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Scopolamine disrupts hippocampal activity during allocentric spatial memory in humans: an fMRI study using a virtual reality analogue of the Morris Water Maze

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Abstract

The role of the septohippocampal cholinergic system in memory disorders is well established. The effects of cholinergic challenge in animals have been extensively studied using the Morris Water Maze (MWM) which engages allocentric spatial memory. The present study investigated the effect of the centrally active muscarinic antagonist scopolamine on allocentric spatial memory in humans using a virtual reality analogue of the MWM task, the Arena task. Twenty right-handed healthy male adults with a mean age of 28 years (range 23–35 years) were studied using functional MRI in a randomized double-blind cross-over design with scopolamine bromide (0.4 mg i.m.) or placebo (saline) administered 70–90 min before the beginning of the functional scan. Scopolamine induced a significant reduction in the activation of the hippocampus/parahippocampal gyrus compared with placebo. Furthermore, there was dissociation between hippocampus-based and striatal-based memory systems, which were significantly more activated in the placebo and scopolamine conditions, respectively. The activation of the striatal system under scopolamine challenge was accompanied by the activation of the amygdala. In conclusion, the study extends the well-documented finding in animals of the attenuating effect of scopolamine on hippocampal activity during allocentric spatial memory to humans. Furthermore, the results call for further investigation of the dissociation between the hippocampal and neostriatal memory systems during allocentric spatial processing under cholinergic blockade in humans.

Keywords

Alzheimer's Disease, acetylcholine, allocentric spatial memory, caudate nucleus, fMRI, hippocampus, Morris Water Maze, scopolamine

Introduction

Early and sensitive biomarkers of disorders such as Alzheimer's disease (AD) are needed, particularly with the development of neurobiological treatments that have the potential to slow or halt disease processes (Lanctôt, 2009). In AD, neurocognitive changes in the prodromal or early stages reveal specific brain correlates that might be targeted by neurobiological markers to monitor disease progression. Specifically, empirical studies reveal that the conversion from mild cognitive impairment (MCI) to Alzheimer's is 100% in the presence of hippocampal atrophy (Apostolova et al., 2006).

Testing hippocampal activity linked to episodic memory function is one of the procedures that could be used for early detection of MCI/AD, prior to evident structural aberrations of the hippocampus and associated mnemonic decline. A robust test of hippocampal function could also aid the identification of psychopharmacological compounds for the prevention and early treatment of AD. In addition, bridging the gap between models and concepts employed in preclinical models of cognitive processes and human cognitive systems will provide a valuable link in the development of therapeutically efficacious treatments.

One of the mnemonic functions associated with hippocampus that could be measured reliably in both animals and

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humans is allocentric spatial memory (O'Keefe and Nadel, 1978). Allocentric or view-independent spatial memory (O'Keefe and Nadel, 1978) requires a functionally and/or structurally intact hippocampus in animals and humans (review, Burgess et al., 2002; Burgess, 2006). Furthermore, the hippocampus has been proposed to support a certain type of cognitive or mnemonic processing that supports allocentric memory, namely cognitive mapping, in which external Euclidian geometry is represented physiologically by assemblies of cells within the hippocampus. The latter is indicated in rodents by the presence of place cells (O'Keefe and Nadel, 1978) and, more recently, spatial view cells in non-human primates (Rolls, 1999). Hippocampal involvement in allocentric spatial memory has been extensively studied using the Morris Water Maze (MWM) (Morris, 1981), one of the most widely used behavioural paradigms for studying hippocampal involvement in memory.

We have developed a functional magnetic resonance imaging (fMRI)-compatible human analogue of the MWM, called the *Arena* task. We have demonstrated bilateral hippocampal activation during allocentric, but not egocentric, spatial memory acquisition in healthy male participants using this task (Parslow et al., 2004). More recently, we have reported attenuated hippocampal activation during both acquisition and recall during an allocentric memory task in healthy aged adult males (Antonova et al., 2009). As the next step in the task development as a functional biomarker for early diagnosis and rapid identification of compounds showed promising promnesic effects for the treatment of AD, we have investigated the effect of cholinergic blockade on hippocampal activity during performance on the *Arena* task measured using fMRI.

The cholinergic system is of particular relevance in human memory disorders. The degeneration of neurons that release acetylcholine is one of the hallmarks of Alzheimer's-type dementia (e.g. Whitehouse et al., 1982). The septohippocampal cholinergic afferent projection, which provides modulatory input to fronto-limbic brain regions, has been strongly implicated in hippocampus-dependent memory (review, Everitt and Robbins, 1997) and its dysfunction (review, Kasa et al., 1997). The blockade of muscarinic, rather than nicotinic, acetylcholine (ACh) receptors has a particularly strong association with memory impairment (Bymaster et al., 1993; Ohno et al., 1994).

The MWM procedure has been employed extensively in investigating the role of the septohippocampal cholinergic system in hippocampus-dependent memory in animals (reviews, Brandeis et al., 1989; McNamara and Skelton, 1993), with scopolamine bromide being the most commonly employed compound. Scopolamine bromide is a centrally active muscarinic antagonist, which has high specificity for muscarinic receptors (Frey et al., 1992). It is thought to selectively impair learning of new information through blockade of the physiological effect of ACh on calcium-dependent potassium currents (Madison and Nicoll, 1984), resulting in a decreased spiking response of neurons and preventing sustained activity within the hippocampus (Hasselmo and Wyble, 1997). Rogers and Kesner (2003) demonstrated that scopolamine disrupts encoding in both CA3 and CA1 subregions of the hippocampus. As would be expected from the proposed mechanism of its action, scopolamine has been found to impair spatial learning when administered before acquisition of the platform's location, but not during the recall stage of the MWM procedure (reviews, Brandeis et al., 1989; McNamara and Skelton, 1993).

Despite extensive literature using the MWM to study scopolamine-induced allocentric spatial memory impairment in animals, there are no published studies of scopolamine effects on MWM performance in humans. Therefore, the aim of the present study was to examine the effect of cholinergic challenge using scopolamine on hippocampal function during an animal-to-human translation paradigm using fMRI. We predicted that scopolamine would attenuate hippocampal activation during allocentric spatial learning.

Method

Participants

Twenty healthy right-handed male volunteers (mean age = 28.1 years old, SD = 3.4, range 23–35) were recruited to meet the following criteria: (i) body weight more than 60 kg; (ii) no abnormality on clinical examination, including a history or presence of cardiac, ophthalmologic, gastrointestinal, hepatic, or renal disease, or other condition known to increase risk of side effects; (iii) no abnormality on clinical chemistry, haematology or electrocardiogram examination at screening; (iv) negative urine drug screen at the clinical screening and on the day of the scan; (v) no abuse of alcohol (defined as an average intake >21 units per week or 3 units per day); and (vi) no history or presence of neurological or psychiatric conditions (e.g. stroke, traumatic brain injury, epilepsy, space-occupying lesions, multiple sclerosis, Parkinson's disease, vascular dementia, transient ischaemic attack, schizophrenia, major depression, etc). Participants were excluded if they fulfilled any of the following criteria: (i) had received prescribed medication within 14 days prior to the first dosing (drug or placebo), which might interfere with the study procedures or compromise safety; (ii) had received over-the-counter medicine within 48 h before the scanning days; (iii) had participated in a trial with any drug within 84 days before the first scan; or (iv) had a caffeinated drink within 24 h of dosing.

All participants gave signed consent prior to taking part in the study. The study was approved by the Ethical Committee of King's College London, UK.

Design and procedures

The study used a randomized double-blind cross-over design with scopolamine bromide and saline (placebo) as within-subject conditions. Scopolamine (0.4 mg) dissolved in 0.5 ml of saline or saline alone (0.5 ml) was administered subcutaneously. The scopolamine dose was selected based on previous research examining dose-related effects for this drug (Robbins et al., 1997), showing that 0.4 mg provides an optimal balance between its deleterious effect on memory and sedation. Each substance was administered 70–90 min before the beginning of the functional scan based on the known pharmacokinetic properties of scopolamine (Ebert et al., 1998). Each subject

was scanned twice; half of the subjects received placebo first and the other half received scopolamine first. These two subgroups of participants were matched on full-scale ($p = 0.842$) and performance ($p = 0.865$) intellectual quotient as estimated using the Wechsler Abbreviated Scale of Intelligence (Wechsler, 1999). The interval between two scanning sessions for each subject was 3–4 weeks. A block-design fMRI experiment was conducted during each scanning session. Two blocks of trials that were equivalent in difficulty were used for the two scanning sessions in counterbalanced order to control for possible learning effects.

Experimental task

The design of the *Arena* task is presented in Figure 1. The virtual reality environment consists of a circular arena adorned with abstract colour patterns on the walls. The task is to navigate successfully within this arena using a joystick. At the beginning of each trial the participants have to move towards the pole until they reach it (Figure 1, Acquisition: Start), and the participant has to move towards the pole until they reach it (Figure 1, Acquisition: Finish). This is followed by a 30-s presentation of a blank screen (Figure 1, Retention), during which participants are instructed to actively rehearse the remembered position of the pole. The participants are then moved to a different starting position within the arena with the pole removed (Figure 1, Recall: Start). They then have to use the patterns around the arena as cues to navigate towards the previous location of the pole (Figure 1, Recall: Finish). The different starting position ensures that only allocentric memory can be used to accurately locate the original pole position. After recall, participants are presented with 15 s of a blank screen (Figure 1, Rest I), followed by a 30-s presentation of a static picture with abstract coloured patterns resembling the *Arena* walls (Figure 1, Visual Control); and finally a 15-s presentation of a blank screen (Figure 1, Rest II). The Acquisition, Retention, Recall, and visual control conditions lasted for 30 s. A standard game joystick was used to navigate the *Arena* and allow the participant to indicate the pole's location during the recall phase via a response button. The joystick velocity was set to reach the pole no quicker than 22–26 s from the beginning of the recall epoch (depending on the starting position relative to the pole). If the participant has made no response, the location at the end of this recall epoch (i.e. at 30 s) was taken as the response position. A single trial lasted 2.5 min, with six trials presented during a single fMRI experiment. The total duration of the experiment was 15 min.

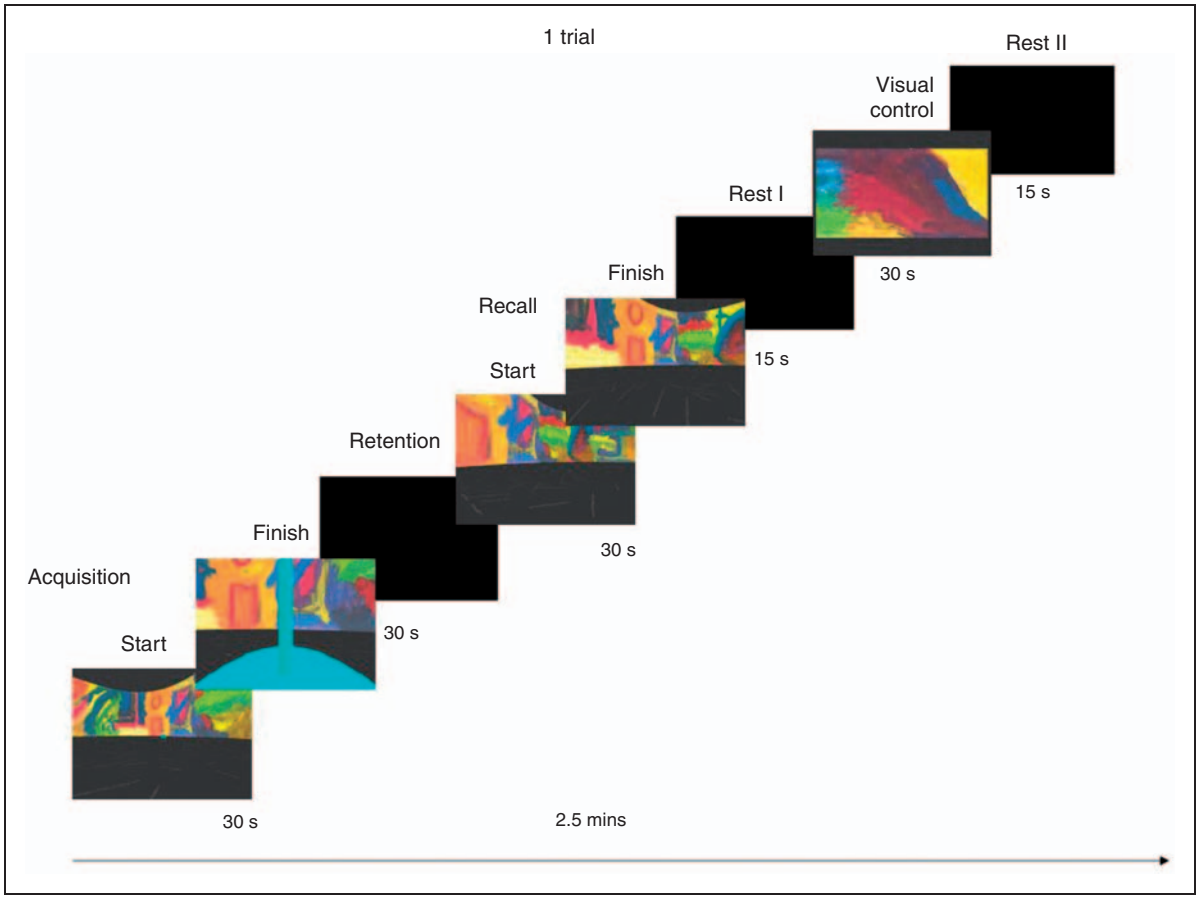

Figure 1. Functional magnetic resonance imaging design of one trial of the *Arena* task with snapshots of the *Arena* environment.

Off-line task training

Each participant was extensively trained on the task and had completed a block of six trials before each scanning occasion. The environment of the *Arena* (i.e. the patterns on the wall) was held constant between off-line training and scanning sessions, with the pole location varied between each training session, and between the training session and the subsequent scanning session. Each pole location was equivalent in difficulty. The extensive training was applied to ensure that participants not only became comfortable with the motor demands of the task, but had 'mapped' the *Arena* environment (i.e. learned multiple cue configurations) prior to scanning to facilitate allocentric spatial processing during on-line performance.

fMRI acquisition

Images were acquired using a 3.0-Tesla, General Electric Medical Systems Excite II scanner (General Electric, Milwaukee, WI, USA). Three-hundred image volumes (each consisting of 53 near-coronal slices) were collected using a gradient-echo echo planar imaging sequence with a repetition time of 3000 ms, an echo time of 30 ms and a 90° flip angle. The slices were positioned perpendicular to the main axis of the hippocampi. The body coil was used for RF transmission and an 8-channel head coil for RF reception. Each image slice was acquired using a 64×64 image matrix over a 24-cm field of view. The resulting in-plane pixel size of the images was 3.75 mm × 3.75 mm. The image slices had a thickness of 3.0 mm with a 0.3 mm gap. We chose thin slices to maximize our ability to identify small brain structures and to reduce the signal dropout owing to through-plane de-phasing effects. The total acquisition time of the time series was 15 min. Head movement was limited by foam padding within the head coil and a restraining band across the forehead. At the same session a 60-slice high-resolution gradient-echo echo planar sequence was acquired in both the coronal and axial planes with the same acquisition parameters apart from a 128×128 matrix, giving 1.875×1.875 in-plane resolution.

Subjective stress and arousal

Subjective stress and arousal, as well as the symptoms of dry mouth, were rated on present/not present basis by the clinician based on participants' verbal report 15 min pre-placebo/scopolamine administration, 45 min post-administration (before scanning) and 95 min post-administration (after scanning).

Data analysis

Behavioural data analysis. Performance accuracy was assessed using a mean displacement error, the mean distance in arbitrary units from the participant's finishing position during recall from the actual position of the pole during acquisition across six experimental trials. The significance of the difference in performance accuracy between placebo and scopolamine conditions was ascertained using a paired samples *t*-test in SPSS version 15.

fMRI data analysis. At the individual subject level, a standard general linear modelling approach was used to obtain estimates of the response size (beta) to each of the components of the *Arena* task (Acquisition, Retention, Recall, and Visual control). Following transformation of the fMRI data for each individual into standard space and smoothing with a 3D 7 mm FWHM Gaussian filter, the experimental model was convolved for each condition with gamma variate functions having peak responses at 4 and 8 s following stimulus onset to accommodate variability in blood-oxygen-level dependence (BOLD) response timing. By fitting these convolved model components to the time series at each voxel, beta estimates were obtained for each effect of interest. The standard errors of these beta estimates were computed non-parametrically using a bootstrap procedure designed to operate on time series data, containing serial dependencies, with repeated deterministic (experimentally determined) effects. This method is outlined in detail in Politis (2003). Parameter standard errors were estimated using 200 bootstraps at each voxel. Using the combined parameter estimates over all conditions, the mean fitted time series was also computed and, from the combined bootstrap parameter estimates for each bootstrap, the 95% confidence limits on the fitted time series.

The second-level analysis proceeded by computing the placebo minus scopolamine difference in effect size for each subject at each voxel and the standard error of this difference (using the bootstrap estimates derived above). The significance of these differences was then tested in three ways: (1) a simple parametric random effects (paired *t*-test), using only the placebo–scopolamine effect size differences; (2) a permutation test of the same random effects *t* statistic in which the null distribution was estimated by randomly swapping the signs of the differences; 40,000 permutations per voxel were used to obtain a confidence limit of 0.0007 to 0.0013 on an uncorrected *p*-value of 0.001; and (3) a mixed effects test using both the effect size differences and their subject-level standard errors to accommodate first (subject) level heteroscedasticity (Thirion et al 2007). This was also conducted using 40,000 permutations per voxel.

Results

Behavioural analysis

There was no significant difference in performance accuracy, as measured using the mean displacement error when comparing the placebo and scopolamine conditions (placebo: Mean = 12.73, SD = 6.21; scopolamine: Mean = 14.23, SD = 7.09; $t = -0.859$, df = 19, $p = 0.401$).

fMRI analysis

Acquisition versus rest. For both placebo and scopolamine conditions, a widespread neural network was activated during acquisition relative to rest, including dorsolateral prefrontal cortex (DLPFC), lateral and medial parietal and occipital cortices bilaterally, as well as brainstem and cerebellum on the left with placebo and bilaterally with scopolamine

(see Supplementary Materials for Tables S1 and S2, and Figure S2).

The main differences in the activation sites observed in the two conditions were large bilateral activation clusters centred in the fusiform gyrus and extending to hippocampus/parahippocampal gyrus in the placebo condition, and the activation of the right caudate nucleus and left cingulate gyrus (BA 24 and 31) in the scopolamine condition. Further difference included left-sided insula activation following placebo and right-sided activation of this region following scopolamine administration, as well as left-sided thalamus activation following placebo and bilateral activation of this region following scopolamine administration.

Recall versus rest. Following both placebo and scopolamine administration, similar brain networks were activated to that seen during acquisition, with the addition of frontal opercula areas (BA 10 and 11) of the right hemisphere (see Supplementary Materials for Tables S1 and S2, and Figure S2).

As during acquisition, significant hippocampal activation was only observed following placebo administration, whereas the caudate nucleus activation was only observed following scopolamine administration. Bilateral activation of the insula was observed with placebo and left-sided activation with scopolamine. Right-sided thalamic activation was seen following placebo administration whereas there was bilateral activation of this region with scopolamine. Finally, and in contrast to the pattern of activation in the acquisition condition, similar areas of the cingulate gyrus were activated bilaterally during recall with both placebo and scopolamine.

Visual control versus rest. The visual control task activated the frontal eye-field areas (BA 6 and 8) and occipital cortex bilaterally. There was no hippocampal activation following either placebo or scopolamine administration.

Placebo versus scopolamine. The detailed results of this analysis are presented in Table 1 and Figure 2.

A significantly stronger activation of the right hippocampus/parahippocampal gyrus and bilateral fusiform gyrus (BA 19/18) following placebo than scopolamine administration was observed during acquisition only ($p = 0.00055$). In addition, the left lateral temporal cortex (BA 20/21) was activated significantly more strongly under placebo than scopolamine during both acquisition and recall.

The right caudate nucleus and thalamus were activated significantly more strongly during both acquisition and recall following scopolamine than placebo administration.

Table 1. Brain regions showing differential activation in placebo and scopolamine conditions during acquisition and recall (mixed effects analysis, $t > [3]$, $p > 0.001$ uncorrected)

	Subregion (Brodmann Area)	Side	No. of voxels	Maxima voxel *x y z*	Subregion (Brodmann Area)	Side	No. of voxels	Maxima voxel *x y z*
	Acquisition				Recall			
Brain region	Placebo > Scopolamine				Placebo > Scopolamine			
Temporal Lobe	Superior Temporal Gyrus (21)	L	5	−62 −15 −3	Middle Temporal Gyrus (20)	L	14	−47 −30 −13
Limbic Lobe	Hippocampus/ Parahippocampal Gyrus (36)	R	15	22 −37 −10	–	–	–	–
Occipital	Fusiform Gyrus (19)	R	7	40 −63 −10	–	–	–	–
	Fusiform Gyrus (18)	L	9	−25 −85 −13	–	–	–	–
	Scopolamine > Placebo				**Scopolamine > Placebo**			
Frontal Lobe	Medial Frontal Gyrus (10)	R	5	7 59 −7	Middle Frontal Gyrus (46)	L	8	−43 37 30
	–	–	–	–	Medial Frontal Gyrus (6)	R	49	11 11 63
	–	–	–	–		R	6	4 −15 66
Basal Ganglia	Putamen	R	51	18 11 −10				
	Caudate Body	L	6	−18 4 −10				
		R	44	11 11 13	Caudate Body Anterior Cingulate (24) Amygdala	R	79	14 1 17
Limbic Lobe	–	–	–	–		L	5	−1 7 40
	–	–	–	–	Posterior Cingulate (23)	R	66	18 −7 −10
	–	–	–	–		R	71	7 −26 33
Thalamus					Ventral Lateral Nucleus	R	153	7 −11 7
Parietal Lobe	–	–	–	–	Inferior Parietal Lobule (40)	L	8	−51 −26 26
	–	–	–	–	Precuneus (7)	R	211	11 −59 43
	–	–	–	–		L	21	−11 −70 36
Cerebellum	–	–	–	–	Anterior Lobe	R	10	7 −44 3
	–	–	–	–	Posterior Lobe	R	16	29 −44 −33

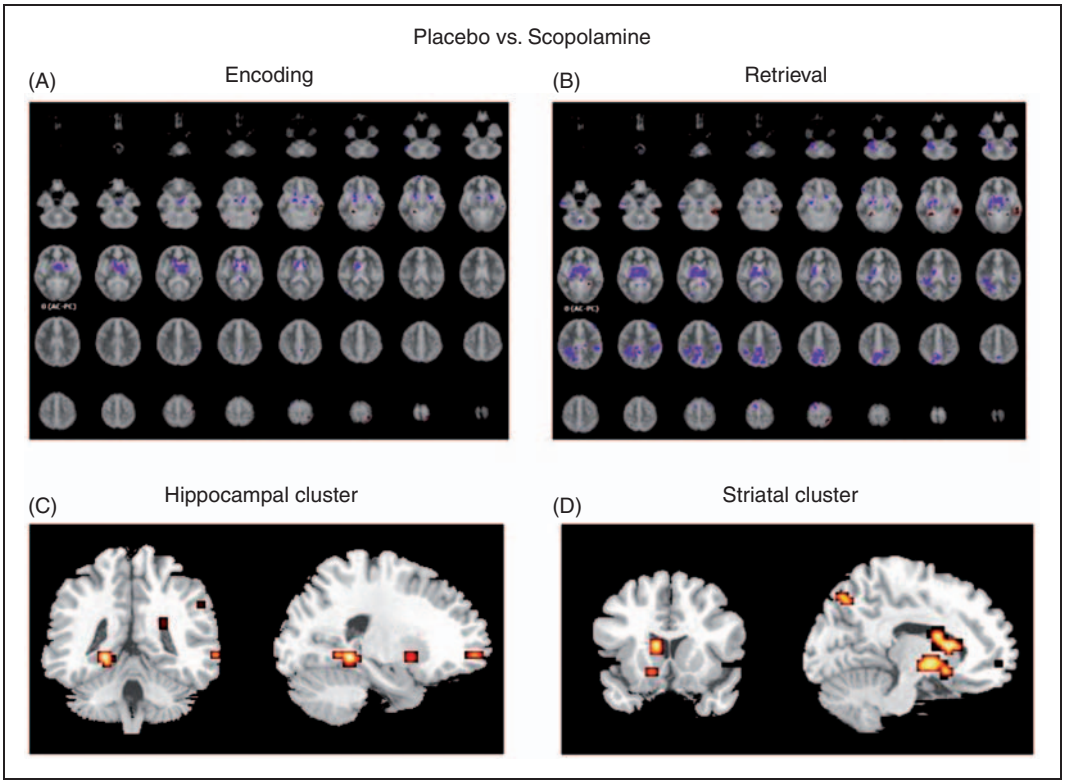

Figure 2. Top row: activation maps thresholded at $t > [3]$ ($p < 0.001$ uncorrected) of the difference between placebo and scopolamine conditions during (A) acquisition and (B) recall overlaid on axial slices of 3.3 mm thickness throughout the brain (Red: placebo > scopolamine; Blue: scopolamine > placebo; left = right). Bottom row: (C) hippocampal/parahippocampal cluster significantly more strongly activated during acquisition following placebo administration and (D) caudate nucleus cluster significantly more strongly activated during acquisition and recall following scopolamine administration overlaid on a coronal and sagittal slice (left = right).

The same was also observed for bilateral putamen during acquisition, and left DLPFC (BA 46), inferior parietal lobule (BA 40), right medial frontal gyrus (BA 6), amygdala, cingulate gyrus (BA 24 and 23), precuneus (BA7), and cerebellum during recall.

In order to characterize these effects in more detail, we extracted time series information from the most strongly differentially (placebo–scopolamine) activated voxels in the hippocampus/parahippocampal gyrus, caudate nucleus and amygdala. As can be seen from the fitted time series and their 95% confidence limits (Figure 3) hippocampal activation was attenuated following scopolamine administration during acquisition, whereas caudate nucleus and amygdala were deactivated with placebo, but either relatively inactive or activated following scopolamine administration during both acquisition and recall.

Subjective stress and arousal

All participants in the scopolamine condition reported subjective experiences of arousal, stress and symptoms of a dry mouth. In addition, four participants who received placebo first were noted to have the same experience in the placebo condition. The mean accuracy for these participants was 20.98 (SD = 6.48) under placebo and 13.58 (SD = 6.82) under scopolamine treatment, compared with the remaining group means of 10.67 (SD = 4.23) and 14.39 (SD = 7.37) for placebo and scopolamine, respectively. Consequently, we reanalysed the behavioural data, removing these participants. With these participants removed from the analysis the mean accuracy of the scopolamine group was largely unchanged (scopolamine: Mean = 14.39, SD = 7.37). By contrast, the mean accuracy of the placebo group was markedly improved (placebo: $N = 20$ Mean = 12.73, SD = 6.21 to $N = 16$ Mean = 10.67, SD = 4.23); the latter result being consistent with the behavioural effect of 0.4 mg dose of scopolamine on memory task performance in previous human studies. A t-test of these means revealed a significant effect of treatment showing that the scopolamine significantly decreased accuracy ($t = -2.146$, df = 15, $p = 0.049$). (Figure 4 presents a bar chart of means and standard errors of performance accuracy for the whole group, for four participants who experienced placebo effect, and the remaining 16 participants.)

We reanalysed the fMRI data excluding these four participants. The overall pattern of brain activations during placebo and scopolamine was unchanged, only the power of the effects was affected. Importantly, all four participants showed significant ($p < 0.001$) activation of the hippocampus during acquisition under placebo.

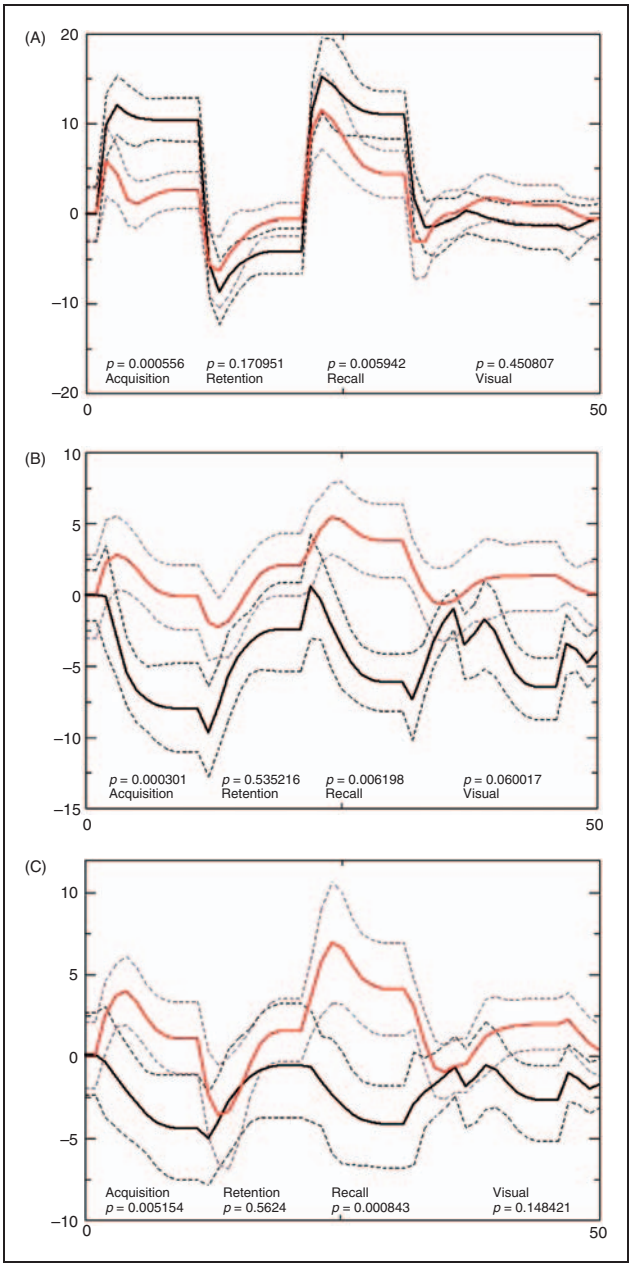

Figure 3. Mean fitted time series extracted from: (A) right hippocampus/parahippocampal gyrus [22,−37,−10]; (B) right caudate nucleus [18, 4,−10]), and (C) right amygdala [18, −7, −10] during the experiment in placebo and scopolamine conditions. (Placebo: Black solid line = mean fitted values, Black dashed line = 95% Confidence Interval; Scopolamine: Red solid line = mean fitted values, Red dashed line = 95% Confidence Interval).

Discussion

The main objective of the present study was to investigate the effect of the centrally active muscarinic antagonist scopolamine on neural activity during allocentric spatial memory in humans using a virtual reality analogue of the MWM. A widespread neural network comprising DLPFC, lateral and medial parietal cortex, thalamic and cerebellar areas

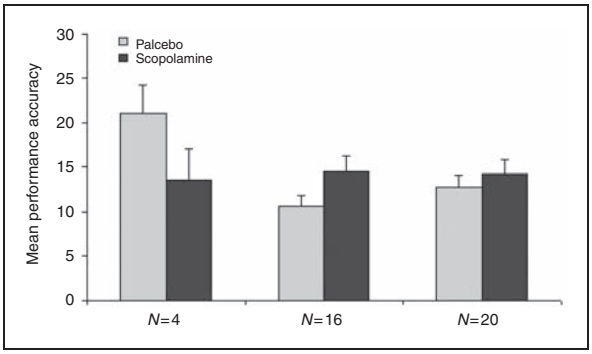

Figure 4. A bar chart comparing the means and standard errors of *Arena* performance accuracy during placebo and scopolamine conditions for the whole sample ($N = 20$), four participants who have experienced the side effects of scopolamine when administered with placebo, and the remaining 16 participants.

was activated following both placebo and scopolamine administration. As predicted, hippocampal/parahippocampal gyrus activation was significantly disrupted by scopolamine challenge, and this effect was particularly strong during acquisition. A network of other brain regions was significantly more activated by scopolamine than placebo, including striatum during both acquisition and recall, orbitofrontal cortex during acquisition, and DLPFC (BA 46), supplementary motor area (SMA, BA 6), amygdala, thalamus, ventral anterior and posterior cingulate, precuneus, and cerebellum during recall.

The differential effect of scopolamine on hippocampal activity during the MWM procedure observed in the present study extends this well-documented finding in animals to humans. The mechanism of the effect of scopolamine on information acquisition has been simulated in a network model of hippocampal function (Hasselmo and Wyble, 1997), suggesting that scopolamine impairs learning of new information by decreasing spiking activity within the hippocampus. Furthermore, Cornwell et al. (2008) have demonstrated greater theta oscillations in human hippocampus and parahippocampal gyrus during spatial learning of a hidden platform in a virtual reality MWM using magnetoencephalography, with hippocampal theta activity being highly predictive of performance. Interestingly, the observed posterior hippocampal activity during virtual spatial navigation in the study of Cornwell et al. peaked at around 0.25–1.25 s after trial onset. In our study, the time-course of hippocampal activation measured by BOLD signal was very similar. The BOLD response measured with fMRI has been previously shown to correlate with local field potentials (input and its intracortical processing), but not multi-unit spiking activity (output) of the neurons (Logothetis et al., 2001). Future studies should employ simultaneous electroencephalography/fMRI measurement to investigate the effect of scopolamine on hippocampal spiking activity and theta oscillations during allocentric spatial memory in humans.

The current study has revealed dissociation between hippocampus-based and striatum-based memory system activations following placebo and scopolamine administration. Hippocampal activation under placebo was accompanied by

striatal deactivation, whereas attenuated hippocampal activation under scopolamine was accompanied by a significantly stronger activation of the striatum. A differentiation between the role of the hippocampus-based memory system (associated with place or spatial learning) and the striatum-based memory system (associated with distinct cue/landmark learning) has been shown in the MWM task in rats (Packard and McGaugh, 1992). A similar dissociation between the hippocampus and dorsal striatum in rats was also observed using a T-maze task, where successful performance could be achieved either via use of extra-maze spatial information or a simple motor response (Packard and McGaugh, 1996). Relative ACh levels in hippocampus and dorsal striatum were found to predict the choice strategy in a T-maze task (McIntyre and Gold, 1999), with high hippocampal ACh relative to dorsal striatum predicting a place response, and high striatal ACh relative to hippocampus predicting a distinct cue response strategy. This dissociation between two memory systems in place learning has been demonstrated in humans using fMRI and an eight-arm maze virtual reality task (Bohbot et al., 2004; Iaria et al., 2003). Both studies have observed that the spatial strategy was associated with significant hippocampal activity, whereas the distinct cue response strategy was associated with sustained activity in the caudate nucleus. In the *Arena* task, the use of a cue response strategy was expected to be minimized by shifting the starting position of the participant between acquisition and recall. It was thought that the pole location could not be straightforwardly retrieved by direct reference to either egocentrally encoded direction or proximal place cues. Nevertheless, the task can be solved in an egocentric fashion using spatial manipulation of the visual display at the recall stage. For example, the spatial array at recall could be continuously rotated using a combination of the depth cues and pattern recall in order to recreate egocentric geometric coordinates. This is a similar process to the one suggested by Wang (2003) in relation to human memory, in which humans are able to solve allocentric tasks by updating their representations while moving, with positions coded learned using associative memory (Shelton and McNamara, 1997; Wang, 1999). It should be noted that in humans there is substantial evidence that non-mnemonic allocentric manipulation and representation, such as mental rotation ability, is not reliant on hippocampal function, but thought to be reliant on parietal lobe functioning (Abrahams et al. 1999; Feigenbaum et al. 1996; Worsley et al. 2001), such that this type of process might not be affected by hippocampal modulation.

Little is known about the neurobiological mechanisms mediating interactions between hippocampal and striatal memory systems. Possible mechanisms include direct anatomical projections or indirect modulatory influences of other brain structures (Poldrack and Packard, 2003). The cholinergic blockade of the hippocampus following scopolamine administration in the present study might have directly disinhibited the striatum. Alternatively, activation/disinhibition of the striatum could have been modulated by inhibition/activation of other brain regions. Thus, the parahippocampal (and fusiform) gyrus activity was significantly attenuated following scopolamine administration. The parahippocampal cortex is thought to have a role in cognitive mapping of the environment by providing spatial scene information to the hippocampus (e.g. Kohler et al., 2002; Maguire et al., 1998). In the absence of such input from the parahippocampal cortex following the cholinergic blockade, the striatal memory system might have been engaged to compensate using a simpler encoding strategy based on a single distinctive cue/landmark. Furthermore, we observed dissociation between hippocampal and amygdala activity following scopolamine administration. Recent evidence suggests that the amygdala may exert a modulatory influence on hippocampal and neostriatal memory systems (review, Packard and Cahill, 2001).

Along with striatal activity we observed the activation of the fronto-thalamic-cerebellar circuitry during recall under scopolamine. This neural network is known to associate with working memory for both verbal and spatial stimuli (Smith and Jonides, 1998). Its activation suggests the use of a verbally based response strategy during spatial navigation (distinct cue/platform location) following scopolamine administration and perhaps more effortful processing during recall, possibly as a compensatory mechanism due to disrupted hippocampus-based allocentric processing. It was not in the aims of the present study to investigate the effect of scopolamine on formation of different strategies, nor did we have any hypothesis about the differential affect of scopolamine on acquisition and recall during allocentric spatial learning and, therefore, the functional significance of the differential effect of cholinergic blockade on different memory systems and memory processes could not be established from the design of the present study. These findings deserve further investigation given the role of cholinergic dysfunction in memory impairments.

In the current study, the effect of muscarinic blockade on allocentric spatial memory and hippocampal function was investigated using an analogue of the working memory version of the MWM. In the original reference memory version of the MWM procedure (Morris, 1981), an animal is trained over a number of days on the same platform location and tested on this location on day 5–6. The recall of the platform location under these circumstances can be insensitive to hippocampal lesions (Eichenbaum et al., 1990; Olton, 1977), presumably due to memory consolidation (Morris et al., 2003). In the working memory version, the platform is moved to a novel location each day and the animal is re-tested on their memory for the location within a timescale of seconds to minutes, with a maximum timescale of hours (e.g. Whishaw, 1985). No consolidation is required and the successful recall would depend on sustained spiking activity within the hippocampus. Earlier behavioural studies of the effect of scopolamine on hippocampal-based learning and memory in rodents have generally observed that procedures requiring working rather than reference memory are more sensitive to central muscarinic blockade (Bartolini et al., 1992; Givens and Olton, 1994; Moran, 1993). More recent studies employing the reference memory version of the MWM have, however, shown scopolamine-induced disruption of platform location acquisition (Herrera-Morales et al., 2007; Von Linstow Roloff et al., 2007). It remains to be investigated whether scopolamine disrupts place acquisition in the reference memory version of the MWM in humans.

Despite the robust fMRI findings, it appears that the behavioural performance of a number of participants on the task may have been affected by some aspects of the procedure, such as the MRI enclosure or the stress of receiving a drug of which they have no experience of. Specifically, four participants that received placebo as their first treatment reported subjective feelings of stress that may have had a significant impact on the accuracy of their performance. In the absence of the fMRI data it may have been concluded that the drug and/or procedure lacked sensitivity. However, the fMRI data show robust activation of the hippocampus by the task under placebo and reduced activation under scopolamine with these four participants included in the fMRI analysis. In this procedure the fact that the hippocampus activations are relatively unaffected by the subjective feeling of arousal, stress and symptoms of a dry mouth suggests that greater power may be required to detect behavioural changes than brain activations. Further work will be required to confirm these observations, but clearly the combination of fMRI and behaviour significantly aids the interpretation of each individual dataset.

In conclusion, with the resurgence of interest in the scopolamine-reversal model for treatment development in AD (Buccafusco et al., 2008), there is a strong drive to develop human tests of allocentric spatial memory with procedures analogous to those used in animal experiments in order to aid the rapid translation of animal to human clinical trials of compounds showing promising cognition-enhancing effects. This study is the first demonstration of septohippocampal cholinergic system involvement in hippocampus-based allocentric spatial memory in humans using the working memory MWM analogue, lending credence to the use of the MWM in scopolamine-reversal approaches to drug discovery in animals for the treatment of memory impairment associated with AD. Furthermore, the study is the first to demonstrate the dissociation between the hippocampal and striatal memory systems under cholinergic blockade in allocentric spatial memory in humans. Further studies are needed to investigate the effect of cholinergic blockade on the reference memory version of the MWM, and to address the interaction between septohippocampal and nucleus basalis–amygdala cholinergic projections in allocentric spatial memory in humans.

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