Research report

Replication and further studies of neural mechanisms of spatial mnemonic processing in humans

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Abstract

Changes in neuronal firing rates during periods of time when subjects are required to remember information (retention delays) have been reported in non-human primates. In humans, tests for such functional changes using hemodynamic markers of neural activity have typically relied on cognitive subtraction. However, the temporal resolution of fMRI allows a more direct test than that afforded by cognitive subtraction of the idea that certain brain regions may increase their neural activity during retention delays in humans. Using a method that exploits this temporal resolution, increased functional activity attributable to a retention delay for spatial information in regions proximate to/within the right frontal eye field and the right superior parietal lobule were detected (in four out of four and three out of four subjects, respectively; this is an internal replication of the results of [E. Zarahn, G.K. Aguirre, M. D’Esposito, Temporal isolation of the neural correlates of spatial mnemonic processing with fMRI, Cognit. Brain Res., 7 (1999) 255–268.]). Second, a model in which ventral and not dorsal prefrontal cortex in humans is involved in simply maintaining spatial information was tested. The results disputed this model as increases in fMRI signal attributable to the retention delay were detected more frequently in dorsal than ventral prefrontal cortex. Third, a model which posited that the intensity of neural activity is causally related to the accuracy of spatial mnemonic representation was tested by comparing retention delay signal between correct and incorrect trials. The results did not support this model in any of the regions tested. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

In non-human primates, a neural phenomenon temporally associated with retention of spatial information across a delay is the presence of changes in firing rate [6,25,43,56]. Such changes in firing rate during spatial retention delays have been hypothesized by some to be the activation of relevant perceptuo-motor representations [26]. Others have, perhaps similarly, hypothesized that they are “on-line” representations of spatial information [29]. In this report, we assume that local neural activity changes during retention delays indicate involvement of that part of cortex in some aspect of mnemonic processing and/or motor preparation relevant to the task at hand.

It is of general interest to see which brain regions evidence neural activity changes during retention delays for spatial information in humans. Some previous efforts to address this question have not directly tested for functional activity changes during the retention delay, but rather have used cognitive subtraction [3,14,32,39,40,45,66,68]. In this context, however, cognitive subtraction has been shown to be susceptible to artifact that could lead to invalid inference [74]. In contrast, we have previously observed functional changes directly attributable to a retention delay for spatial information in humans with fMRI using an analysis method that is not susceptible to such artifact [74]. These changes occurred in all subjects in a region proximate to/within the frontal eye fields (FEF) and in the superior parietal lobule (SPL). One purpose of the current study was to see if this finding could be internally replicated. Replication (first internal, then in other laboratories) is a well recognized necessity of scientific research for the demonstration of the reliability of results.

A model put forth by Petrides [52] and Owen et al. [45] states that in humans, ventral prefrontal cortex (PFC) is involved in the maintenance of information (including spatial information) to be used in comparisons while dorsal PFC is only invoked during monitoring and/or manipula-
tion of information. In our previous study, subjects were presented with a brief visual stimulus whose location was to be compared with that of a second stimulus presented after a retention delay [74]. The models of Refs. [52,45] predict no activation associated with the retention delay in dorsal PFC in this type of paradigm. Counter to this prediction, increases in functional activity attributable to the retention delay were detected within the dorsal PFC of a subset of the subjects. Data from ventral PFC were not acquired. A second purpose of the current study was to more fully test the models of Refs. [52,45] by collecting data from both the ventral and dorsal PFC during the maintenance of spatial information to be used in a comparison.

A third purpose of the current study was to test a model that relates the intensity of neural activity (on a spatial scale of millimeters) to the accuracy of spatial representation in a set of selected brain regions. Although this model resides in a gross temporal (s) and spatial (mm) domain, it is of interest because it considers a mechanism of neural computation, therefore going beyond models which solely regard an unspecified nature of involvement in spatial mnemonic representation. The test of this model involved comparing the intensity of fMRI signal change attributable to the retention delay of correct trials to that of incorrect trials.

2. Methods

2.1. Subjects

Four healthy, right handed volunteers with normal vision participated in this study. They will be referred to as subjects JB (M; age 22 years), JR (F; age 19 years), CS (F; age 19 years), and ST (M; age 19 years). All gave informed consent.

2.2. Behavioral paradigm

The behavioral paradigm (implemented using PsyScope v1.1b software; [8]) involved positional discrimination of two visual stimuli that were presented either simultaneously [the ND (no-delay) condition] or separated in time [the D (delay) condition]. Thus, the D condition was designed to rely on the mnemonic maintenance of spatial information (i.e., in the absence of stimulus energy), while the ND condition was designed to be performable without it. The spatial and temporal structures of the D and ND conditions are described directly below.

2.2.1. ND condition

The time line of an ND trial is schematized in Fig. 1 (top). Each trial began with a blank screen (gray background) from $t = 0$ to $t = 3600$ ms (not shown in Fig. 1). Then at $t = 3600$ ms, a centrally located (fixation) line segment appeared. The subject was instructed to maintain fixation on this line segment for as long as it remained on the screen. At $t = 3900$ ms, a line segment (referred to as the target; Fig. 1) in addition to a pair of collinear line segments separated by a distance equal to the length of the target line (referred to as the vernier, Fig. 1) appeared in the subject’s upper left quadrant, and both remained there for 1100 ms. On each trial, the vernier was either more or less eccentric than the target. During this 1100 ms, the subject made a response based on judging the relative eccentricities of the target and vernier stimuli: If the vernier was more eccentric (i.e., farther away from the fixation line) than the target line, the correct response was to press the right button of a gamepad interface with a consistent finger of the right hand. Likewise, the left button was to be pushed with a consistent finger of the left hand if the vernier was less eccentric than the target stimulus. The a priori probability of the correct motor response being left or right on any given trial was 50%. The response had to occur during the 1100 ms of the simultaneous target/vernier stimuli presentation ($t = 3900$–5000 ms after the beginning of the trial), or else it was not recorded. Thus, the response was required while the stimuli forming the basis of the judgment were still present. A blank screen was shown from the offset of the fixation line until the start of the next trial.

2.2.2. D condition

The time line of a D trial is schematized in Fig. 1 (bottom). Each trial began with a blank screen (gray background) from $t = 0$ to $t = 3600$ ms (not shown in Fig. 1). A fixation line then appeared, and remained on the screen for a period of 13.4 s. The subject was instructed to maintain fixation on this line for as long as it remained on the screen (as in the ND condition). At $t = 3900$ ms, the target appeared in the subject’s upper left quadrant for 100 ms (stimulus presentation in Fig. 1). A 12-s delay ensued during which only the fixation line was present (delay in Fig. 1). At $t = 16$ s, the vernier stimuli appeared for a duration of 1000 ms (discrimination/response in Fig. 1). The same response rules as in the ND trials determined the correct response during D trials. The response had to occur while the vernier stimulus was present ($t = 16,000$–17,000 ms after the beginning of the trial), or else it was not recorded. Thus, the response was required while one of the stimuli that formed the basis of the judgment was not present (i.e., the target), while the other one was present (i.e., the vernier). Thus some mnemonic representation of
the location of the target was presumably required to respond correctly at an above chance level. The target stimuli for both the ND and D conditions were presented at eccentricities of roughly 6–12°. All black line stimuli (fixation, target, and vernier) were approximately 0.2° thick and 1.7° long. The target and vernier stimuli on a given trial (either ND or D) were separated by approximately 1.1° along a radius emanating from the center of the fixation line at approximately a 45° angle from horizontal, and always in the upper-left visual field. The purpose of this constraining of stimuli to the upper-left visual field was to maximize signal:noise of the fMRI signal in the case of topographical representation of spatial information

Each trial in both the ND and D conditions was 30 s. This duration includes any effective inter-trial interval. The ND and D trials were presented pseudo-randomly in runs of 10 total trials. The duration of a run was thus 5 min. The actual number of ND and D trials presented per run was random, following a hypergeometric distribution with the pool of ND trials being 10, the pool of D trials being 10, and the sample size being 10. Consequently, the number of D and ND trials per run was not always equal. Subjects performed four runs of the paradigm, with the expected number of ND and D trials per subject each being 20.

All subjects were trained on both ND and D trials prior to the day of scanning. This training was performed on two days, with the second day being the day prior to the scan, and the first day being separated by at least 1 day from the second day. On the first training day, the subjects were provided with a verbal and schematic overview of the temporal and spatial structure of the ND and D trials, the instructions to maintain fixation while the fixation line was present, and the response rules. They subsequently performed 10 ND trials and 10 D trials on each training day. The fixation instructions were reiterated on the second training day and on the day of the scan.

2.2.3. Behavioral measures
Behavioral measures were only tabulated for performance during scanning (not during practice days). On each trial (of both ND and D types), the accuracy of the positional discrimination (correct or incorrect; failures to respond were tabulated as incorrect), reaction time from onset of vernier stimuli, and the laterality of response were recorded by the testing program and analyzed off-line. Differences in accuracy and reaction time between ND and D trials were assessed via paired t-tests. In addition, a test of bias in laterality of response was performed. Such bias would not yield an optimal strategy for the subjects to maximize % correct as the a priori probability which could have been estimated by the subject from training of a left or right response was 0.5.

2.3. fMRI scanning methods
2.3.1. fMRI data acquisition
Imaging was carried out on a 1.5-T SIGNA scanner (GE Medical Systems) equipped with a fast gradient system for echo-planar imaging. A standard radiofrequency head coil was used with foam padding to comfortably
restrict head motion. High-resolution sagittal and axial T1-weighted images were obtained in each subject. Using the BOLD [44] technique, a total of 150 gradient echo-planar images per slice ($T_R = 2000\text{ ms, } T_E = 50\text{ ms};$ 18 contiguous 5-mm axial slices with no skip between slices) were then obtained in each of 4 scans at a resolution of $64 \times 64$ pixels in a 24-cm field of view. This yielded a total of 600 data points in time per voxel per subject. During these scans, the subject was performing the behavioral paradigm described above. Twenty seconds of “dummy” gradient and RF pulses preceded the actual data acquisition to approach steady-state tissue magnetization.

### 2.3.2. Data processing

Off-line data processing was performed on SUN Sparc workstations using programs written in Interactive Data Language (Research Systems, Boulder, CO). After image reconstruction and prior to motion correction, the data were corrected in time for the interleaved fMRI slice acquisition sequence by using sinc interpolation. The data were then subjected to two iterations of least-squares, six parameter, rigid-body realignment (part of SPM96b package; [21]) without corrections for spin history [23] which would not be expected to be necessary for the long TR used here [31]. The performance of this motion correction varied. In the loss of portions of the top and bottom slices of the functional data (due to z-motion out of the field of view).

### 2.4. Analysis of fMRI time series

#### 2.4.1. Multiple, single-subject analyses

The voxel-wise data from each subject were statistically analyzed individually within the framework of the general linear model modified for serially correlated errors [70]. As detailed below, both Talairach coordinates from observed suprathreshold voxels from individual subjects, as well as the local gross neuroanatomy of said activations were used to assess inter-subject variability in the locations of activations.

The rationale for multiple, single-subject analyses as opposed to a group analysis approach is that information can be lost when data from subjects are combined at an early stage of analysis and subject-by-task interactions are ignored. Most analyses de facto test for activation patterns that are consistent enough across subjects in a standard space to be detected after group-averaging. Such analyses typically assume that any aspect of brain organization that is not consistent across subjects is either artifact, or not of interest [30]. To test for the possibility of inter-subject variability one needs to either explicitly model subject-by-task interactions or perform multiple, single-subject analyses and observe patterns of variability / consistency. As the latter method allows maximal preservation of information regarding structure:function relationships, it has been used here. It is also relevant to note that even from a pure signal-detection standpoint, group neuroimaging analyses are not expected to be as powerful as single subject designs given the same total number of observations (due to spatial variability of activations across subjects; [71]).

![Fig. 2. The logic of the hypothesis tests regarding functional change corresponding to the retention delay is illustrated.](image)
Fig. 3. Shown is a schematic representation of a probabilistic model that relates the intensity of neural activity to the variability of spatial mnemonic representations in a causal fashion. There are different probability densities (univariate Gaussian here, but could be multivariate and of arbitrary form) of the spatial representation that can be drawn upon in a particular trial for a first stimulus. Three (but could be any number > 1) probability densities which differ only in their variance are shown. The areas of the filled, black tails of the densities are directly related to proportion of errors in a forced choice paradigm involving a positional discrimination of a second stimulus located at an arbitrary, fixed distance to either one side or the other of the true position of the first stimulus. It can be seen that the ratio of the black to white area of the density increases as the variance of the density increases. For the case shown, the choice of density (and thus variability of spatial representation) on any given trial would be determined by a discrete random variable (perhaps indicative of some behavioral state), though more generally the variance could be a function of a continuous random variable. To be clear, all that has been described of the model so far is a probabilistic nature of the accuracy of spatial representation with no reference to neural activity. Now, associated with each variance is an intensity of neural activity (indicated by the dark bars) such that smaller variances are associated with more intense neural activity. Thus, variance [distribution A] < variance [distribution B] < variance [distribution C] implies $I_a < I_b < I_c$ (where the $I_i$ is the intensity of neural activity corresponding to probability density $i$). If the variance of the random variable which determines the density function is non-zero over trials (here indicated by the presence of all three trial types in the pie diagram), then this model predicts that correct trials will have a greater average neural signal intensity than incorrect trials.
Finally, standard random effects models applied in standard space assume a Gaussian distribution of subject-wise effects; this is an assumption that is difficult to test with the relatively small numbers of subjects typically used. An alternative method (and the one adopted here) is to use the binomial distribution [34] to test the hypothesis that the probability of an effect in a defined region across subjects is greater than a common false-positive rate applied to each subject’s data. This method is valid regardless of the underlying distribution of subject-wise effects.

2.4.2. Principle of test for changes attributable to retention delay

This test is based upon the separation in time between the various behavioral components of the D trial types relative to the temporal resolution of fMRI. Conceptually,

Fig. 4. Shown are t-statistic maps corresponding to fMRI signal increases attributable to the retention delay of D trials of the four individual subjects. Specifically, the t-statistic of each voxel is that associated with the sum of the least-squares coefficients of the middle two shifted impulse response function covariates [73]. These t-statistic maps are overlaid on their corresponding, spatially normalized T1-weighted images, and thresholded at $\alpha = 0.05$ per search region per subject. The maps have been masked with the right FEF search region (green), right SPL search region (blue), and the bilateral PFC search region (pink) such that only results from within these regions are visible. This masking is appropriate as the hypotheses of the current study were limited to these regions. The suprathreshold voxels themselves are in yellow. (A) subject CS and subject ST, (B) subject JB and subject JR.
the D condition comprises the temporal components of stimulus presentation, delay, and discrimination/response periods (and implicitly, inter-trial interval; Fig. 1 bottom). Each component is temporally contiguous with the other, such that no absolute boundary can be established that delimits the neural processing associated with a given component. However, as the duration of the delay period is long both with respect to neural dynamics and the fMRI response, it is theoretically possible to statistically isolate functional changes attributable exclusively to the delay period from those attributable to the other task components. In brief, a basis set of shifted BOLD fMRI impulse response functions modeled the fMRI response associated with each trial (Fig. 2). A subset of these shifted impulse response functions (the middle two) would be sensitive to fMRI signal changes attributable to neural activity changes during the retention delay only (Fig. 2). Their least-squares coefficients were used to generate t-statistics [70] which allowed statistical tests of the relevant hypotheses. For comprehensive statistical and methodological details, see Ref. [73].

It is essential to keep in mind that the measure provided by BOLD fMRI is not of neural activity but of hemodynamically transformed neural activity. Hemodynamics in-
duce a delay and dispersion of neural activity changes [4,22]. Therefore, any neural activity changes during the retention delay will not manifest as fMRI changes exactly during the retention delay. Instead fMRI signal changes corresponding to retention delay neural activity changes will begin a few seconds after the onset of the retention delay. The analysis method used explicitly accounts for this delay and dispersion [73].

2.4.3. Replication tests in FEF and SPL

The designation of particular voxels from the previous study as being part of right FEF or right SPL was based upon both the location of voxels on individual neuroanatomy as well as their Talairach coordinates (see Ref. [74]). Once so designated, the within-subject, mean centers of mass of these voxels in Talairach coordinates (in mm) were determined. The approach taken to determine neuroanatomical regions in which to test for retention delay activity in the new subjects was based on a method used by Liang et al. [35]. In brief, a sphere was generated in Talairach space around the center of mass for each region from the data of the previous study with a radius equal to sqrt(10 [largest eigenvalue of the coordinates from the previous study]). If the centers of mass were normally distributed with homogeneous variance along each axis, then this sphere would contain approximately 98% of the centers of mass of suprathreshold voxels [35]. This search region was then transformed into the space of each of the subjects of the current study [21].

Statistical thresholds for replication were obtained by Bonferroni correction based upon the size (in voxels) of the search regions (yielding \( \alpha = 0.05 \), one-tailed, per search region per subject). That is, the probability of a single suprathreshold voxel or more per region was 0.05 under the null hypothesis. So, it would be incorrect to hold the notion that more than one voxel per region would be required for statistical significance when using such thresholds.

2.4.4. Tests for retention delay activity in PFC

PFC regions of interest were defined on the anatomical images of each subject by one of the authors (EZ). All brain regions anterior to the precentral sulcus extending ventrally to the orbito-frontal regions and dorsally to the top of the brain were included with the constraint that no voxels included in the FEF search region described above (reflected across the Y–Z plane to yield a bilateral FEF representation) would also be included in the PFC search region. Statistical thresholds for rejecting the null hypothesis in the PFC were obtained by Bonferroni correction based upon the size of the PFC search regions within each subject (yielding \( \alpha = 0.05 \), one-tailed, per bilateral search region per subject).

2.4.5. Test of model depicted in Fig. 3

Fig. 3 describes a model in which the intensity of neural activity is causally related to the accuracy of spatial mnemonic representation. This model predicts that correct trials will tend to have greater fMRI signal changes attributable to the retention delay than incorrect trials. This is equivalent to an interaction of retention delay and accuracy.

Tests for interactions of retention delay and accuracy were performed in fMRI time series averaged over clusters (i.e., 1 or more voxels that were contiguous in 3-D) of voxels within search regions for right FEF, right SPL, and bilateral PFC that were significant for the main effects (i.e., summed across correct and incorrect trials) of retention delay. The false positive rate of this test was controlled within each of the three search regions of each subject at \( \alpha = 0.05 \) (two-tailed) by Bonferroni correction based on the number of clusters searched. The main effect of retention delay and the interaction of retention delay with accuracy were orthogonal comparisons.

3. Results

3.1. Behavioral measures

Accuracy and reaction time measurements for ND and D trials were obtained from all subjects (\( n = 4 \)) during fMRI data acquisition. Accuracy on ND trials (mean = 95%; range = 90–100%) was significantly better (\( t = 9.17, \text{df} = 3, \text{two-tailed} \ p = 0.0027 \text{by paired t-test} \) than performance on D trials (mean = 74%; range = 67–86%). Reaction times for delay trials (mean = 680 ms) were not significantly different (\( t = 1.49, \text{df} = 3, \text{two-tailed} \ p = 0.23 \text{by paired t-test} \)) from those for no-delay trials (mean = 591 ms). A bias for side (i.e., left or right) of motor response was not detected (\( t = -0.33, \text{df} = 3, \text{two-tailed} \ p = 0.76 \text{by paired t-test} \)). This supports the idea that if there was any systematic (across subjects) bias in the remembered, egocentric spatial representation of the target relative to its presented location (or purely in motor output), that such a bias was small relative to the difference in eccentricity between the presented location of the target and the vernier stimulus. Therefore, the paradigm seemed more sensitive to variable errors in spatial representation than systematic.

3.2. Replication tests in FEF

Suprathreshold fMRI signal increases attributable to the retention delay of the spatial memory test were observed in four of four subjects in the right FEF search region. Examination of the neuroanatomic location of the suprathreshold voxels within the FEF search region (Fig. 4A,B) revealed that subjects JB, JR, and ST evidenced such voxels at the junction of the precentral sulcus and superior frontal sulcus (as previously observed by [74]).
Fig. 5. (A) Shown is the trial-averaged time series from a representative voxel from right FEF (subject JB) that exhibited suprathreshold signal increases attributable to the retention delay of D trials. The large symbols connected by sinc-interpolation (for ease of viewing) are the actual data; the smaller symbols are fits (or predicted values) generated by the linear model used in analysis. The symbols overlap at some points. (B) The group averaged z-score signals for the suprathreshold contiguous (in 3-D) clusters of the right FEF search region are shown. The value at $t = 0$ was subtracted from the ND and D time series shown in (B) for display purposes. The error bars are standard deviations (S.D.) (not standard errors (S.E.) which reflect variability in the normalized responses across subjects. These error bars are smaller than the symbols in some cases. In both (A) and (B), the arrows indicate when a motor response was required during ND (left arrow) and D (right arrow) trials while the gray bar indicates the retention delay period during D trials.

while subject CS evidenced a suprathreshold voxel within a distance of one voxel to this junction. In subjects CS, JB, and ST, there were also suprathreshold voxels more anterior to this junction, lying in the superior frontal sulcus (Fig. 4A,B). In subjects JB and JR, suprathreshold voxels were located dorsally (Fig. 4B) relative to subject CS (Fig. 4A), while the suprathreshold voxels of subject ST (Fig. 4A) had the greatest ventral–dorsal extent within this search region. The across-subject mean (±S.D.) coordinates (in mm) of the centers of mass in Talairach space for

Fig. 6. (A) Shown is the trial-averaged time series for a representative cluster of voxels (of subject ST; cluster size = 2 voxels) from right SPL that exhibited suprathreshold signal increases attributable to the retention delay of D trials. Conventions are the same as in Fig. 5A. (B) The group averaged z-score signals for the suprathreshold contiguous (in 3-D) clusters of the right SPL search region are shown. Conventions are the same as in Fig. 5B.
the suprathreshold voxels for the right FEF search region were \([29 \pm 4], -9 \pm 7, 58 \pm 10\). The corresponding mean \((\pm \text{S.D.})\) coordinates observed in Ref. [74] were \([32 \pm 3], -3 \pm 4, 52 \pm 4\).

Visual inspection of the trial-averaged fMRI time series from the suprathreshold voxels within the right FEF search regions provided confirmation of the results of the statistical analysis. To properly visually interpret these time series, one must recall that fMRI signal changes are delayed and dispersed versions of neural activity changes. Therefore, neural activity changes occurring during a particular time in the trial will be manifested as smooth fMRI signal changes at later points in time. The analysis method used explicitly accounted for this delay and dispersion [73].

In the suprathreshold FEF search region voxels one sees signal increases (relative to inter-trial interval) during D trials that are protracted relative to those present during ND trials. As a representative example, the trial-averaged time series (of both ND and D trials) from a single cluster in the right FEF of subject JB which evidenced suprathreshold signal increases attributable to the retention delay is shown in Fig. 5A. This region, like most others with suprathreshold signal increases attributable to the retention delay during D trials, also evidenced a suprathreshold signal increase with respect to the inter-trial interval during ND trials. 

The trial-averaged ND and D signals for the suprathreshold contiguous (in 3-D) clusters of the right FEF search region of each subject were averaged to yield a regional average in each subject. The \(z\)-scores (to eliminate the effects of intersubject variability in baseline offsets and scaling but to retain shape information) of these subject-wise regional averages were, in turn, averaged across subjects. The resulting right FEF trial-averaged time-series are displayed in Fig. 5B as a representation of the group response. The same pattern as seen in the single cluster from the right FEF of subject JB is seen in the signal averaged across all clusters and subjects. There is a transient response during ND trials, and a more protracted response during D trials.

3.3. Replication tests in SPL

Suprathreshold fMRI signal increases attributable to the retention delay of the spatial memory test were observed in the right SPL search region of three of four subjects (in subjects JB, CS, and ST; not in subject JR; Fig. 4A,B). Examination of the gross neuroanatomical structures inhabited by these suprathreshold voxels did not allow an obvious refutation of the idea that they should be classified as SPL. Though it could be argued that a subset of the voxels might be classifiable as precentral, the suprathreshold voxels seemed to somewhat avoid the medial wall of the parietal lobe (Fig. 4A,B). The across-subject mean \((\pm \text{S.D.})\) coordinates (in mm) of the centers of mass in Talairach space for the suprathreshold voxels for the right SPL search region were \([14 \pm 3], -67 \pm 4, 63 \pm 6\). The corresponding mean \((\pm \text{S.D.})\) coordinates observed in Ref. [74] were \([19 \pm 4], -62 \pm 4, 64 \pm 2\).

A representative trial-averaged time series from a single cluster in the right SPL of subject ST which evidenced suprathreshold signal increases attributable to the retention delay is shown in Fig. 6A. The same general pattern is seen in this cluster as was evident in the right FEF. The trial-averaged signals averaged across regions and subjects for the right SPL is shown in Fig. 6B. Again, a similar pattern is seen here as in right FEF with a transient response during ND trials (as well as an apparent off-response upon the extinguishing of the fixation line during ND trials) and a more protracted response during D trials.

3.4. Tests for retention delay activity in PFC

There were fMRI signal increases attributable to the retention delay of the spatial memory test in the PFC of subjects JB, CS, and ST; not in subject JR; Fig. 4A,B).

Table 1

<table>
<thead>
<tr>
<th>Subject</th>
<th>3-D cluster size (in voxels; 1 voxel = 0.07 cm(^3))</th>
<th>Coordinates of center of mass (mm in Talairach space)</th>
<th>Brodmann’s area (BA)</th>
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<td>1 ([-41, 26, 50]) left BA 8</td>
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<tr>
<td></td>
<td>2 ([-38, 45, 32]) right BA 9</td>
<td></td>
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<tr>
<td>JR</td>
<td>3 ([-38, 55, 12]) left BA 10/46</td>
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<tr>
<td></td>
<td>2 ([-26, 52, 12]) right BA 10/46</td>
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<td>ST</td>
<td>1 ([-52, 7, 5]) left BA 44/45</td>
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<td>1 ([-26, -4, 65]) right BA 6</td>
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three of four subjects (in subjects JB, JR, and ST; Fig. 4A,B). The anatomical locations (in terms of both Talairach coordinates and corresponding BA) of the centers of mass of all suprathreshold clusters (i.e., voxels contiguous in 3-D) in the PFC search region of each subject are presented in Table 1. The summary results reported in a cytoarchitectonic study of human PFC [57] were used to designate BA 9 and 46 (see legend for Table 1). The locations of the suprathreshold voxels in the PFC were apparently quite variable across subjects: there was at least one suprathreshold voxel present in at least one of the three subjects in the regions of Talairach space corresponding to BA 8, 9, 10, 44, 45, and 46.

Despite this overall variability in location of retention delay activity, all three subjects did evidence suprathreshold voxels within BA 9/46 (i.e., dorsolateral PFC). A representative trial-averaged time series from a single cluster in the left PFC of subject JR which evidenced suprathreshold signal increases attributable to the retention delay is shown in Fig. 7A. The average signal of such clusters across left and right hemisphere PFC search regions and subjects is shown in region Fig. 7B. Only one subject (ST) evidenced suprathreshold voxels in ventral PFC (one cluster at the border of left BA 9 and 44, and another in left BA 44/45; Table 1).

3.5. Accuracy of spatial representation and intensity of neural activity

The model illustrated in Fig. 3 predicts that the magnitude of functional change during the retention delay would vary with accuracy. Only clusters of voxels found in either the right FEF search region, the right SPL search region, or the bilateral PFC search region that were suprathreshold for the main effects of retention delay were subjected to a test of this model. Of this set, there were none that evidenced t-values significantly different from 0 (for α = 0.05, two-tailed, per search region per subject) for the interaction of retention delay by accuracy. In Fig. 8, we show representative fMRI time series corresponding to D trials (from clusters significant for the main effects of retention delay) in the right FEF, right SPL, and (right) PFC classified according to the correctness of the behavioral response. 3

It may be argued that this test for interactions was invalid due to lack of sensitivity. This is a valid concern in general, and affects interpretation of negative results in a probabilistic sense (see Section 4). The main problem in ascertaining sensitivity is that what constitutes an a priori meaningful effect size is difficult to objectify. However, we might provisionally take as a meaningful neural interaction effect size to be equivalent to that of the main effect of retention delay. If so, then we could examine the efficiency [34] of the estimator of the interaction effect relative to that of the main effect of retention delay to determine relative sensitivity. Doing so, we see that the efficiency of the interaction estimator is actually greater

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3 It should be kept in mind that there were different numbers of trials contributing to correct group and incorrect group time series. This is because the number of correct trials > the number of incorrect trials in all subjects.

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Fig. 7. (A) Shown is the trial-averaged time series for a representative cluster of voxels (of subject JR; cluster size = 1 voxel) from left PFC SPL that exhibited suprathreshold signal increases attributable to the retention delay of D trials. Conventions are the same as in Fig. 5A. (B) The group averaged z-score signals for the suprathreshold contiguous (in 3-D) clusters of the bilateral PFC search region are shown. Conventions are the same as in Fig. 5B.
Fig. 8. Shown are representative adjusted, trial-averaged time series from D trials, categorized by accuracy. As all subjects performed above chance (see Section 3), there were more correct than incorrect trials. (A) Time-series from a cluster (size = 3 voxels) from the right FEF of subject JR. (B) Time-series from a cluster (size = 2 voxels) from the right SPL of subject CS. (C) Time-series from a cluster (size = 1 voxel) from the right PFC of subject ST. There were no statistically significant differences between correct and incorrect trials in the signal changes attributable to the retention delay in any of the clusters whose constituent voxels evidenced a main effect of retention delay. The arrow indicates when a motor response was required during D trials while the gray bar indicates the retention delay period.

than that of the main effect estimator (by a factor of 1.3). In addition, the t-statistic thresholds applied in the interaction test (an average of 2.4 across subjects) ended up being lower than the corresponding main effect thresholds (3.8) due to the smaller number of comparisons performed in the former case. Since suprathreshold main effects were observed in every subject, we can conclude that if any interactions of retention delay with accuracy exist at all, then they are very likely to be much smaller in magnitude than the main effect.

To further test the accuracy–intensity model, data collected from a separate set of five subjects performing the same behavioral paradigm [74] were subjected to a similar analysis (the only difference being that the criterion for
significance of main effects of retention delay in this supplementary analysis was based upon mapwise thresholds as opposed to search region-wise thresholds. Of the clusters from right FEF, right SPL, or (bilateral) PFC whose voxels had significant main effects for the retention delay period in the second set of subjects, none were significant for the interaction of retention delay with accuracy. Thus, in sum, none of nine independent datasets evidenced clusters of voxels (initially defined based on main effects of retention delay) with a significant dependence of signal change during the retention delay on accuracy in the right FEF, right SPL, or (bilateral) PFC.

4. Discussion

4.1. Replication tests in FEF and SPL

In a previous study [74], increases in functional signal attributable to a retention delay for positional information were observed in five of five subjects in both right FEF and right SPL. These results were replicated here in a different set of subjects (right FEF: four of four subjects; right SPL: three of four subjects). Considering that the false positive rate (corrected for multiple comparisons) was controlled at $\alpha = 0.05$ in each region in each subject, the probabilities of these numbers of positive results or greater in the current study due to chance alone are low ($p = 0.0000062$ in right FEF and $p = 0.00049$ in right SPL, from the binomial distribution). Therefore, we are led to the conclusion that these results are reliable across both subjects and data collection periods in our laboratory (from our selected population of 18–25-year olds).

Sustained fMRI signal changes attributable to retention delays have been observed in other laboratories in the context of behavioral paradigms designed to engage various (not necessarily spatial) types of memory processes [9,13,15,61]. Taken together with the current results, they support the idea that protracted synaptic activity on a spatial scale of mm is a neural mechanism involved in activating manifold types of representations. These data also complement the numerous results from electrophysiological studies which have directly demonstrated sustained neural activity during retention delays at the single-cell level in non-human primates (for example, Refs. [6,18,24,25,27,41,55,56,63,69]).

It should be reiterated that the dextral laterality of the FEF and SPL search regions for tests of replication follows from the fact that the most reliable (i.e., in five of five subjects) activations in the previous study were on the right. However, all of the task-relevant stimuli for this and the previous study [74] were either presented foveally (the fixation line) or in the upper-left visual field ( vernier and target stimuli). The purpose of this lateralization of stimuli was to maximize signal:noise of the fMRI signal in the case of topographical representation of spatial information. Therefore, neither the current study nor the previous study could test hypotheses regarding whether any laterality of functional localization is related to the laterality of stimuli. Varying the laterality of stimuli presentation within a study could test such hypotheses in the future.

As neural activity associated with a retention delay for spatial information appears to be a reliable finding in FEF and SPL, we will now attempt to integrate this observation with previous information gathered about the function of these regions. Before doing so, we feel it would be helpful and apposite to remark that one can discuss the results of brain imaging studies from two non-exclusive perspectives. One is a region-based perspective in which one tries to consider all of the functions attributed to a region based on neuroimaging and other data. The second is a cognitive process-based perspective in which one is interested in the neural substrates of a particular cognitive process. Our primary inference from this latter perspective is that, on account of their exhibiting fMRI signal change attributable to the retention delay, the FEF and SPL are involved somehow with mnemonic spatial representation. In Sections 4.2 and 4.3, we will speak more from a region-based perspective.

4.2. Previous experimental results regarding the function of the FEF

Electrical stimulation of the region hence referred to as the FEF in man [59] and non-human primates [7] elicits eye movements in a topographic fashion, while neuroanatomically concordant regions activate during performance of self-paced [33,49,51] or visually-guided eye movements [2,19,50,68] as measured with positron emission tomography. Lesions involving FEF impair voluntary eye movements in non-human primates [64,65]. There is thus rather convincing evidence that the so-named FEF is involved in executive aspects of eye movements. However, it does not logically follow from these observations that the FEF, or every region in its vicinity, is involved exclusively in the executive aspects of eye movements (however, see the opinion expressed in Ref. [48]). On the other hand, it is possible that in this study, as well as most other reported neuroimaging studies of spatial working memory, that eye movements did occur (as they were not measured) and were the cause of the signal changes observed.

Beyond the possibility of the FEF being involved in more than simply eye movements, there is lesion [16,17,42,53,62] and electrophysiological [6,25] evidence supporting a role of the FEF in aspects of visual attention and spatial memory in human and non-human primates. Furthermore, several previous group-averaged, cognitive subtractive neuroimaging studies of maintenance of spatial information have reported suprathreshold signal change in the [spatial memory requiring condition]—[non-memory requiring, control condition] subtraction at locations in proximity to/within the FEF [3,32,45,66,68]. However, it
should be reiterated that eye movements were not controlled in these neuroimaging studies.

Courtney et al. [13] provided a definition of what they would consider to be an area “specialized” for spatial mnemonic processing. Their criteria included that such an area must be distinct from the functionally defined FEF. Using event-related fMRI methods, they found that within the vicinity of the FEF, the centroids of voxel clusters whose fMRI signal change correlated with saccades were more posterior to the centroids of voxel clusters whose fMRI signal change correlated with the retention delay. This result supported the authors’ hypothesis that there was a region anterior to the FEF that was specialized for spatial mnemonic processing. Interestingly, however, it was found in that study that there were correlations with the retention delay in voxels lining the precentral sulcus (considered by those authors to be the FEF proper) as well as the superior frontal sulcus (considered by these authors to be a region specialized for spatial mnemonic processing; see note 26 from that work). Though the results of the current study cannot directly speak to the hypothesis of functional segregation within the vicinity of the FEF, they do externally replicate the general finding [13] of functional change temporally associated with maintenance of spatial information in the neighborhood of the FEF. A comparison of the mean Talairach coordinates of centers of activation corresponding to the retention delay in the vicinity of the (right) FEF between these two studies demonstrates reasonable agreement (mean ± S.D., in mm: [32 (±3), −3 (±4), 52 (±4)] in the current study, and [27 (±5), −5 (±4), 49 (±5)] in Ref. [13]).

4.3. Previous experimental results regarding the function of the SPL

Similar to the FEF, lesion evidence supports a role for the posterior parietal cortex in aspects of oculomotor function and spatial attention in humans [5,47,53,54,60] and non-human primates [36,37]. Corbetta et al. [12] reported that the SPL evidenced functional changes (measured with PET) that correlated with spatial attention allocated to the contralateral hemisphere during an experiment which controlled for eye movements. Previous group-averaged, cognitive subtractive neuroimaging studies of egocentric spatial memory in humans have reported activation in BA 7 (i.e., SPL [14,45]), though others have reported activation in BA 39/40, i.e., inferior parietal lobule/supramarginal gyrus [32,66,68]).

Posterior parietal areas 7a and LIP in macaques may be functionally analogous to parts of the SPL in humans (posited in Ref. [53]). Neurons in areas 7a and LIP display memory-correlated activity during delayed saccade tasks [1,10,28] which seems to be related, in separate neuronal sub-populations, to either the planning for an intended eye movement or to the maintenance of sensory information [11,38,67]. Both of these functions concern the representation of spatial information over time (one in motor coordinates, the other in sensory coordinates).

Muri et al. [42] used transcranial magnetic stimulation (TMS) to putatively induce a transient lesion in human subjects during the performance of a memory-guided saccade task. It was reported that such lesions of the SPL only affected contralateral, memory-guided saccadic amplitudes when they were administered 260 ms following saccade target presentation, not when lesions were administered during the midst of the retention delay. Another TMS study in human subjects showed maximal effects of transient lesion in posterior parietal cortex on the variable error of memory-guided saccades at 100 ms following the extinction of the target (tests were performed at 0, 50, 100, and 150 ms post-extinction; [46]). The results from both of these studies support the necessity of the posterior parietal cortex for early mnemonic spatial processing (i.e., within a few hundred ms after stimulus energy has been extinguished). However, if the lesions administered in the study of Muri and colleagues effectively included that part of the SPL observed to be active during the midst of the retention delay in the current study, then this would suggest that such activity, although extant, is not representative of necessary neural processing for accurate memory-guided saccades in an otherwise normal subject. Future studies could test this idea more directly by guiding TMS with functional maps of retention delay activity obtained with fMRI from the same subject. We note that the behavioral paradigms of Refs. [41,45] are different from the paradigm used in the current study: in the memory-guided saccade tasks of these two studies, subjects could rely on motor coordinates to perform the task correctly, while in the current study subjects could not rely exclusively on motor coordinates (as the task required the remembered stimuli to be accessible in sensory coordinates).

4.4. Tests for retention delay activity in PFC

The presence of suprathreshold signal change attributable to the retention delay in the PFC of three of four subjects provides support for the hypothesis that parts of the PFC of primates are engaged during processing associated with maintenance of egocentric spatial information [29]. A model put forth by Petrides [52] and Owen et al. [45] posits that in humans ventral PFC (BA 44, 45, and 47) is involved in the maintenance of information (including spatial information) to be used in “active comparisons” (like that type of information remembered by subjects in the current study), while dorsal PFC (BA 9 and 46) is only invoked during “monitoring” and/or “manipulation” processes. In the current study, positive relationships with the retention delay were not observed at all in BA 47 and in only one of four subjects in BA 44/45. In contrast, all subjects (from both the current study and a previous one using the same method; [74]) that exhibited suprathreshold voxels in PFC contained at least a subset in the expanse of
BA 9/46 [57], i.e., dorsal PFC. Thus, the results of this study do not support the models of by Petrides [52] and Owen et al. [45]. Therefore, either these models should be modified to account for these results or some argument should be generated to dispute the ability of this experiment to test these models.

4.5. Accuracy of spatial representation and intensity of neural activity

No differences between correct and incorrect trials in the magnitude of signal change attributable to the retention delay were observed in those voxel clusters (of the right SPL, right FEF, and PFC search regions) whose constituent voxels evidenced main effects of retention delay. It is worth subjecting this result to a careful analysis as it well exemplifies the seeming problem of inference based on negative results. First we must decompose the problem of inference into two independent components which we must be careful to avoid conflating: statistical and logical. The statistical issue is that of sensitivity. In this case, sensitivity to the main effects of retention delay was less than the sensitivity to a same-sized interaction of retention delay and accuracy. In addition the thresholds applied to the interaction were lower than those applied to the main effect of retention delay. This information coupled with the lack of a detected interaction allows us to state that if the effect predicted by the accuracy–intensity mechanism exists, it is likely to be appreciably smaller than the main effect of retention delay.

The logical issue involves asking what, if anything, is implied if the negative result is not a statistical false negative. When applied to a specific region, the model specified in Fig. 3 comprises the conjunction of two propositions. The first is implicit (and easily overlooked), and is that the region is involved in spatial representation. The second is that accuracy of spatial representation is a function of the intensity of neural activity in that region *4* (see Fig. 3 for details). Together they predict a difference in fMRI signal change between correct and incorrect trials. Therefore, if this prediction is not observed then the disjunction of the negations of the two propositions is true. So, it could be that (possibility 1) none of the regions examined have anything to do with spatial representation whatsoever, or (possibility 2) that one or more of them does but just not through the specified accuracy–intensity mechanism. We need not stop there, however, because of the manner in which we selected the voxels in which to test the model. Only voxels that had significant associations with the retention delay were tested. If we take such an association with the retention delay to be indicative of involvement in spatial representation, then we would discard possibility 1 and refine our conclusion to state that the regions of the FEF, SPL, and PFC involved in spatial representation do not possess the accuracy–intensity mechanism.

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*4* This is a bit subtle because the second proposition implies the first, but the converse is not true.


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