orienting is dissociated from target detection in human posterior parietal cortex. *Nature Neuroscience* **3**, 292–297.

Davidson MC, Marrocco RT (2000). Local infusion of scopolamine into intraparietal cortex slows covert orienting in rhesus monkeys. *Journal of Neurophysiology* **83**, 1536–1549.

Ellis JR, Ellis KA, Bartholomeusz CF, Harrison BJ, *et al.* (2006). Muscarinic and nicotinic receptors synergistically modulate working memory and attention in humans. *International Journal of Neuropsychopharmacology* **9**, 175–189.

Fan J, McCandliss BD, Fossella J, Flombaum JI, Posner MI (2005). The activation of attentional networks. *Neuroimage* **26**, 471–479.

Fan J, McCandliss BD, Sommer T, Raz A, Posner MI (2002). Testing the efficiency and independence of attentional networks. *Journal of Cognitive Neuroscience* **14**, 340–347.

Freedman R, Adams CE, Leonard S (2000). The alpha7-nicotinic acetylcholine receptor and the pathology of hippocampal interneurons in schizophrenia. *Journal of Chemical Neuroanatomy* **20**, 299–306.

Freedman R, Adler LE, Leonard S (1999). Alternative phenotypes for the complex genetics of schizophrenia. *Biological Psychiatry* **45**, 551–558.

Freedman SB, Beer MS, Harley EA (1988). Muscarinic M1, M2 receptor binding. Relationship with functional efficacy. *European Journal of Pharmacology* **156**, 133–142.

Friedman JI (2004). Cholinergic targets for cognitive enhancement in schizophrenia: focus on cholinesterase inhibitors and muscarinic agonists. *Psychopharmacology* (*Berlin*) **174**, 45–53.

Furey ML, Pietrini P, Haxby JV, Drevets WC (2008). Selective effects of cholinergic modulation on task performance during selective attention. *Neuropsychopharmacology* **33**, 913–923.

Green A, Ellis KA, Ellis J, Bartholomeusz CF, *et al.* (2005). Muscarinic and nicotinic receptor modulation of object and spatial n-back working memory in humans. *Pharmacology, Biochemistry and Behavior* **81**, 575–584.

Haerting C, Markowitsch HF, Neufeld H, Calabrese P, *et al.* (1999). *WMS-R* (*Wechsler Gedächtnistest – Revidierte Fassung*). Manual. Göttingen: Verlag Hans Huber.

Harries MH, Samson NA, Cilia J, Hunter AJ (1998). The profile of sabcomeline (SB-202026), a functionally selective M1 receptor partial agonist, in the marmoset. *British Journal of Pharmacology* **124**, 409–415.

Helmstaedter C, Lendt M, Lux S (1990). In: *VLTM: Verbaler Lern- und Merkfähigkeitstest*. Manual. Göttingen: Beltz Test.

Herrero JL, Roberts MJ, Delicato LS, Gieselmann MA, *et al.* (2008). Acetylcholine contributes through muscarinic receptors to attentional modulation in V1. *Nature* **454**, 1110–1114.

Jaaskelainen IP, Hirvonen J, Huttunen J, Kaakkola S, Pekkonen E (1999). Scopolamine enhances middle-latency auditory evoked magnetic fields. *Neuroscience Letters* **259**, 41–44.

Koller G, Satzger W, Adam M, Wagner M, Kathmann N, Soyka M, Engel R (2003). Effects of scopolamine on matching to sample paradigm and related tests in human subjects. *Neuropsychobiology* **48**, 87–94.

Konrad K, Neufang S, Thiel CM, Specht K, *et al.* (2005). Development of attentional networks: an fMRI study with children and adults. *Neuroimage* **28**, 429–439.

Langmead CJ, Watson J, Reavill C (2008). Muscarinic acetylcholine receptors as CNS drug targets. *Pharmacology & Therapeutics* **117**, 232–243.

Lehrl S (2005). *Mehrfachwahl-Wortschatz-Intelligenztest MWT-B*. Balingen: Spitta Verlag.

Lencz T, Smith CW, McLaughlin D, Auther A, *et al.* (2006). Generalized and specific neurocognitive deficits in prodromal schizophrenia. *Biological Psychiatry* **59**, 863–871.

Levin ED, McGurk SR, Rose JE, Butcher LL (1990). Cholinergic-dopaminergic interactions in cognitive performance. *Behavioral and Neural Biology* **54**, 271–299.

Li Z, Bonhaus DW, Huang M, Prus AJ, *et al.* (2007). AC260584 (4-[3-(4-butylpiperidin-1-yl)-propyl]-7-fluoro-4H-benzo[1,4]oxazin-3-one), a selective muscarinic M1 receptor agonist, increases acetylcholine and dopamine release in rat medial prefrontal cortex and hippocampus. *European Journal of Pharmacology* **572**, 129–137.

Loudon JM, Bromidge SM, Brown F, Clark MS, *et al.* (1997). SB 202026: a novel muscarinic partial agonist with functional selectivity for M1 receptors. *Journal of Pharmacology and Experimental Therapeutics* **283**, 1059–1068.

MacDonald 3rd AW, Cohen JD, Stenger VA, Carter CS (2000). Dissociating the role of the dorsolateral prefrontal and anterior cingulate cortex in cognitive control. *Science* **288**, 1835–1838.

Marrocco RT, Davidson MC (1998). Neurochemistry of attention. In: Parasuraman RE (Ed.), *The Attentitive Brain* (pp. 35–50). Cambridge: MIT Press.

Ochoa EL, Lasalde-Dominicci J (2007). Cognitive deficits in schizophrenia: focus on neuronal nicotinic acetylcholine receptors and smoking. *Cellular and Molecular Neurobiology* **27**, 609–39.

Pekkonen E, Hirvonen J, Jaaskelainen IP, Kaakkola S, Huttunen J (2001). Auditory sensory memory and the cholinergic system: implications for Alzheimer's disease. *Neuroimage* **14**, 376–382.

Perfetti B, Saggino A, Ferretti A, Caulo M, *et al.* (2007). Differential patterns of cortical activation as a function of fluid reasoning complexity. *Human Brain Mapping*. Published online: 19 December 2007. doi:10.1002/hbm.20519.

Pinheiro JC, Bates DM (2000). Mixed-effects models in S and S-PLUS. New York: Springer.

Plakke B, Ng CW, Poremba A (2008). Scopolamine impairs auditory delayed matching-to-sample performance in monkeys. *Neuroscience Letters* **438**, 126–130.

Posner MI, Petersen SE (1990). The attention system of the human brain. *Annual Review of Neuroscience* **13**, 25–42.

Reitan RM (1958). Validity of the trail making test as an indicator of organic brain damage. *Perceptual and Motor Skills* **8**, 271–276.

Riedel WJ, Hogervorst E, Verhey FRJ, Jolles J (1997). Measuring cholinergic functions and cognitive abilities. In: Hindmarch I, Stonier PD (Eds), *Human Psychopharmacology: Measures and Methods* (pp. 29–56). Chichester: Wiley.

Sabri O, Kendziorra K, Wolf H, Gertz HJ, Brust P (2008). Acetylcholine receptors in dementia and mild cognitive impairment. *European Journal of Nuclear Medicine and Molecular Imaging* **35** (Suppl. 1), S30–45.

Sarter M, Hasselmo ME, Bruno JP, Givens B (2005). Unraveling the attentional functions of cortical cholinergic inputs: interactions between signal-driven and cognitive

modulation of signal detection. *Brain Research Reviews* **48**, 98–111.

Schmidt M (1996). In: *Rey Auditory Verbal Learning Test: A Handbook*. Los Angeles, CA: Western Psychological Services.

Schwarz RD, Callahan MJ, Coughenour LL, Dickerson MR, *et al.* (1999). Milameline (CI-979/RU35926): a muscarinic receptor agonist with cognition-activating properties: biochemical and in vivo characterization. *Journal of Pharmacology and Experimental Therapeutics* **291**, 812–822.

Shallice T (1982). Specific impairments of planning. *Philosophical Transactions of the Royal Society of London, Series* B **298**, 199–209.

Shannon HE, Bymaster FP, Calligaro DO, Greenwood B, *et al.* (1994). Xanomeline: a novel muscarinic receptor agonist with functional selectivity for M1 receptors. *Journal of Pharmacology and Experimental Therapeutics* **269**, 271–281.

Shekhar A, Potter WZ, Lightfoot J, Lienemann J, *et al.* (2008). Selective muscarinic receptor agonist xanomeline as a novel treatment approach for schizophrenia. *American Journal of Psychiatry* **165**, 1033–1109.

Sherman SJ, Atri A, Hasselmo ME, Stern CE, Howard MW (2003). Scopolamine impairs human recognition memory: data and modeling. *Behavioral Neuroscience* **117**, 526–539.

Sitaram N, Weingartner H, Gillin JC (1978). Human serial learning: enhancement with arecholine and choline impairment with scopolamine. *Science* **201**, 274–276.

Slotnick SD, Moo LR, Segal JB, Hart Jr. J (2003). Distinct prefrontal cortex activity associated with item memory and source memory for visual shapes. *Cognitive Brain Research* **17**, 75–82.

Specht K, Lie CH, Shah NJ, Fink GR (2008). Disentangling the prefrontal network for rule selection by means of a non-verbal variant of the Wisconsin Card Sorting Test. *Human Brain Mapping*. Published online: 26 August 2008. doi:10.1002/hbm.20637.

Sperling R, Greve D, Dale A, Killiany R, *et al.* (2002). Functional MRI detection of pharmacologically induced memory impairment. *Proceedings of the National Academy of Sciences USA* **99**, 455–460.

Thiel CM, Friston KJ, Dolan RJ (2002). Cholinergic modulation of experience-dependent plasticity in human auditory cortex. *Neuron* **35**, 567–574.

Thiel CM, Henson RN, Morris JS, Friston KJ, Dolan RJ (2001). Pharmacological modulation of behavioral and neuronal correlates of repetition priming. *Journal of Neuroscience* **21**, 6846–6852.

Thienel R, Voss B, Kellermann T, Reske M, *et al.* (2009). Nicotinic antagonist effects on functional attention networks. *International Journal of Neuropsychopharmacology*. Published online: 9 September 2009. doi:10.1017/S1461145709990551.

Tucha O, Lange KW (2004). *TL-D Turm von London – Deutsche Version*. Manual. Göttingen: Hogrefe Verlag.

Tzourio-Mazoyer N, Landeau B, Papathanassiou D, Crivello F, *et al.* (2002). Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain. *Neuroimage* **15**, 273–289.

Weiner DM, Meltzer HY, Veinbergs I, Donohue EM, *et al.* (2004). The role of M1 muscarinic receptor agonism of N-desmethylclozapine in the unique clinical effects of clozapine. *Psychopharmacology* (*Berlin*) **177**, 207–216.

Witte EA, Marrocco RT (1997). Alteration of brain noradrenergic activity in rhesus monkeys affects the alerting component of covert orienting. *Psychopharmacology* (*Berlin*) **132**, 315–323.