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ORIGINAL PAPER

Cholinergic blockade under working memory demands encountered by increased rehearsal strategies: evidence from fMRI in healthy subjects

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Abstract The connection between cholinergic transmission and cognitive performance has been established in behavioural studies. The specific contribution of the muscarinic receptor system on cognitive performance and brain activation, however, has not been evaluated satisfyingly. To investigate the specific contribution of the muscarinic transmission on neural correlates of working memory, we examined the effects of scopolamine, an antagonist of the muscarinic receptors, using functional magnetic resonance imaging (fMRI). Fifteen healthy male, non-smoking subjects performed a fMRI scanning session following the application of scopolamine (0.4 mg, i.v.) or saline in a placebo-controlled, repeated measure, pseudo-randomized, single-blind design. Working memory was probed using an n-back task. Compared to placebo, challenging the cholinergic transmission with scopolamine resulted in hypoactivations in parietal, occipital and cerebellar areas and hyperactivations in frontal and prefrontal areas. These alterations are interpreted as compensatory strategies used to account for downregulation due to muscarinic acetylcholine blockade in parietal and cerebral storage systems by increased activation in frontal and prefrontal areas related to working memory rehearsal. Our results further underline the importance of cholinergic transmission to working memory performance and determine the specific contribution of muscarinic transmission on cerebral activation associated with executive functioning.

Keywords Cholinergic system · Scopolamine · Muscarinic antagonist · Working memory · fMRI · n-back task

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Introduction

The cholinergic system constitutes one of the most important transmission systems for mediating cognitive processes in humans. It consists of two subsystems: the muscarinic system and the nicotinic system, with cholinergic projections originating in the nucleus basalis of Meynert and the substantia innominata in the basal forebrain [21, 39, 54]. By projecting to the hippocampus and to frontal areas, they mediate fundamental cognitive processes [55]. Among others, learning and memory as well as

attention and processing speed are critically modulated by cholinergic transmission via acetylcholine [16, 55].

Evidence for the impact of cholinergic transmission on cognitive processes mainly derives from studies in patients with neuropsychiatric disorders like Alzheimer's disease or schizophrenia. Besides other symptoms, these patients present cognitive deficits while at the same time exhibiting specific alterations of the cholinergic system as evidenced by post-mortem studies [20, 25, 33, 42, 50, 56].

In addition, research also focuses on a more profound understanding of the association of cholinergic transmission and cognitive processes in healthy samples. Depending on the concentration and task, an increase in cholinergic transmission, for example via smoking, produces improvements of cognitive functions like memory and attention [2, 7, 14, 37, 58, 67], whereas blocking cholinergic transmission impairs cognition [15, 32, 35, 43, 51, 65, 73].

Previous behavioural studies have consistently shown that scopolamine, a powerful selective muscarinic antagonist, impairs cognitive functions like verbal fluency ([1], but also see [49]), memory [35, 52, 60], working memory [30], and, to a lesser extent, attention ([35], for reviews see [36, 48]). Only few studies examined the influence of blocking cholinergic transmission using imaging techniques, and existing studies mainly concentrated on memory functions and attention processes. For example, Sperling et al. [64] compared the effects of lorazepam (a benzodiazepine drug) and scopolamine on episodic memory performances in a study using fMRI. In their study, scopolamine differentially impaired behavioural performance accompanied by decreased activations in inferior prefrontal, hippocampal and fusiform areas. Thienel et al. [68] could demonstrate that scopolamine impairs the functional and behavioural correlates of attention. In this fMRI study, the Attention Network Task (ANT) was used and besides increases in reaction time, results showed decreased activations in the anterior cingulate cortex for conflicting stimulus processing and decreased activations in left superior and left middle frontal brain areas in the orienting condition of the task.

Regarding the effects of muscarinic blockade on higher cognitive functions, for example working memory, the available literature is even smaller. Nevertheless, a study published by Thomas et al. [70] compared the effects of placebo, scopolamine and donepezil (an acetylcholinesterase inhibitor, increasing ACh transmission) on working memory performances in elderly healthy subjects. Results demonstrated that blocking the muscarinic transmission with scopolamine induced large impairments across all aspects of working memory performance, which were partially ameliorated by the simultaneous administration of donepezil. Administration of donepezil alone improved performances.

Furey et al. [23] showed that enhancement of cholinergic transmission via physostigmine—an indirectly acting cholinomimetic drug—improves performance on a working memory task. Using fMRI, the authors revealed that increased cholinergic transmission resulted in enhanced neural processing in ventral visual cortical regions and at the same time reduced activity in the anterior prefrontal cortex.

Furthermore, a recent study using positron emission tomography (PET) conducted by the same group specified that cholinergic augmentation via physostigmine compensated increasing working memory loads which under placebo resulted in systematic increases in the magnitude of neural responses in regions of the prefrontal cortex related to working memory [24].

To conclude, working memory processing is influenced by cholinergic transmission that can be modulated by the muscarinic antagonist scopolamine. Although the significance of cholinergic transmission for cognitive processes like memory and attention is well established, the basic mechanisms and principles especially regarding higher cognitive processes have not been fully understood.

Selective blockade of one of the two cholinergic subsystems allows the examination of the contribution of each of the receptor systems to an applied cognitive paradigm, which will lead to a better understanding of the specific contribution of these subsystems.

The specific impact of scopolamine on the neural correlates of working memory integrity has not been investigated in sufficient depth. In order to clarify its cerebral as well as behavioural effects, we applied a well-established verbal n-back paradigm (0- and 2-back) in an fMRI study in healthy subjects. Previous results clearly outline an n-back-related pattern of activation in a fronto-parietal network [12, 46, 57], thus, in areas addressed by the cholinergic system. The verbal n-back paradigm and especially the 2-back task are considered to rely on a number of working memory processes, including maintenance, monitoring, updating and manipulation of remembered information ([26], for a review see [46]). Based on previous behavioural and imaging studies applying different paradigms, we hypothesized that blocking the muscarinic transmission with scopolamine as compared to placebo would result in interferences within the fronto-parietal-cerebellar areas related to working memory performance. Specifically, we expected that challenging the cholinergic system by blocking the muscarinic transmission would require a compensation of this loss via concentrating primarily on brain areas related with active rehearsal of verbal material. Hence, we expected hyperactivations in frontal

areas following application of scopolamine as compared to administration of placebo.

Methods and materials

Subjects

Fifteen healthy male adults, aged 23–29 years ($M = 25.6$, SD $= \pm 2.2$), participated in this fMRI study. We restricted the sample to male subjects to avoid influence due to gender differences in brain activation. Subjects were recruited through advertisements at the local university hospital. All subjects were students and proficient in German. Subjects had a mean IQ of 113.8 (SD = 16.9; verbal IQ estimate) [38].

Participants were intensely screened for medical, neurological and psychiatric history. The Structured Clinical Interview for DSM-IV (SCID-I, German Version) [74] was performed by experienced raters to exclude lifetime diagnosis for axis 1. Subjects with first-degree relatives with psychiatric disorders were excluded. The subjects were included in the study if they were non-smokers, right-handed (Edinburgh Inventory) [45], not currently on any medication and had no history of abuse of alcohol or psychoactive substances. Additional laboratory evaluations (ECG, blood sample analyses, urine drug screening) were completed to rule out medical disorders and current drug abuse habits. Furthermore, the usual exclusion criteria for MRI were applied (e.g. metallic objects in the body, tattoos and disorders that affect cerebral metabolism).

Subjects gave written informed consent prior to taking part in the study, which was approved by the RWTH Aachen Ethics Committee according to the Declaration of Helsinki and by the Federal Institute for Drugs and Medical Devices (BfArM). Subjects were paid for their participation.

Stimuli

The working memory task consisted of a previously established version of the n-back paradigm [34, 57]. Two active conditions and a baseline condition were applied. The participants viewed sequences of single letters (A–Z) and had to react upon two instructions: in the 0-back condition, subjects were asked to react as soon as they saw the letter 'X' on the screen. The 2-back condition required button presses as soon as a letter occurred that matched the last but one presented letter. In the high-level baseline condition, subjects were asked to just concentrate on the occurring letters without any motor response.

Stimuli were printed in red and appeared on a black background. Letters were presented for 500 ms and a reaction had to appear within 900 ms. The response button (LUMItouch) had to be pressed with the right index finger. Baseline, 0-back- and 2-back condition consisted of 19 stimuli with 0- and 2-back comprising 7 targets (=target probability of .37). Conditions were introduced by instruction slides. Stimulus presentation was controlled by the software package PRESENTATION (Neurobehavioral Systems Inc., San Francisco, CA). The whole task consisted of eight 0-back-, seven 2-back- and 15 baseline-conditions and lasted 15 min.

Cholinergic challenge procedure

The study employed a single-blind, double-dummy (oral/intravenous), time-elapsed, repeated measures design. Subjects were tested under three conditions—placebo/placebo (PP), scopolamine/placebo (SP) and mecamylamine/placebo (MP)—which were separated by at least 1 week. On arrival, the participants underwent a medical and neurological examination, followed by the administration of either an oral dose of mecamylamine (0.2 mg/kg, max. 15 mg) plus intravenous placebo (saline) or intravenous scopolamine (0.4 mg) plus oral placebo, or intravenous placebo and oral placebo in the placebo condition. Thus, a maximum of one active substance was given at a time, that is there was no mecamylamine plus scopolamine but a placebo/placebo condition. The dosing and timing of medication were chosen according to the literature to optimize any possible cognitive effects, while minimizing physical side effects [66]. The order of the application of the active substance was pseudorandomized across subjects, and medications were given under single-blind conditions. Since the effects of scopolamine and mecamylamine were not compared directly and due to limited space, mecamylamine results are described elsewhere.

Ninety minutes after drug administration, the scanning session began. Participants were observed for at least 3 h following the administration to make sure that no further side effects occurred.

Side effects

None of the subjects reported side effects following placebo administration. After scopolamine administration, all subjects reported modest side effects that mainly included dry mouth and feelings of sedation. One subjects had to be excluded after scopolamine administration due to excessive sedation during the fMRI (subject fell asleep during the scanning session).

Data acquisition

Imaging was performed on a 1.5 Tesla Avanto MR scanner (Siemens Medical Systems) in the Institute of Neuroscience and Biophysics—Medicine, Research Centre Juelich.

Functional images were obtained with echo-planar imaging (EPI), which is sensitive to blood oxygenation level–dependent (BOLD) changes. Repetition time was 2.8 s with a flip angle of 90° and an echo time of 60 ms. These images consisted of 29 slices of 4 mm thickness (gap between slices 0.4 mm) and had an in-plane resolution of 64 × 64 pixels. The field of view was 200 × 200 mm^2 resulting in a voxel size of 3.125 × 3.125 × 4 mm^3. Slices covered the whole brain and were positioned transaxially parallel to the anterior–posterior commissural line (AC-PC). For each subject, a time series of 338 images was acquired; the first five of which were discarded in order to allow for scanner equilibration.

Behavioural data analyses

Three behavioural variables were assessed for the n-back task: hits, misses and false alarms. The results originate from the logfiles of twelve subjects, two logfiles had to be discarded due to technical failure during the scanning session. Differences were calculated by paired t-tests using SPSS 18 (SPSS Inc., Chicago, USA).

FMRI data analysis

Data analysis was performed with SPM5 (http://www.fil.ion.ucl.ac.uk/spm/). Functional images were realigned using the 'register to mean' option in SPM5 and resliced for timing and motion corrections. Subsequently, they were normalized to Montreal Neurological Institute space (MNI) and smoothed with a 10-mm isotropic Gaussian Kernel. The time series data were high-pass filtered with a high-pass cut-off 1/128 Hz to remove low frequencies.

Statistical analysis was performed in a two-level, mixed-effects procedure. On the first level, the BOLD responses for both conditions were modelled by a boxcar function convolved with the canonical hemodynamic response function employed by SPM5. Contrast images for each subject were calculated contrasting the 2-back and the 0-back condition focusing on working memory processes. The group analysis was based on a random effect model, applying one-sample *t* tests per group (scopolamine and placebo) per contrast. Comparisons between the scopolamine and the placebo condition were performed on these contrasts with two-sample *t* tests for dependent samples.

In order to account for multiple comparisons, we applied a combined height and extent threshold technique based on Monte Carlo simulations of whole-brain activation. Assuming an alpha-error voxel activation of $P < 0.001$, after 10,000 simulations a cluster size of 45 contiguous resampled voxels was indicated to prevent a false positive rate above 5% due to multiple testing [19, 47, 61, 68].

Results

Behavioural data

Analyses for the attention (0-back) condition revealed significant differences between scopolamine and placebo for accuracy and errors, but no significant difference for false alarms (see Table 1). Regarding this condition, scopolamine produced a significant higher number of hits and at the same time a significant number of misses compared to placebo. Analyses for the 2-back condition focusing on working memory showed no group differences between placebo and scopolamine in accuracy and errors, but a significant difference between scopolamine and placebo in terms of false alarms: scopolamine led to a significant increase in false alarms (Table 1).

Imaging data

Under working memory load (2-back versus 0-back contrast), subjects activated a widespread network encompassing the bilateral dorsolateral prefrontal cortex (DLPFC), the inferior parietal lobule and cerebellum (Table 2; Fig. 1).

Table 1 Group comparisons between placebo and scopolamine: group means (M ± SD), *T* values and *P* values of hits, misses and false alarms for the 0-back and 2-back condition

	Placebo	Scopolamine	*T* (*df*)	*P*
Hits 0-back	48.92 (0.277)	52.3846 (5.25259)	−2.427 (12)	0.032*
Hits 2-back	46.92 (2.813)	44.9231 (4.21231)	1.606 (12)	0.134
Misses 0-back	0.08 (0.277)	3.6154 (5.25259)	−2.481 (12)	0.029*
Misses 2-back	2.08 (2.813)	4.0769 (4.21231)	−1.606 (12)	0.134
False alarms 0-back	1.08 (0.760)	1.5385 (1.76141)	−1.032 (12)	0.323
False alarms 2-back	0.08 (0.277)	0.8462 (1.06819)	−2.739 (12)	0.018*

* $P < 0.05$, ** $P < 0.08$ (Bonferroni corrected)

Scopolamine (contrast Placebo > scopolamine) reduced the blood oxygen level–dependent (BOLD) effect mainly in the left precuneus, right inferior frontal lobe, superior temporal lobe and angular gyrus (Fig. 2). Increased activation during blockade of cholinergic transmission by scopolamine (contrast Scopolamine > placebo) was found exclusively in frontal areas: Significantly greater activation during scopolamine compared to placebo was found in the superior medial frontal cortex bilaterally, the gyrus rectus/orbitofrontal gyrus and the inferior frontal gyrus (see Fig. 3).

Table 2 Local maxima for the clusters activated for the contrast 2back versus 0back for scopolamine > placebo and placebo > scopolamine (Monte Carlo-corrected threshold of $P < 0.05$, extend threshold $k > 45$ continuous voxels)

2back versus 0back			
Region	MNI (x,y,z)	Z	*k*
Scopolamine > placebo			
L Rectus (BA 11)	0, 42, −24	3.51	329
L medial frontal gyrus (BA 9)	−6, 60, 24	3.03	207
L Insula (BA 13)	−36, 0, 15	2.66	85
Placebo > scopolamine			
L precuneus (BA 7)	−15, −72, 60	4.82	9,230
R inferior frontal gyrus/pars triangularis (BA 45)	39, 30, 0	2.87	607
R middle frontal gyrus (BA 9)	33, 36, 39	2.38	116
L precentral gyrus (BA 9)	−42, 9, 30	2.24	49

Discussion

We investigated the effect of blocking muscarinic transmission on the neural correlates of working memory in an fMRI study in healthy non-smoking males.

Our study yielded four main results: (1) compared to placebo, blocking cholinergic transmission through the administration of scopolamine, an antagonist of muscarinic receptors, increased the rate of false positives on an n-back task but did not affect hits on the 2-back condition. Regarding the 0-back condition, (2) scopolamine increased the rate of hits as compared to placebo. Neurofunctionally, (3) a decrease in activity was prominent in parietal, occipital and temporal regions under scopolamine compared to placebo whereas (4) increased activity was found exclusively in frontal/prefrontal areas. Results are discussed in the light of a downregulation of cholinergic transmission as a consequence of the muscarinic receptor blockade and compensatory effects to adhere relatively normal performance.

Behavioural results

On the behavioural level, a significant increase in hits and an associated significant decline in misses were observed in the 0-back condition after scopolamine application. This can be interpreted as a form of 'over-compensation' due to the drug effects: The fact that subjects produced fewer correct answers under placebo as compared to the cholinergic challenge condition is most likely due to the fact that the 0-back condition is very easy to solve and does not represent any cognitive challenge for the subjects.

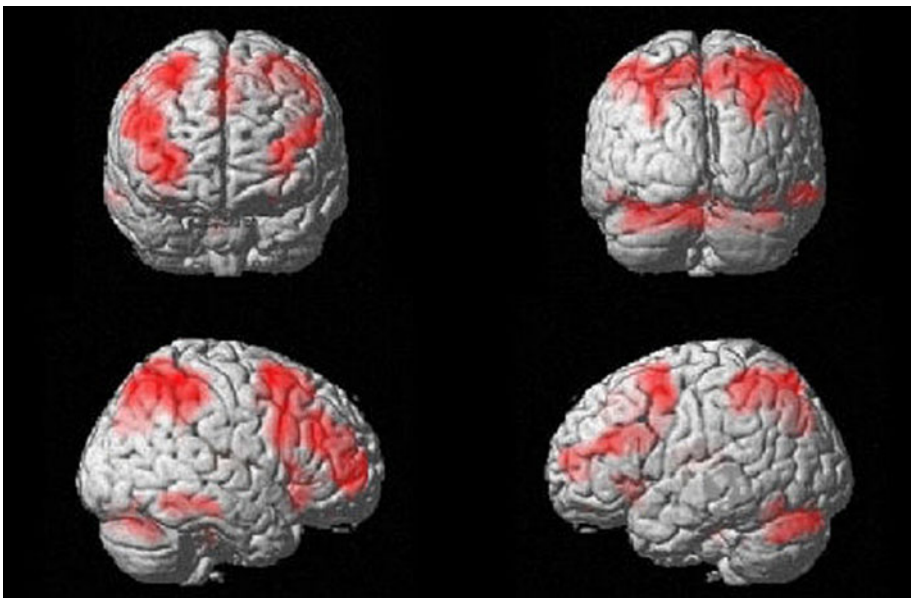

Fig. 1 Group activation (one-sample *t* test, $P < 0.05$) for the working memory contrast (2back vs. 0back) for the placebo condition. Activation emerged in a fronto-parietal-cerebellar network typically found during working memory tasks

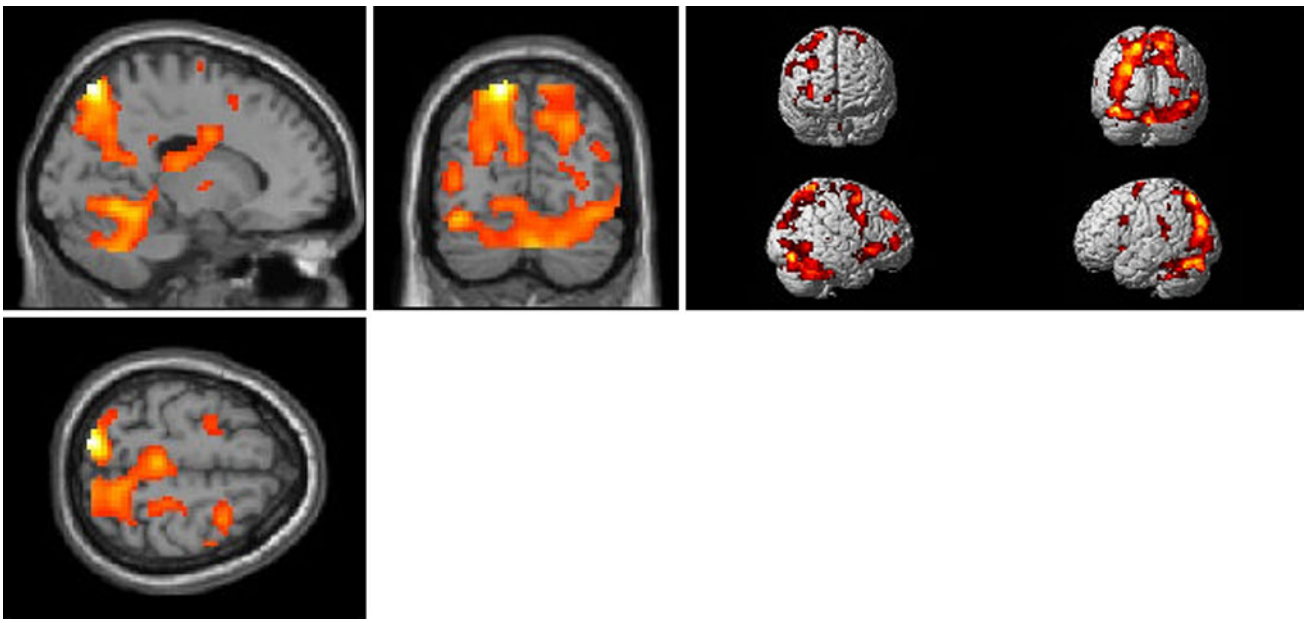

Fig. 2 Significant group differences in fMRI activation (two-sample t test) for the contrast 2back vs. 0back between placebo and scopolamine (uncorrected height threshold, $P = 0.05$; extent threshold, $k = 45$ voxels) showing increased activations under placebo in the left precuneus and precentral gyrus, the right inferior and middle frontal gyrus (projections onto a standard brain)

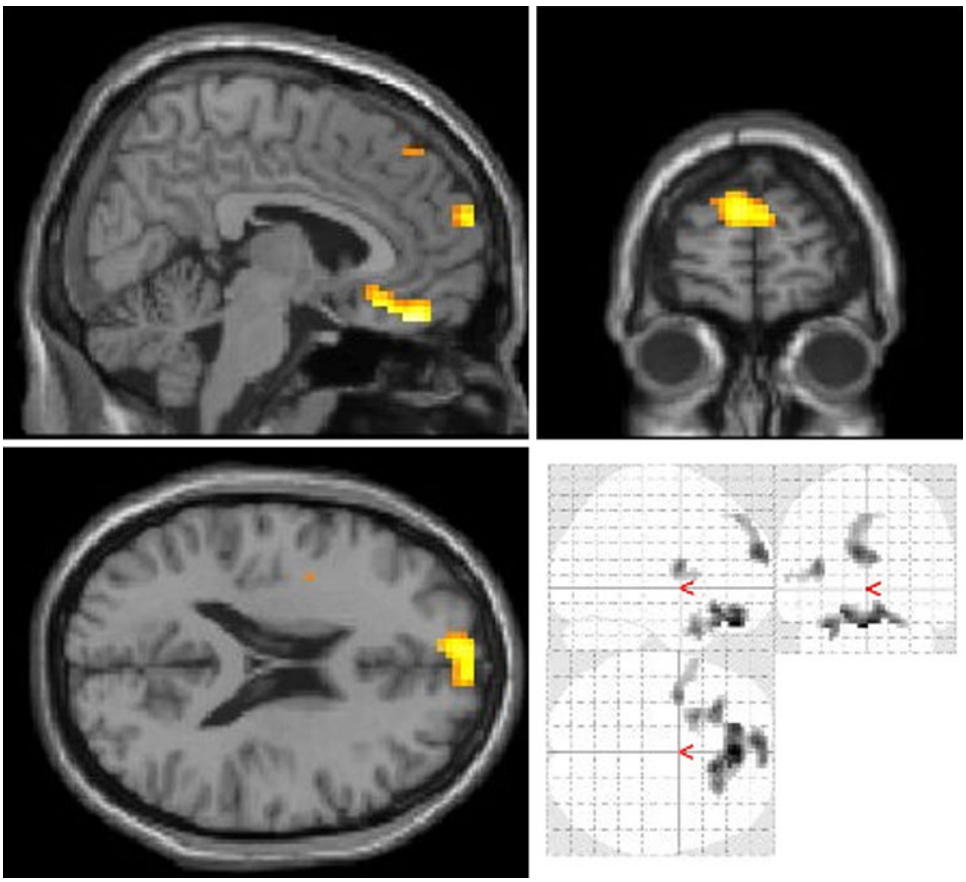

Fig. 3 Significant group differences in fMRI activation (two-sample t test) for the contrast 2back vs. 0back between scopolamine and placebo (uncorrected height threshold, $P = 0.05$; extent threshold, $k = 45$ voxels) showing increased activations under scopolamine in the left gyrus rectus, the left medial frontal gyrus and the left insula (projections onto a standard brain)

Therefore, some careless mistakes can be expected. On the other hand, it is assumed that due to the downregulation of the muscarinic transmission under scopolamine, the subjects had to be more focused to keep their regular level of performance. This additional concentration might have led to a kind of overachievement in this very easy 0-back

condition, but could not be maintained by the subjects as the working memory load accumulated in the 2-back condition. Here, the subjects only achieved a performance comparable to the placebo performance. In contrast, a significant rise in false alarms could be observed in the 2-back condition. This can be interpreted as an indication of frontal disinhibition processes based on the loss of cholinergic transmission. On the other hand, hits were not affected by scopolamine in this condition. This absence of a behavioural effect on hits could be due to a deficiency in titrating the task difficulty appropriately. It can be assumed that a higher working memory load (e.g. with a 3-back condition) would be necessary to produce such an effect in a comparable sample of high performing healthy subjects. Altogether, these results show that even with a task difficulty supposed to be easily solvable by the subjects, the assumed resources used to maintain the performance level were not sufficient to cover the loss of the muscarinic transmission completely and therefore resulted in a significant increase in false alarms in the 2-back condition.

FMRI results

Modulation of cholinergic transmission by blockade of the muscarinic receptors resulted in specific alterations in brain activations, which were clearly dissociable from the effects of placebo on working memory activations.

The overall activation pattern under placebo condition during working memory performance/demands (2-back > 0-back contrast) showed the expected activation pattern during a typical n-back paradigm both in the placebo and in the scopolamine condition. These findings are in line with similar previous studies and meta-analyses on n-back tasks [46], thus confirming the validity of the paradigm and the scanning procedure.

The direct comparison of both conditions revealed specific brain activation differences, resulting in clearly distinguishable activation patterns.

Hypoactivations under scopolamine compared to placebo

Reduced activation under the influence of scopolamine (contrast placebo > scopolamine) was found mainly in parietal areas.

The precuneus was shown to be recruited with increasing cognitive demands in working memory tasks. Cavanna et al. [8], for instance, describe the precuneus as a major association area subserving a variety of higher-order cognitive functions. Its main function, the integration of external and internal information, is based on anatomical connections to cortical areas such as frontal cortex and brainstem, both of which areas are crucially important in cholinergic transmission. In an fMRI study with patients with mild cognitive impairment (MCI), Goekopp et al. [27] reported a significantly stronger activation within the precuneus detected after a period of adjunctive therapy with galantamine (an acetylcholinesterase inhibitor that increases cholinergic transmission). This is in line with our results as on the one hand patients with MCI suffer from cognitive deficits that include impairments in working memory tasks. On the other hand, patients' deficits are considered to be related to cholinergic alterations, for instance a loss of cortical cholinergic receptors and their connections [41]. Taken together, the studies could show that blocking the muscarinic transmission by scopolamine affects brain activation in an area that is also prominently active when cholinergic transmission is enhanced.

Hyperactivation under scopolamine compared to placebo

Greater activation during blockade of cholinergic transmission by scopolamine (contrast scopolamine > placebo) was found exclusively in prefrontal areas.

Previous functional imaging studies investigating working memory maintenance have consistently reported increasing activations in frontal and ventrolateral prefrontal areas depending on rising working memory load [13, 44, 71]. These signal increases within the networks representing working memory processes are interpreted as the cortical response to higher cognitive demands [5, 28, 53]. Compared to our placebo condition, subjects in the scopolamine condition therefore are confronted with the additional demand of compensating the blockade of the muscarinic transmission during the 2-back-performance. This additional required effort resulted in stronger frontal activations. As the placebo condition included the same 0-back- and 2-back conditions as the drug condition, the increase in frontal activations during scopolamine cannot be explained with increased working memory load.

In a review, Fletcher and Henson [18] included studies concerning working memory as well as encoding and retrieval of memory. The authors argue that although a distinction between these different aspects of memory is justified for many reasons, regarding the frontally mediated processes there are most likely considerable overlaps. This is an important argument in the context of our study, because existing fMRI studies using scopolamine to block cholinergic transmission mainly investigate performances on memory tasks whereas fMRI studies investigating working memory performances under scopolamine are less frequent. A review by Thiel [67] integrates the existing literature on effects of cholinergic modulation on learning and memory into two classes of effects: first, cholinergic modulation has an impact on brain areas associated with 'stimulus processing', for example fusiform gyrus or extrastriate areas. Second, cholinergic modulation also affects

the direct learning process referred to as 'learning related networks' by the author, including the prefrontal cortex and hippocampus. The latter effect agrees with our results of increased prefrontal activation in response to the scopolamine challenge. Another important conclusion of this review is that cholinergic effects especially on prefrontal regions seem to be of high importance for memory performance. An explanation of the underlying exact mechanisms of this cholinergic contribution could not be given based on available data. There are still only few studies challenging this area of complex cholinergic interaction and the possible effects of cholinergic alterations on cognitive performance using imaging techniques. The existing literature shows that scopolamine mostly affects the acquisition of new information as compared to the recollection of well learned information [60, 63]. These impairments in acquisition could have affected the performance of the 2-back condition and therefore could be the reason for the additional recruitment of prefrontal areas to maintain an adequate level of performance.

We interpret the additional recruitment of task-relevant brain regions under scopolamine, which resulted in hyperactivations in medial prefrontal areas, as a compensatory mechanism that allowed the participants to keep their behavioural performances as unaffected as possible. Brain activation in exactly these regions has been previously reported in numerous fMRI studies addressing working memory in various clinical research topics, for example attention deficits in healthy siblings of schizophrenic patients [59], visuospatial functioning in youth with foetal alcohol spectrum disorders [63] or cognitively intact APOEε4 carriers [17], which clearly reflects the relevance of such medial frontal areas for our experimental task, a necessary requirement for compensatory processes. In the present study, this additional recruitment of medial prefrontal regions did not allow for an entirely unaffected performance and therefore resulted in an increase in false alarms. Therefore, it can be assumed that a higher working memory load (e.g. by adding a 3-back condition) might have resulted in additional behavioural differences between the scopolamine and the placebo condition.

In working memory tasks, parietal regions are thought to be mainly recruited for increased task demands as well as storage, retrieval and interference resolution [31, 40]. On the other hand, frontal and prefrontal areas in working memory tasks are involved in phonological storage, rehearsal and maintenance functions as well as handling increasing working memory load [5, 62].

Comparable to the placebo condition of the present study, these functions usually do not need specific resources but are processed automatically. The fact that these regions in our study were more strongly activated under influence of scopolamine suggests that the subjects could not rely on these mostly automatic resources the way they could during placebo. Indeed, they relied on a compensatory increase in order to maintain the same level of performance.

Our results can be further discussed in line with the working memory model of Baddeley and Hitch [3]. These authors distinguish between passive storage and active rehearsal. Active rehearsal of verbal material within the phonological loop is considered to prevent the decay of material inside the phonological store. Though usually the 2-back task does not demand a lot of rehearsal within the healthy subjects, blocking the muscarinic transmission obviously resulted in a greater need to maintain the verbal information active in the phonological storage by rehearsing the information. This rehearsal is part of the articulatory control process [4] and takes place within the ventrolateral frontal cortex whereas passive storage predominantly happens in the parietal, temporal and occipital lobes [6, 10].

Summarizing behavioural and brain activation results in the light of the current literature, our study on the one hand shows that the fronto-parietal-cerebellar network is not affected by blocking muscarinic transmission. Behaviourally, scopolamine negatively affected false alarms but not hit rates on the 2-back condition of the n-back task while on the 0-back condition scopolamine resulted in a significantly higher number of hit rates. These findings are interpreted as compensatory cerebral processes leading to an exceedingly accurate performance on the easy task and a regular level of performance on the more challenging task. In particular, subjects seem to have exceedingly recruited task-specific frontal areas in order to maintain the regular level of task performance on cognitively challenging tasks when blocking of muscarinic receptors led to relevant parietal and cerebellar hypoactivations. These additionally recruited (pre)frontal areas have been implicated in the processing of cognitive control and higher cognitive load. According to Chua and Chee [11] or Chee and Chua [9], performance decrements in working memory processes can also be ascribable to reduced attention processes necessary to execute these processes. On a functional basis, these attention deficits can be detected in reduced activations within the parieto-occipital cortex. As fundamental part of almost all 'higher cognitive processes', attention constitutes the foundation of higher-order processes like working memory. Against this background, the hypoactivations under scopolamine seen in parietal areas can be related to reduced attention processes as an effect of the muscarinic blockade. This is furthermore important as scopolamine has been proven to affect attention processes next to memory processes [16, 55, 68, 69]. When interpreting the results of the current study, it is important to keep in mind that the applied paradigm was chosen particularly as it does

to not require a huge amount of cognitive effort. The fact that modest behavioural and distinct cerebral alterations could already be observed during the n-back task lays ground for future investigations, for instance a muscarinic influence on subcomponents of working memory, for other executive functions or studies on relevant neuropsychiatric populations.

Clinical implication

Working memory is a higher-order cognitive function with characteristic deteriorations in a wide range of neuropsychiatric disorders (e.g. schizophrenia, depression, substance use). The separation of distinct contributions of the two cholinergic receptor systems to cognitive performance is aimed to achieve a better understanding of their respective contributions to develop more sensitive strategies of enhancing working memory processes in patients with cognitive impairments associated with the cholinergic system (e.g. schizophrenia). Interestingly, acetylcholinesterase inhibitors (e.g. Galantamine) do not ameliorate the cognitive impairments in schizophrenia in a comparable amount as they do in Alzheimer's disease [72]. The present study in healthy subjects supports the ongoing research regarding the amelioration of cognitive impairments and provides further knowledge concerning the specific impact of the muscarinic receptor system on higher cognitive processes. Based on these findings, future research may develop more specific treatment regimes for cognitive impairments in neuropsychiatric disorders like schizophrenia.

Methodological considerations

We employed the rather simple 2-back task to probe working memory function specifically as it was supposedly easy to execute by a sample of high-functioning healthy young students. The motivation for choosing this task was based on the following considerations: Combining neuroimaging with psychopharmacology is difficult because one scanning session has to integrate three different domains: the process of the functional imaging itself, translating subtle changes of the BOLD response into a cortical representation of the subject, the paradigm that is used to reflect the influence of the conditions on the performance of the subject and last the pharmacological intervention, which is supposed to influence both the cerebral activation recorded by the scanning procedure and the behavioural performance of the subject. The above-mentioned review by Thiel et al. [67] concerning the cholinergic modulation of learning and memory in the human brain illustrated that the effects of cholinergic modulation on brain activity are very specific. Studies using PET to examine the effects of scopolamine on a word list paradigm found attenuated regional cerebral blood flow in bilateral prefrontal cortex compared to placebo [29], whereas in PET studies using physostigmine, an acetylcholinesterase inhibitor that enhances the cholinergic transmission, the same reduced activations in prefrontal areas were found [22]. Thus, to bypass these counterintuitive results, we decided to apply a well-established working memory paradigm to translate the effects of our pharmacological intervention on the performance on a paradigm reliable enough to rule out possible ambiguous interpretations because of the paradigm's theoretical background. The n-back paradigm can be regarded as sufficiently robust to fulfil this request.

Conclusion

The purpose of this study was to investigate muscarinic contributions to functional correlates of working memory in a sample of healthy non-smoking subjects. On the behavioural level, scopolamine did not affect hits, but subjects generated more false alarms under scopolamine compared to placebo in the 2-back condition. This finding is considered to reflect frontal disinhibition processes. Concerning brain function, we identified hyperactivations following application of scopolamine in frontal and prefrontal areas and hypoactivations in parietal and cerebellar areas. In line with the existing literature, our results indicate that these hyper- and hypoactivations can be seen in the light of muscarinic modulation of functional subcomponents and gives furthermore evidence for a compensatory strategy attenuating the induced cognitive challenge that resulted from the muscarinic blockade.

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Conflict of interest The authors report no conflicts of interest.

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