Original Research

Cholinergic enhancement differentially modulates neural response to encoding during face identity and face location working memory tasks

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Abstract

Potentiation of cholinergic transmission influences stimulus processing by enhancing signal detection through suppression and/or filtering out of irrelevant information (bottom-up modulation) and with top-down task-oriented executive mechanisms based on the recruitment of prefrontal and parietal attentional systems. The cholinergic system also plays a critical role in working memory (WM) processes and preferentially modulates WM encoding, likely through stimulus-processing mechanisms. Previous research reported increased brain responses in visual extrastriate cortical regions during cholinergic enhancement in the encoding phase of WM, independently addressing object and spatial encoding. The current study used functional magnetic resonance imaging to determine the effects of cholinergic enhancement on encoding of key visual processing features. Subjects participated in two scanning sessions, one during an intravenous (i.v.) infusion of saline and the other during an infusion of the acetylcholinesterase inhibitor physostigmine. In each scan session, subjects alternated between a face identity recognition and a spatial location WM. Enhanced cholinergic function increased neural activity in the ventral stream during encoding of face identity and in the dorsal stream during encoding of face location. Conversely, a reduction in brain response was found for scrambled sensorimotor control images. The cholinergic effects on neural activity in the ventral stream during encoding of face identity were stronger than those observed in the dorsal stream during encoding of face location, likely as a consequence of the role of acetylcholine in establishing the inherently relevant nature of face identity. Despite the limited sample-size, the results suggest the stimulus-dependent role of cholinergic system in signal detection, as they show that cholinergic potentiation enhances neural activity in regions associated with early perceptual processing in a selective manner depending on the attended stimulus feature.

Keywords: Acetylcholinesterase inhibitors, cholinergic system, face perception, working memory, fMRI, visual cortex

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Introduction

The cholinergic neurons from the basal forebrain have widespread projections throughout the cortex that modulate signal-to-noise (S/N) processing mechanisms.[1,2] Direct application of acetylcholine to the visual cortex in animals results in an enhanced selectivity of neural responses to the target orientation and a decreased response to more dissimilar stimulus orientations.[3] Human neuroimaging studies also showed that enhanced cholinergic function increased in response to task-specific stimuli and reduced in response to non-task stimuli.[4] These findings show that the cholinergic system directly modulates S/N neural processing and may suggest a key mechanism by which acetylcholine directly affects cognitive functions.[5,6]

Working memory (WM) is modulated by cholinergic activity. Generally, agents that block cholinergic neurotransmission impair WM performance,[7,8] while agents that enhance cholinergic activity improve performance.[9,10] These effects likely result from the modulation of the neural representation of task stimuli. Functional studies have shown that increasing cholinergic activity alters the neural response throughout the regions of the brain recruited to perform the WM task[4,11–15] in a manner that correlates with an improvement in task performance.[10,12] Importantly, cholinergic modulation has a larger effect

during the encoding of new information than when viewing stimuli during the retrieval/matching component of a task.[4,16,17]

The cholinergic system is also centrally involved in selective attention processes and likely influences attention via stimulus–processing mechanisms.[18,19] Selectively attending to object identity in a visual WM paradigm preferentially modulated neural response in the ventral visual stream, while attending to the spatial location of similar stimuli–enhanced activity in the dorsal visual stream.[20–23] These effects may be mediated by cholinergic activity, as the cholinergic system is implicated in both top-down, goal-directed and bottom-up, stimulus-driven attention mechanisms.[16,18,19,24–28] Consequently, in the presence of multiple stimuli or multiple stimulus features, top-down and bottom-up attention mechanisms may act through cholinergic modulation of the S/N neural response to preferentially process one stimulus over another.[13,19]

In the current study, we planned to determine the differential influence of pharmacologically induced cholinergic enhancement on cortical activity during a visual WM task, while participants attended selectively to either identity or spatial location features of the same stimuli. We hypothesized that cholinergic potentiation would increase neural activity preferentially during the encoding of the attended stimulus features and that visual regions showing enhanced encoding effects would be evident in the ventral (object based) and dorsal (spatial based) visual pathways, dependent on the attended stimulus feature.

Methods

Subjects

Nine right-handed healthy volunteers (mean age = 27.8 ± 4.6 years; five females/four males) participated in a randomized, double-blind, placebo-controlled study conducted over two sessions. One session included an infusion of physostigmine and the other an infusion of a placebo saline solution. All subjects underwent clinical, neurological and psychiatric examinations and laboratory tests, including routine blood and urine tests, blood pressure, electrocardiogram, chest X-ray and a structural brain MRI scan. All participants were free of medication, including over-the-counter medications, for a minimum of four weeks before entering the study and gave their written informed consent to participate to the study after the procedures and risks involved had been explained.[29] The study was approved by the National Institute of Mental Healthy Institutional Review Board (National Institutes of Health Protocol 00-M-0056). Three subjects were discarded from the analysis; data were incomplete for one subject and extensive head movement (more than one voxel size) during the fMRI sessions required the removal of two additional subjects from the analysis. Thus, usable data were obtained from six subjects.

Experimental design

Subjects participated in two distinct experimental sessions, during which they received i.v. infusions of placebo (saline solution) or drug (physostigmine) in a random order, and subsequently performed a visual WM task. The physostigmine infusion was administered as a 10-min loading dose at 1.93 mg/h followed by a maintenance drip of 0.816 mg/h for a total rate of 1.0 mg/h. Infusions continued for 30 min before beginning the task to obtain stable drug effects.[4] Glycopyrrolate, a cholinergic muscarinic antagonist that does not cross the blood-brain barrier, was administered i.v. (0.02 mg) 2–5 min before beginning the physostigmine infusion to minimize peripheral side effects.[30,31] In the placebo session, saline solution was administered according to the same infusion schedule used for physostigmine. Blood pressure and heart rate were monitored continuously throughout each of the two sessions by using an MRI compatible device.

Task design

Subjects performed two versions of a visual WM task, including a face identity recognition and a spatial location discrimination task. For each WM trial, pictures of three faces were presented for 2-s each in three different locations randomly selected from 24 possible locations on the monitor (the encoding phase), followed by a 9-s delay period (the maintenance phase) and by a 3-s presentation of a test face (the retrieval phase). In the face identity recognition task, participants were instructed to indicate if the test face matched (in terms of identity) one of the three encoded items, regardless of the location in which the stimuli had been presented; during the spatial location discrimination task, participants were instructed to indicate if the test face matched (in terms of spatial location) one of the three encoded locations, regardless of the face identity. In both task conditions, WM trials alternated with sensorimotor control trials in which phase-scrambled faces were presented in the same spatial and temporal manner as the WM items, and participants were instructed to press both response buttons when the 'test' item appeared (see Figure 1). Accuracy and reaction time (RT) data were recorded. The intertrial interval was fixed to 9 s. WM task conditions alternated over 12 scanner runs, resulting in six runs of both the identity and the spatial location WM tasks. The sequence of the runs was randomized across subjects. Overall, each session lasted about 1 h.

Image acquisition

Gradient echo, echo-planar imaging (EPI) data were acquired with a 1.5 Tesla scanner (General Electric, Milwaukee, WI). In each of 12 runs, 98 whole-brain volumes, comprised 24 contiguous, 5-mm, axial slices were obtained (field of view = 24 cm, echo time = 40 ms, flip angle = 90°, repetition time = 3000 ms, image in-plane resolution = 64 × 64 pixels, voxel size 3.5 × 3.5 × 5 mm) resulting in a total of 1176 volumes. High-resolution T1-weighted spoiled grass images were collected for each subject to provide detailed brain anatomy. During the fMRI experiment, stimuli were presented using a rear-projection screen, and subjects were responded using right-held response buttons. MRI data were acquired at the National Institute of Health.

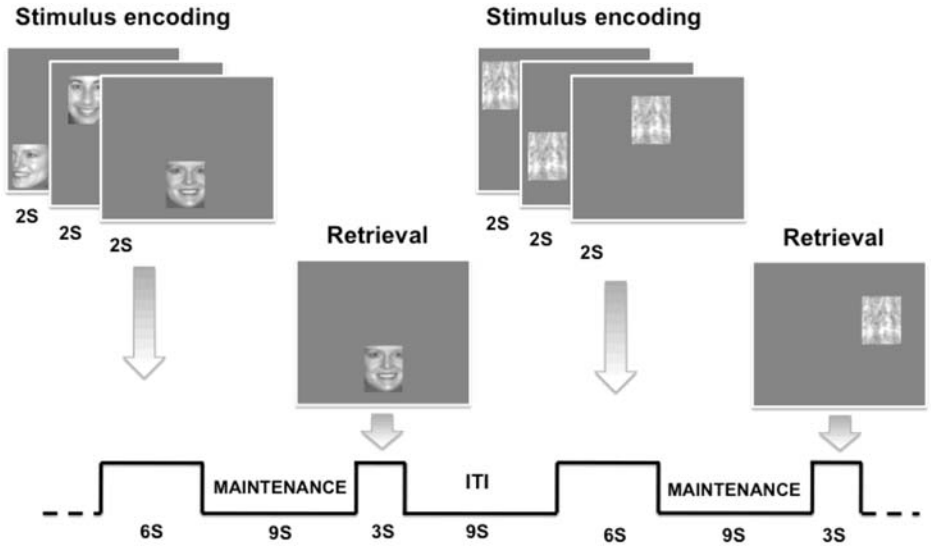

Figure 1 Task design. Pictures of three faces were presented in 3 of 24 possible locations for 2 s each (encoding), followed by a 9-s delay (maintenance), and by a 3-s presentation of a test face (retrieval). During the face identity WM task, subjects indicated if the test item matched in identity of one of the three encoding items, irrespective of its location. During the spatial location WM task, participants indicated if the test item matched in spatial location one of the three encoding items, irrespective of its identity. Sensorimotor control items consisted of phase-scrambled faces presented in the same spatial and temporal manner as the WM items.

Data analysis

Behavioural data (accuracy and RT) were analysed using Statistical Package for the Social Sciences (SPSS Inc., Chicago IL). A repeated measures ANOVA was performed to assess differences between the placebo and drug sessions for WM conditions and the sensorimotor control task.

Functional imaging data were analysed using the AFNI and SUMA packages and related software plugins – http://afni.nimh.nih.gov/afni.[32] Prior to standard preprocessing, the first four volumes and the last volume acquired were discarded (resulting in a total of 1116 volumes) to allow the MR signal to remain at steady state. Volumes from all runs were concatenated and coregistered (*3dvolreg*), temporally aligned (*3dTshift*) and spatially smoothed (*3dmerge*, Gaussian kernel 8 mm, full-width half maximum). Data were normalized individually by calculating the mean intensity value for each voxel and by dividing each time point in the time series within each voxel by its average to estimate the percent signal change at each time point and were spatially normalized to the Talairach and Tournoux Human Brain Atlas.[33] Each phase of the task was modelled separately (encoding, maintenance, retrieval) for each experimental condition (face identity WM, spatial location WM and the control task). The stimulus input functions were convolved with the hemodynamic response function to create predicted responses, and multiple regression was used to analyse the data. Movement parameters were included in the multiple regression analysis as regressors of no-interest. We included general linear tests (GLTs) within the multiple regression model to identify the specific responses related to each component of each WM task, as compared to the relative component of the control task. As the control stimuli alternated with task stimuli, the sensorimotor control stimuli that alternated with face identity stimuli served in the comparison for the identity task, and the control stimuli that alternated with spatial location stimuli served in the comparison for the spatial task. The results of the GLTs were used in subsequent group analyses.

Face identity recognition versus spatial location discrimination WM tasks

A two-tailed paired *t*-test contrasting the BOLD responses during the placebo session of the face identity and the spatial location tasks was assessed ($P < 0.05$ uncorrected, minimum cluster size 5000 μL) to highlight differences in neural responses between the two tasks in the absence of any drug modulation.

Drug effects within task components

Brain regions that showed a significant percent signal change during each task component (i.e. encoding, maintenance and retrieval phases, separately) combined over the tasks type (face identity and spatial location WM) and the drug condition (saline solution and physostigmine) were identified ($P < 0.0001$, uncorrected) and then used to mask subsequent analyses (thus, three inclusive masks were created, one for each task phase and combined across experimental conditions). Monte Carlo simulations were conducted using *AlphaSim* to estimate the minimum cluster size for a small volume correction (SVC) for each of the three inclusive masks.

For each task phase, a three-way repeated measures ANOVA was conducted, including the drug condition (saline solution and physostigmine) and task type (face identity and spatial location WM) as fixed factors and the subjects as random factor (voxel-level significance $P < 0.05$; SVC $P < 0.05$). Drug-induced changes across- and within-task type, together with drug condition × task interaction, were assessed.

Spheres with a 4-mm radius were centred on *t* or *F* cluster peaks, and task condition coefficients and mean BOLD

signal time series were extracted. For striate and extrastriate regions, where clusters were large and extended bilaterally, the *Maxima* AFNI plugin was used to identify peaks and place spheres of 4-mm radius spaced a minimum of 30 mm apart. For visualization purposes, the BOLD signal time series are presented after removing the influence of the six motion correction parameters (derived from the volume registration and the polynomial regressors that account for baseline shifts) from the voxel raw time series (using *3dSynthesize*).[34] Subsequently, data were averaged over voxels within each sphere using the *Reorder* AFNI plugin, thus obtaining the hemodynamic activity during face identity, spatial tasks and control condition for all of the WM phases (encoding, maintenance, retrieval). All statistical results were anatomically localized on Talairach-transformed T1-weighted images and visualized using normalized SUMA surface templates.

Results

Behavioural results

Performance accuracy was at ceiling level in both face identity and spatial location WM tasks, during both the Placebo and the Drug sessions, with no significant difference between tasks or sessions ($P > 0.2$-).

Compared to saline solution (Placebo), physostigmine (Drug) administration resulted in a selectively significant reduction in RT during the WM conditions ($P = 0.001$), with no significant change in RT during the control task ($P > 0.4$). Significant reductions in RT also were observed in the face identity (mean Placebo RT $= 1572 \pm 222$ ms; mean Drug RT $= 1429 \pm 209$ ms; $P < 0.001$) and spatial location (mean Placebo RT $= 1376 \pm 239$ ms; mean Drug RT $= 1235 \pm 134$ ms; $P < 0.05$) conditions when considered separately.

Face identity versus spatial location WM tasks

During the Placebo session, the comparison of the two WM tasks revealed an increased BOLD response in the ventral occipital, ventral temporal and inferior prefrontal regions in the face identity task compared to the spatial location task, while during the spatial location task, a significantly greater response was observed in the dorso-occipital and parietal cortical areas compared to the facial identity task ($P < 0.05$ uncorrected, minimum cluster size 5,000 μL) (see Figure 2).

Drug effects on encoding

Significant BOLD responses during the encoding phase (relative to control and combined across the Placebo and Drug sessions) for both face identity and spatial location WM tasks were seen in ventral occipital, ventral temporal and dorso-occipital cortical areas, intraparietal sulci and prefrontal regions ($P < 0.0001$, uncorrected), and, as described in the 'Methods' section, comprised the inclusive mask for subsequent analyses of the neural responses during the encoding phase (minimum cluster size of 4185 μL, SVC corrected P value <0.05).

Figure 3 shows the significant overall effects of physostigmine administration during encoding combined across the face identity and spatial location WM tasks. An increased BOLD response was observed in the bilateral visual striate and extended extrastriate cortical regions, including the fusiform and lingual gyri ($P < 0.05$, SVC). No cluster survived SVC in the task by drug interaction.

Physostigmine administration selectively enhanced the encoding response during the face identity WM task in the bilateral fusiform (Figures 4a and 4b and 5a and 5b) and lingual gyri, left middle occipital, right cuneus (Figures 4c and 5c) and in the right premotor (Figures 4d and 5d) and the middle prefrontal, bilateral inferior frontal and left insula areas (Figures 4e and 5e). In contrast, no change in

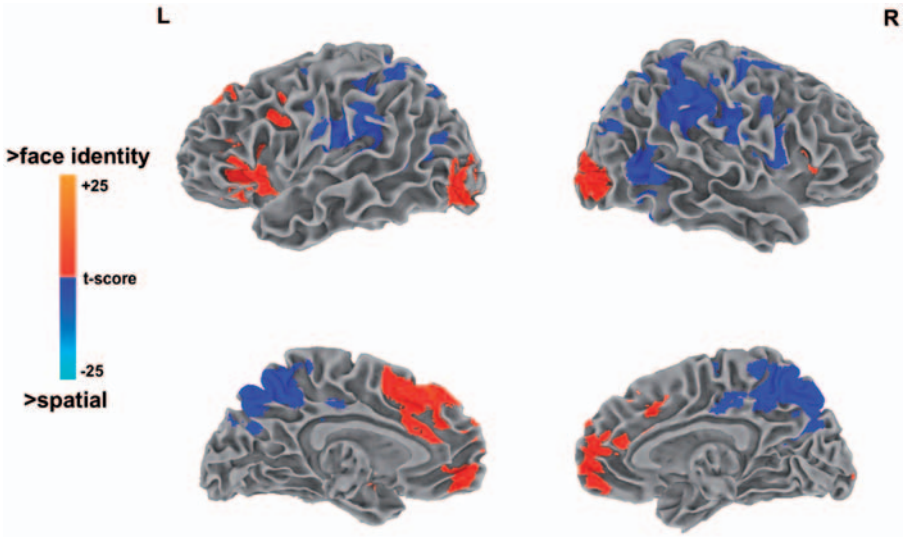

Figure 2 A comparison of the two WM tasks (face identity and spatial location tasks) to each other during the placebo session. The face identity task increased BOLD in ventral occipital, ventral temporal and inferior prefrontal regions (shown in red), while the spatial location task preferentially recruited dorso-occipital and parietal cortices (shown in blue). T-score maps are projected onto a standard Talairach template, and results are thresholded at a significance level of P < 0.05, with a minimum cluster size 5000 μL. (A colour version of this figure is available in the online journal)

BOLD response in these cortical areas was detected during the spatial location WM task ($P < 0.05$, SVC; Table 1).

During the spatial location WM task, a dorsal region of left cuneus exhibited a selective increase in BOLD during encoding for the spatial task (Figures 4g and 5g), with no effect during the face identity WM task. Additionally, physostigmine enhanced the encoding response during the spatial location WM task in the left middle occipital

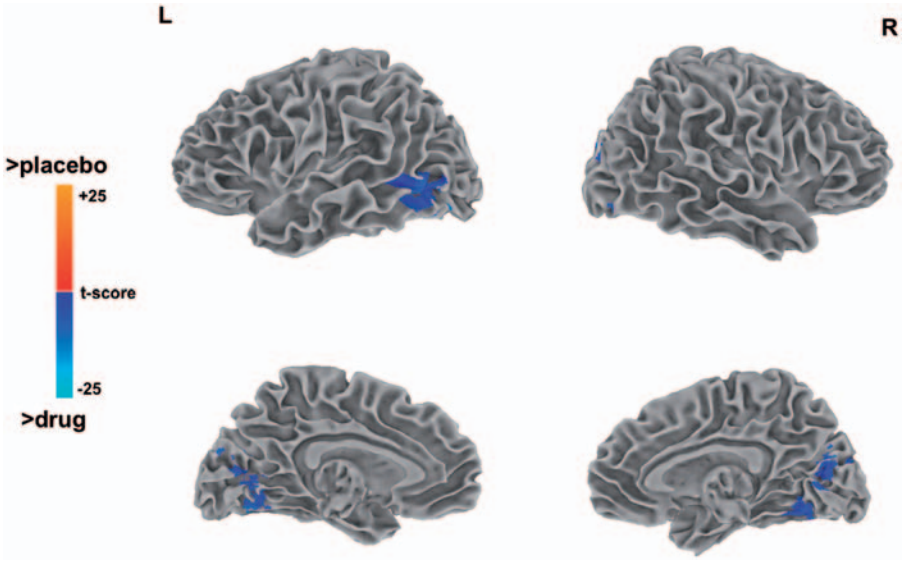

Figure 3 Brain areas with a significant overall drug effect on encoding across the face identity and spatial location WM tasks combined. An increased BOLD response during the drug session was found in the bilateral visual striate and extended extrastriate regions. Results are thresholded at a significance level of $P < 0.05$, small volume corrected. (A colour version of this figure is available in the online journal)

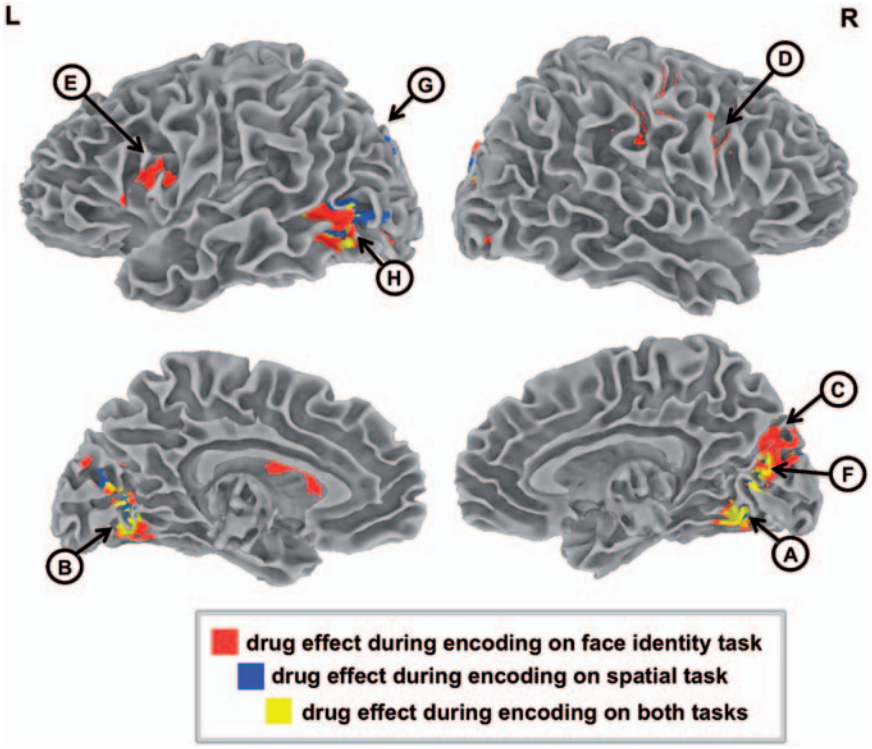

Figure 4 Drug effect for encoding of face identity (red), spatial location (blue) or both tasks (yellow). Results are thresholded at $P < 0.05$ (SVC). BOLD signal time series and further details on brain regions identified with capital letters are reported in Figure 5, Tables 1 and 2. (A colour version of this figure is available in the online journal)

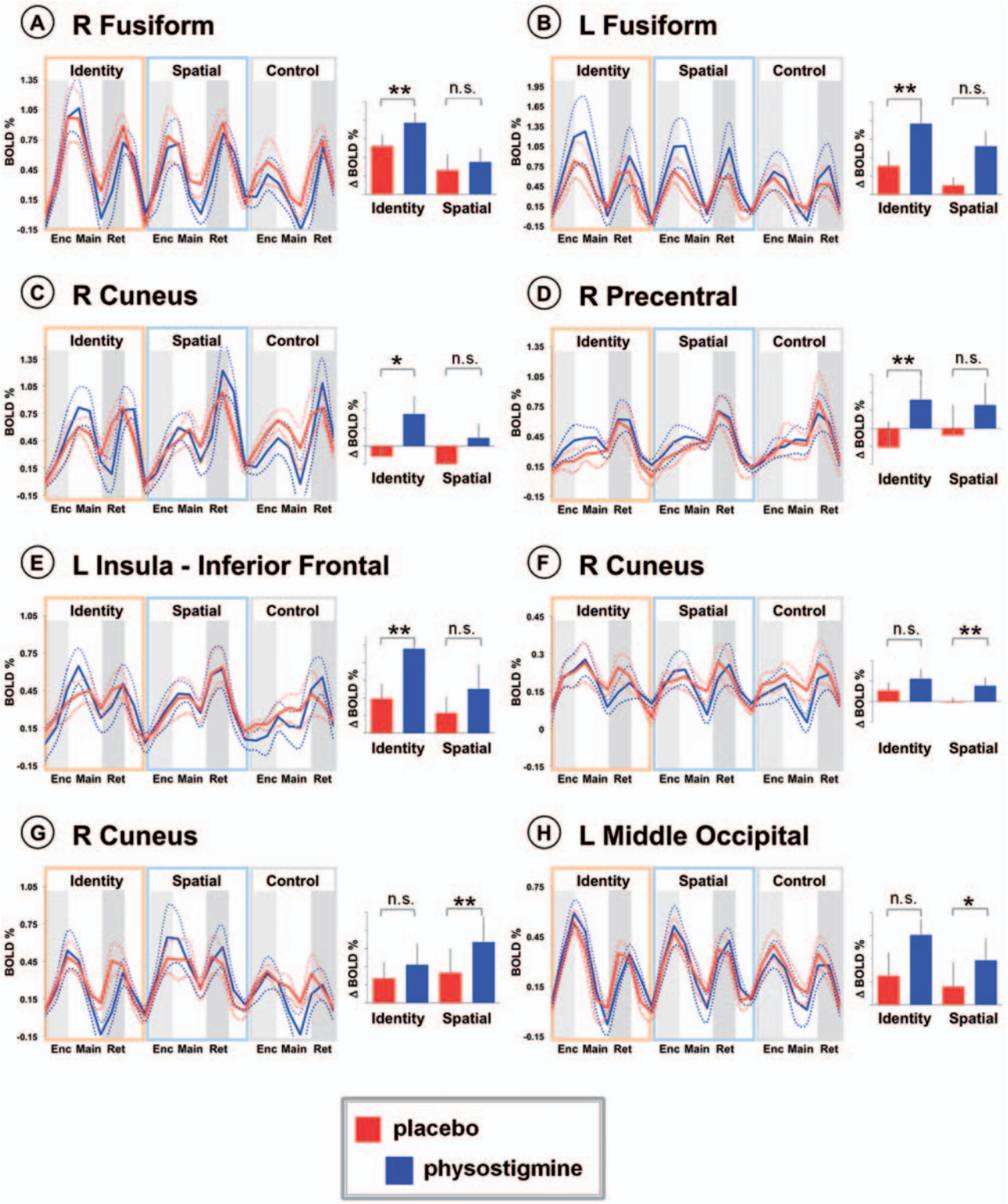

Figure 5 BOLD signal time series (encoding, maintenance and retrieval) and BOLD estimates during encoding for specific regions of interest. See Figure 4, Tables 1 and 2 for further details on ROI selection. Averaged time series depict the BOLD response for each of the WM phases (encoding, maintenance and retrieval) and each task type (face identity WM, spatial location WM and sensorimotor control task), during placebo (red) and physostigmine (blue). Dotted lines depict BOLD signal SEs. Bars reflect mean BOLD response for the identity and spatial WM tasks (relative to the control condition). The statistical comparisons show the significance between placebo and drug conditions during encoding for each WM task using paired t-tests. Error bars reflect SE; n.s. not significant, *$P < 0.05$ uncorrected, **$P < 0.05$ corrected (Bonferroni) for multiple comparisons. (A colour version of this figure is available in the online journal)

Table 1 Regions showing a drug effects on encoding response selectively during the face identity WM task

Region Name	Hemisphere	Brodmann Area	X	Y	Z	Volume (μL)	t-value	Label
Fusiform	R	BA 19	25	−64	−9	6077	−3.43	A
Fusiform	L	BA 19	−30	−63	−6	13832	−3.01	B
Cuneus	R	BA 18	9	−77	19	5720	−2.73	C
Precentral	R	BA 6	41	−10	36	6011	−3.13	D
Insula - Inferior Frontal	L	BA 13	−37	8	14	4494	−3.49	E

For each significant cluster, estimated Brodmann's areas from Talairach and Tournoux Atlas, coordinates of the local maxima, volume of the region and *t*-values of significance (saline solution minus physostigmine) are provided. The last column indicates the regions showed in Figures 4 and 5.

Table 2 Regions showing drug effects on encoding response selectively during the spatial location WM task

Region Name	Hemisphere	Brodmann Area	X	Y	Z	t-value	Label
Cuneus	R	BA17	19	−73	10	−3.08	F
Cuneus	L	BA19	−24	−83	32	−3.14	G
Middle Occipital	L	BA37	−35	−66	2	−2.85	H
Cuneus	L	BA18	−8	−68	5	−3.51	

For each significant cluster, estimated Brodmann's areas from Talairach and Tournoux Atlas, coordinates of the local maxima and *t*-values of significance (placebo minus physostigmine) are provided. The regions were extracted from a unique extended bilaterally cluster of 12,715 μL, using the Maxima AFNI plugin. The last column indicates the regions showed in Figures 4 and 5.

and right cuneus regions ($P < 0.05$, SVC; Table 2, Figures 4f and 4h and 5f and 5h), although these regions also showed a trend of level increases in BOLD response during encoding in the face identity WM task.

Drug effects on maintenance

Significant BOLD responses during the maintenance of WM items were observed in the inferior temporal, superior parietal, intraparietal sulci and prefrontal regions ($P < 0.0001$, uncorrected) and thus comprised the mask for subsequent analyses of the neural responses during the maintenance phase (minimum cluster size of 4309 μL, SVC corrected P value of <0.05). No significant drug effect was observed during the maintenance component of either the face identity or the spatial location WM tasks.

Drug effects on retrieval

Significant increases during retrieval across the WM tasks were observed bilaterally in the occipital, ventral temporal, intraparietal sulci, superior parietal and frontal regions, including the precentral gyri ($P < 0.0001$, uncorrected) and thus comprised the mask for subsequent analyses of the neural responses during the retrieval phase (minimum cluster size of 7529 μL, SVC-corrected P value of <0.05). Significant drug effects (decreases in the BOLD estimates of the task response relative to the sensorimotor control condition during physostigmine infusion) were observed in the left occipital, cingulate and precuneus brain regions ($P < 0.05$, SVC) (see Figure 6), but no region was significant in the task × drug interaction, or in analyses evaluating the individual face identity and spatial location retrieval components separately, indicating that the drug effects did not differ by task.

Discussion

The results of this preliminary study indicate that enhancing cholinergic function while subjects perform a WM task increases neural responses preferentially during the encoding phase and that the specific brain regions showing enhanced encoding effects are dependent on the attended stimulus feature. These observations suggest that attention mechanisms are influenced by cholinergic modulation of S/N neural response to preferentially process one stimulus over another, when multiple stimuli or multiple stimulus features are present. Specifically, while participants performed the face identity WM task, widespread effects were primarily observed during encoding within the ventral visual stream, which preferentially processes object-based or 'what' information.[22,35] When participants performed the spatial location WM task, a selective, though more limited, effect of cholinergic modulation during encoding was observed in the cortical regions that belong to the dorsal visual stream, which preferentially processes spatial or 'where' information.[21,22,36] Thus, the nature of the task, where that pattern of regional recruitment is dependent on the attended stimulus feature, offers a unique opportunity to evaluate the differential influence of cholinergic modulation based on stimulus feature. While the results here may be preliminary due to the limit in sample size, the findings are novel in nature, and the experimental approach may inform interested researchers for future studies.

Cholinergic enhancement also modulated neural responses during the retrieval phase in both the face identity and the spatial location WM tasks, by leading to a decrease in BOLD responses. These effects, however, were less pronounced and more limited than those observed during encoding, consistent with the previously reported

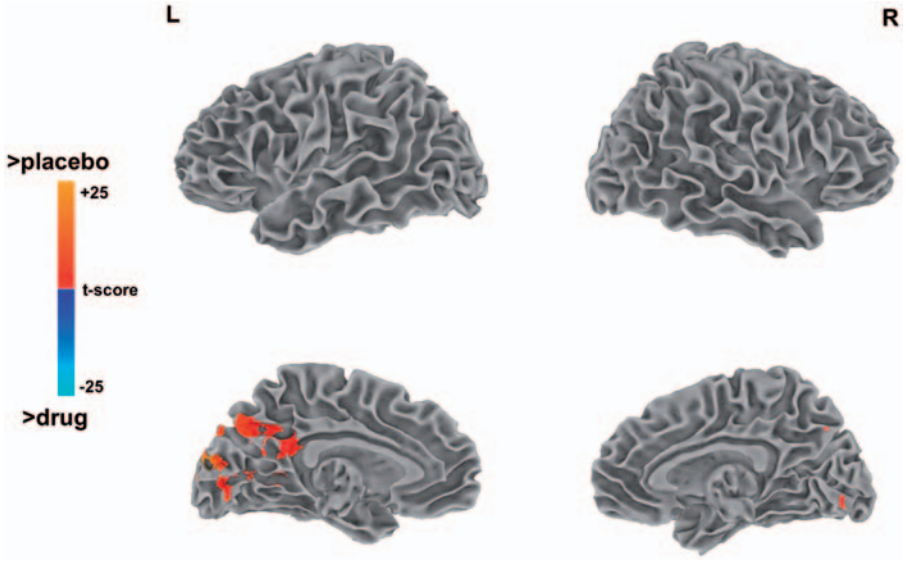

Figure 6 Brain areas with a significant overall drug effect on retrieval across the face identity and spatial location WM tasks combined. A decrease in BOLD response relative to the sensorimotor control condition during the drug session was found in the left occipital, cingulate and precuneus brain regions. Results are thresholded at a significance level of $P < 0.05$, small volume corrected. (A colour version of this figure is available in the online journal)

overall larger influence of cholinergic enhancement during early phases of stimulus processing.[4,16] These reductions in BOLD response are also in agreement with the hypothesis that acute cholinergic enhancement of the encoding of new information is associated with a reduction in neural response during the retrieval phase, as described both at a neuronal and a regional level.[17,37–39] Kukolja *et al.*[16] demonstrated that cholinergic enhancement was associated with an increased neural response during encoding relative to a placebo and an attenuation of retrieval-related activity in the medial temporal lobe in a spatial-episodic memory task. In contrast, in a previous study of ours,[4] we found increases in BOLD response during WM retrieval in visual processing areas. While at the moment, there is some divergence in the literature regarding the cholinergic effects on retrieval that might be specifically related to the stimulus content (e.g. face identity vs. spatial information), cognitive function (e.g. episodic vs. WM) or to the experimental design (i.e. behavioural/motor responses affect the retrieval phase), the effects on encoding processes are rather robust and uncontroversial.[5,40]

Cholinergic enhancement by physostigmine administration selectively improved performance (i.e. reduced RTs) during WM, with no change in performance during the sensorimotor control task. This finding indicates that the influence of cholinergic potentiation on performance is specific to task conditions and does not reflect a generalized, unspecific facilitatory effect on response time. Equally specific effects of cholinergic potentiation on performance during WM but not during the sensorimotor control task have been reported in conjunction with similar, though not identical, visual WM paradigms.[4,12,14]

The enhanced encoding effects observed for both the face identity and spatial location WM tasks are consistent with the hypothesized role of cholinergic modulation in improving S/N processing.[4,5,18,19,41] For both the tasks, increased neural responses were seen during encoding (i.e. signal processing), with no change or a reduced response associated with the control task (i.e. noise). Thus, the current observations support the role of cholinergic activity in S/N processing and additionally show that increased neural activity occurs in selective brain regions that depend on the attended stimulus feature. Importantly, the results further suggest that the enhancement in S/N occurs in a functionally specific manner at a network level, as the effects during the encoding phase were observed selectively within the ventral visual stream when subjects attended selectively to face identity, and within the dorsal visual stream when participants attended selectively to the spatial location feature of the stimuli.

The cholinergically mediated effects on encoding were more prominent for the identity task in the ventral stream than in the dorsal pathway for the spatial task that may suggest that cholinergic modulation has a greater influence on the encoding of identity information. Faces as 'identity stimuli' might result in a more salient stimuli and exert a stronger influence on attention than faces as 'location stimuli', whose fine details are irrelevant to the task. Thus, the interactive effects of the cholinergic system on top-down and bottom-up mechanisms may result in a more relevant enhancement in the encoding of identity information.

The mechanism by which cholinergic enhancement preferentially modulates encoding in the ventral stream may be related to the influence of cholinergic activity on the visual receptive fields.[42–44] Increasing cholinergic function reduces the spatial spread of visual responses in the human early visual cortex[42,45] and decreases the size of the visual receptive fields.[43,44] These effects on the cholinergic system translate into the enhancement of fine stimulus details. Thus, the expectation might be that cholinergic augmentation would preferentially enhance encoding during the face identity WM task in which the visual angle

subtended by each face picture is smaller as compared to the spatial location WM task, and the intrinsic features of the stimulus must be examined in order to perform the task.

The main limitation of current study is the small sample size. Three datasets from the original participants were unusable, two due to excessive head movement during scanning and the third because of drop-out due to side effects from drug administration. We cannot exclude the possibility that specific observations on the effect of cholinergic enhancement (e.g. differences in the modulation between the ventral and dorsal stream and the divergent effect on retrieval) may be influenced by low-statistical power resulting form the limited sample size. Further studies in larger populations will ultimately improve the characterization of cholinergic enhancement. Nonetheless, the overall patterns of neural response associated with the object and spatial-based WM tasks in our study were consistent with the regional patterns reported in previous fMRI studies that adopted an identical WM task,[20,21] suggesting that our task and methods produced comparable activation and thus lending additional support to the overall findings. In addition, the effects of cholinergic modulation for the face identity WM task were equivalent to those reported in a distinct study by Furey *et al*.,[4] in which the same drug infusion criteria and a similar – though not identical – WM for the faces task was adopted, thus indicating that effects on neural activity were statistically significant in spite of the limited number of participants. Thus, while preliminary in nature due to the small sample size, sufficient replication of previous findings supports the validity of the new results reported here. While confirmatory studies that are larger in nature would be important, the new findings take the current literature into a novel direction and thus contribute to the developing insight of cholinergic impact on cognition and stimulus processing.

In future experiments, using content information analysis, we will explore the effects of cholinergic potentiation at the level of multivoxel response patterns.[46] The differences in BOLD patterns following cholinergic enhancement suggest that task-specific changes in neural activity engage distributed representative patterns across ventral visual processing areas rather than producing changes in discrete brain regions.[47]

In summary, the results of this preliminary study support the hypothesis that attention mechanisms act through cholinergic modulation during WM processes to preferentially affect stimulus encoding (top-down) and suggest that these encoding effects are dependent on the attended stimulus feature (bottom-up). Specifically, cholinergic enhancement occurred selectively in the ventral visual stream when the subjects encoded object-based information and occurred preferentially in the dorsal visual stream when the participants encoded spatial-based stimulus information during a WM task. Finally, the effects on encoding were more widespread for the face identity WM task, suggesting that increasing cholinergic function may preferentially enhance the processing of object-based features over spatial location-based stimulus features. Follow-up studies will clarify whether such an effect may be a consequence of a differential 'neural load' due to a need for a finer detail analysis of the attended stimuli in the face identity task as compared to the spatial location task.

Author contribution: PP and MF conceived and designed the experiment; MF, PP and JS performed the experiment; GH, MF and ER analysed the data; GH, MF, ER and PP reviewed and interpreted the results; GH, MF and ER wrote the paper; JS and PP integrated and approved the final version of the manuscript.

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