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# Directional Selectivity of BOLD Activity in Human Posterior Parietal Cortex for Memory-Guided Double-Step Saccades

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**Medendorp, W. Pieter, Herbert C. Goltz, and Tutis Vilis.** Directional selectivity of BOLD activity in human posterior parietal cortex for memory-guided double-step saccades. *J Neurophysiol* 95: 1645–1655, 2006. First published November 16, 2005; doi:10.1152/jn.00905.2005. We used functional magnetic resonance imaging (fMRI) to investigate the role of the human posterior parietal cortex (PPC) in storing target locations for delayed double-step saccades. To do so, we exploited the laterality of a subregion of PPC that preferentially responds to the memory of a target location presented in the contralateral visual field. Using an event-related design, we tracked fMRI signal changes in this region while subjects remembered the locations of two sequentially flashed targets, presented in either the same or different visual hemifields, and then saccaded to them in sequence. After presentation of the first target, the fMRI signal was always related to the side of the visual field in which it had been presented. When the second target was added, the cortical activity depended on the respective locations of both targets but was still significantly selective for the target of the first saccade. We conclude that this region within the human posterior parietal cortex not only acts as spatial storage center by retaining target locations for subsequent saccades but is also involved in selecting the target for the first intended saccade.

## INTRODUCTION

The posterior parietal cortex (PPC) is engaged in spatial processing, in both humans and non-human primates. Over the years it has become clear, mainly from monkey studies, that the PPC is anatomically segregated into various regions that have specialized spatial functions, including spatial attention, memory and action planning.

A prominent and well-studied region within the monkey PPC is the lateral intraparietal area (LIP). The functional role of LIP, however, is controversial. That is, some have associated LIP with the planning of saccades, whereas others have suggested that the activity of neurons in the region describes salient spatial locations, independent of the generation of any specific movement (see Andersen and Buneo 2002; Colby and Goldberg 1999; Gottlieb et al. 2002 for reviews).

In humans, neuroimaging studies on the posterior parietal cortex have recently identified a bilateral region that topographically represents targets for saccades (Sereno et al. 2001). In subsequent experiments, we have exploited the topography of the region to gain more insight into its functional organization. In so doing, we established that the region has an eye-centered organization that is updated when the eyes move (Medendorp et al. 2003). Furthermore, using memory anti-

saccades, we have demonstrated that the topography is linked to the location of saccadic goals and not to the coordinates of the visual stimulus (Medendorp et al. 2005a). Finally, we showed that the region is activated for movements of the eyes and either hand, but that the activation is modulated by which effector is selected to act on the targets (Medendorp et al. 2005b). Based on the collective weight of this evidence, this human PPC region may correspond to the monkey area LIP (Duhamel et al. 1992; Snyder et al. 1997; Zhang and Barash 2000).

Would this region, which we will refer to as retinotopic IPS (or retIPS), have the capacity for storing multiple targets for successive saccades? Monkey LIP has been demonstrated to lack such resources. That is, Mazzoni et al. (1996) recorded from LIP neurons using a memory double-saccade paradigm in which a monkey had to memorize the locations of two stimuli and subsequently made saccades to both locations. They found that the majority of LIP neurons coded the next planned eye movement even though the animals must hold in memory two cued locations. Their results suggest that the second target (or movement plan) must be stored outside of LIP, perhaps in frontal areas (Schall and Hanes 1993; Tian et al. 2000). However, a recent fMRI study by Todd and Marois (2004) has suggested that the human PPC does indeed have storage capacity for multiple target locations in a delayed visual matching-to-sample task. More specifically, the authors showed that activity in their region, which exhibits some overlap with human retIPS, is tightly correlated with the limited amount of target representations that can be stored in memory.

To address this issue, we tracked fMRI signal changes in retIPS while subjects were presented with two brief visual targets, separated by a long delay, in either the same or different visual hemifields. Subjects were required to memorize these two target locations and after another delay saccade to them in sequence. Our results suggest that retIPS is involved in storing multiple target locations, but with a significant bias toward the target for the first saccade.

## METHODS

Ten subjects (6 male/4 female), aged between 23 and 40, gave informed consent to participate in the experiments. All subjects were naïve with respect to our experimental goals. Each subject practiced

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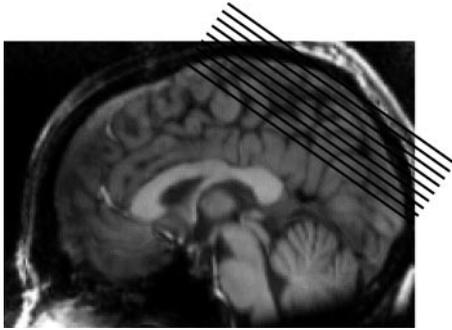


FIG. 1. The location of the nine functional slices in a sagittal image. The functional volume covers the region of interest, retinotopic IPS (retIPS).

all tasks extensively before imaging to ensure that the tasks were performed correctly. Moreover, kinematic recordings and psychophysical measures were taken to confirm correct behavior as described in the following text. Details about the fMRI setup and methods used to measure kinematics have been described in Medendorp et al. (2005a) and will be briefly summarized here.

### MRI scanning

Data were collected with a 4-Tesla Varian (Palo Alto, CA) Unity Inova whole-body MRI scanner equipped with a Siemens Sonata Gradient system (Siemens, Erlangen, Germany). We used a quadrature radio-frequency surface coil, centered on the posterior parietal lobe, to image nine contiguous slices that instantiated a functional volume coinciding with the known locations of the parietal regions-of-interest (see Fig. 1). Functional data were obtained using navigator echo corrected T2\*-weighted spiral imaging (TE = 15 ms; FA = 45°; FOV = 19.2 × 19.2 cm; TR = 1 s; in-plane pixel size = 3 × 3 mm; THK = 4 mm). Functional data were superimposed on high-resolution inversion prepared three-dimensional T1-weighted anatomical images of the brain (typically 128 slices, 256 × 256, FOV = 19.2 × 19.2 cm, TE = 5.5 ms, TR = 10.0 ms). In separate sessions, full brain anatomical images were acquired using a high-resolution inversion prepared 3D T1-weighted scan sequence (FA = 15°; voxel size: 1.0 mm in-plane, 256 × 256, 164 slices, TR = 0.76 s; TE = 5.3 ms).

### Stimulus presentation and eye-movement recording

Stimulus presentation was performed using a Silent Vision SV-4021 projection system (Avotec, Stuart, FL). This system includes an MEyeTrack-SV (Silent Vision) eye tracker (Sensomotoric Instruments GmbH, Teltow, Germany). This device uses fiber optics housed in dual stalks that sit in front of a subject's eyes, allowing presentation of visual stimuli and simultaneous CCD video-based infrared eye tracking. The visual display subtends 30° horizontally by 23° vertically with a resolution of 800 × 600 pixels and a refresh rate of 60 Hz. Eye position was sampled at 60 Hz with an accuracy of ~1°. Before scanning, we calibrated the system with a five-point calibration, with one point in each corner of the visual display as well as a central point. All experimental targets were within the range of the calibration. Analysis of the eye movement traces was performed off-line.

### Location of retinotopic IPS

To localize retIPS, we used a delayed-saccade task, which was incorporated in a block-design paradigm, as described in detail by Medendorp et al. (2005a). In separate blocks, subjects either made delayed saccades or maintained fixation. In the saccade blocks, subject viewed a brief peripheral dot, the target, while they fixated centrally. Then, a band of distractors, consisting of similar dots, blinked during a 2.5-s period, while the subjects maintained fixation. At distractor offset, subjects made a saccade to the remembered target location and then immediately back to center. The time between successive movements was 5 s. Subjects made no movement during the fixation (*F*) task. Each scan consisted of 14 blocks (each 20 s): first two blocks of fixation, then 10 blocks in which four targets in the left visual field were alternated with four targets in the right visual field, and finally two fixation blocks that concluded the scan. Thus in each run, subjects made 20 delayed-saccades into the left hemifield and 20 in the right hemifield. Subjects were tested in four runs, each lasting for 4.67 min. Subjects were given one minute of rest between runs, so that the total time devoted to the localizer was ~22 min.

### Memory-delayed double-step saccades

An event-related paradigm was used to test the response of the retIPS region for memory-delayed double-step saccades. Figure 2A illustrates the paradigm. While subjects fixated centrally on a gray square (Fix), a brief peripheral gray dot (Tar) flashed for 200 ms, either left or right of central fixation, at a random eccentricity between

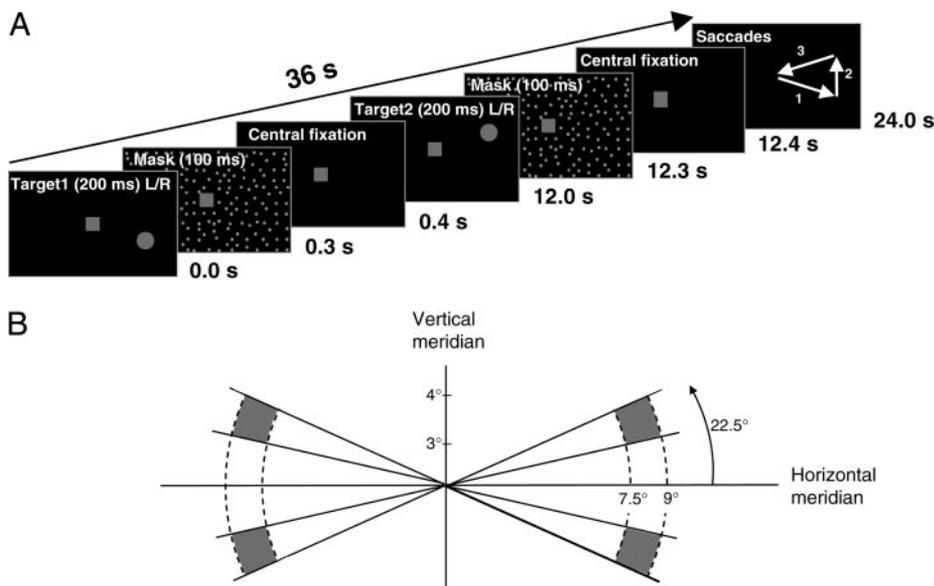


FIG. 2. Memory-guided double saccade event-related paradigm. A: sequence of stimuli and the subject's instructions. After a brief peripheral dot was presented (Tar), either in the left or right hemifield (L/R), a horizontal band of distractors was flashed briefly (Msk). After a delay of 11.6 s, a 2nd target was cued, either in the left or right hemifield, again followed by the band of distractors. Then after a further 12 s, the central fixation square was turned off, signaling the subject to look successively toward the remembered locations of the 2 targets. Stimuli and instructions related to the psychophysical measures taken (see text) are not shown. B: potential locations of the stimuli (gray areas).

7.5 and 9° and at a random angular elevation within a range of 3 and 4°, in either the lower or upper visual field. Figure 2B illustrates the potential stimulus locations, which were also used in the retIPS localizer task. Subsequently, a masking pattern (30° horizontal × 15° vertical, dot's eccentricity 0.36°, density 0.3 dots/deg<sup>2</sup>) blinked for 100 ms to disrupt iconic visual memory. This was followed by a long 11.6-s memory delay during which the subject maintained fixation. Subsequently, 12 s after the trial began another peripheral gray dot (Tar) was flashed for 200 ms, either in the left or right visual field, followed by the same masking pattern (Msk). Subjects had to memorize the locations of both targets. At all times, it was ensured that when the first target was flashed in the upper visual hemifield, the second was presented in the lower and vice versa. This was randomized across trials, conditions, and runs. Next, 12 s after cueing of the second target, the fixation square was turned off, prompting the subject to make saccades to the remembered locations of the targets, in the same sequence as their appearance. To motivate the subjects to saccade to the correct location at the right time, a small letter, either c or o, was flashed briefly, 300 and 600 ms after fixation point offset, respectively, at either remembered location (not shown in Fig. 2A). Due to their very small size (0.3°), the letters were distinguishable only if the subject fixated at the correct location at the right time. Thus the letter task provided a strong incentive for the subjects to remember the precise position of the two targets and, as such, may also have facilitated the formation of a preparatory set. Subsequently, the central fixation square (Fix) was turned on again, 1.2 s after its offset, instructing the subject to make a saccade back to the center of the screen and fixate till the end of the trial. During this period, subjects had to report whether they had seen two identical letters (c-c, or o-o) or different ones (c-o, or o-c), by pressing one of two buttons on a key pad using their right hand. The subject's actual performance was determined from the eye-movement recordings described in the following text.

Essentially, this event-related paradigm had four different conditions regarding the locations of the two targets. Both targets were presented in the right visual field (RR), or both were cued in the left visual field (LL), or the first fell in the right and the second target in the left visual field (RL) or vice versa: the first was flashed in the left and the second target in the right visual field (LR). Each scan run contained 10 epochs (36 s each), in which the four conditions were pseudorandomly interleaved, starting and finishing with a fixation block. Seven subjects were tested in four runs, each lasting 6 min.

### Control experiment

Three naïve subjects and one of the subjects that participated earlier in the double saccade task (described in the preceding text) participated in a control experiment without saccades. In this experiment,

subjects viewed the same set of sensory stimuli as above, presented at the same times but were instructed to keep fixation at all times. This control experiment served to measure the neural response that can be attributed to sole sensory processing, thus excluding the processing that follows this stage in the double saccade paradigm.

### Behavioral analysis

We recorded the button presses during scanning from five of seven subjects who participated in the double-saccade paradigm; their performance was >94% correct. Eye movements were recorded in all seven subjects. An example of one subject's eye traces [(horizontal component (black) and vertical component (gray))] during the four testing conditions is shown in Fig. 3B in relation to the temporal order of events (Fig. 3A). As shown, this subject maintained fixation during the presentation of the cues and made eye movements in the correct directions after the fixation spot was turned off. Figure 3C shows the trajectories of the saccades from this subject for all conditions separately. As shown, the responses were fairly accurate even though the subject had memorized the respective locations for 24 and 12 s. We used the following criteria to distinguish error trials from correct trials. First, during the fixation periods, eye movements should be restricted to <1°, i.e., they should remain less than the noise of our recording system. Second, after the go signal (fixation point offset), saccades should be aimed in the correct direction. We did not put a criterion on saccade amplitude, but as shown (Fig. 3C), saccade metrics were typically of correct size. Eye-movement recordings in all seven subjects confirmed that they generally performed the double saccade task correctly: only in <5% of the trials, did the subjects either break fixation or make their saccades in the wrong direction. We excluded these error trials from further analysis. Furthermore, reaction time analysis revealed that the saccades occurred with a latency of 275 ± 9 (SE) ms after being prompted by fixation point offset. This confirms that the saccades were driven by memory of the previously viewed targets and not simply guided by the visual appearance of the letters. There were no statistically significant differences in saccade latencies among the four conditions [ANOVA  $F(3,128) = 0.26, P = 0.85$ ].

Finally, eye-movement data from the four subjects that were tested in the control experiment confirmed correct fixation for virtually all trials (>97% correct).

### Image analysis and regions of interest (ROIs)

Analysis was performed using Brain Voyager 4.8 and BrainVoyager QX 1.4 software (Brain Innovation, Maastricht, The Netherlands) and Matlab software (The Mathworks). Surface coil images were aligned manually to head-coil images. For functional data analysis,

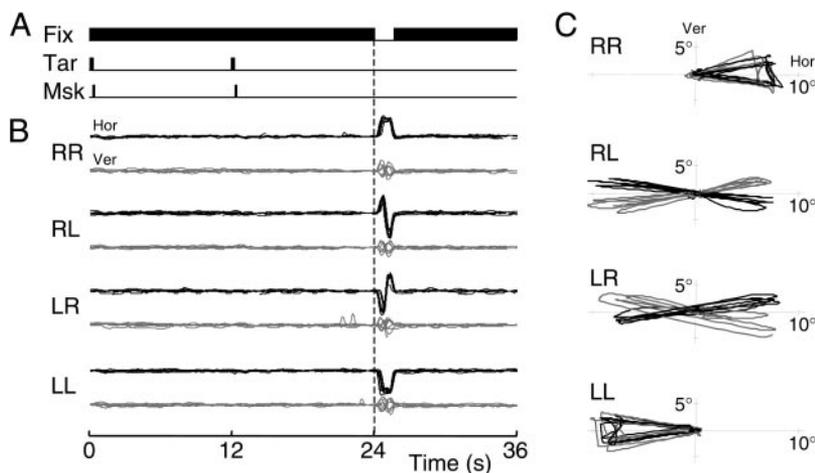


FIG. 3. A: temporal order of events in the double saccade paradigm. B: eye position traces (horizontal, black; vertical, gray) during the 4 different conditions of 1 subject. C: saccade trajectories in the 4 conditions of the same subject (black/gray, 1st target cued in upper/lower visual field). RR, RL, LR, LL signify 4 possible conditions of our paradigm; the 1st letter signifies initial location of the 1st target (R, right hemifield; L, left hemifield); the 2nd letter refers to the location of the 2nd target.

scans were corrected for linear drift and scans with motion artifacts were excluded. Anatomical and functional images were transformed to Talairach space (Talairach and Tournoux 1988).

Using the localizer scans, the parietal region of interest (retIPS) was identified by contrasting the blocks devoted to leftward saccades with the blocks for rightward saccades. We also used the anatomical criteria about the location of retIPS reported by previous studies (Medendorp et al. 2003, 2005a,b; Sereno et al. 2001<sup>1</sup>). We used the false discovery rate (FDR) controlling procedure to correct for multiple comparisons, with a maximum threshold value of 0.05, thus  $q(\text{FDR}) < 0.05$  (Genovese et al. 2002). Our ROIs contained all contiguous voxels within a cubic cluster of  $8 \times 8 \times 8$  mm centered on the point of peak activation that exceeded a threshold of  $q(\text{FDR}) < 0.05$  ( $t = 2.74$ ,  $P < 0.0065$ ).

Using this independently defined bilateral retIPS region, fMRI time courses corresponding to all activated voxels within this region were extracted for each of the subsequent event-related scans and then averaged. The average percent signal change for the four conditions, RR, LL, RL, and LR, was computed using the precueing fixation periods as a baseline. We took this baseline so that the most of the activations reported could be directly compared with the activation when no targets are stored in memory. For each condition, a mean signal and SE were computed.

## RESULTS

Using a block-design paradigm, we first identified human retIPS: a region that becomes selectively activated for single saccades to memorized targets in the right and left visual hemifields. Figure 4 shows this region in one subject, in all three slice views, which is located along the IPS within the posterior parietal cortex. The region in the left IPS (yellow voxels) shows stronger activation for a remembered target location in the right visual field than in the left, whereas the region in the right IPS (blue voxels) represents the opposite pattern.

Figure 5 (*top left*) shows the retIPS region rendered onto an inflated representation of the cortical surface of this subject, illustrating the location of the region relative to other anatomical landmarks. Again, yellow regions indicate a stronger activation for rightward targets, whereas blue regions show

increased activation for targets on the left. The other panels of Fig. 5 demonstrate the regions in three other subjects (*S2*, *S3*, *S5*) who participated in the double-step saccade paradigm. These subjects demonstrate an equivalently organized area in their PPC, mostly located within a small sulcus running medially from the intraparietal sulcus.

For all seven subjects in the double-saccade task, the Talairach coordinates (in mm), peak  $t$ -values and statistical significance of their retIPS regions are presented in Table 1. The mean Talairach coordinates (means  $\pm$  SE) of the retIPS region are  $-24.6$  (1.1),  $-61.4$  (3.0),  $44.7$  (1.6) for the left retIPS and  $21.6$  (2.2),  $-59.4$  (2.4),  $46.1$  (2.8) for the right retIPS, which corresponds to previous studies (Koyama et al. 2004; Medendorp et al. 2003, 2005a,b; Sereno et al. 2001).

Thus retIPS was identified as a neural structure that encodes the location of a target for a single saccade within a topographic map. Does this map have the capacity to store multiple targets for successive saccades? If so, this would implicate retIPS as a spatial storage center, and its activity would be expected to increase with the number of targets to be stored. Alternatively, the map in retIPS may only retain the working memory of the single target selected as the goal of the next saccade. In that case, adding additional saccade targets to working memory should not modulate the activity of retIPS.

To test between these hypotheses, we used an event-related fMRI paradigm in which subjects performed a double-delayed double-saccade task. In this task, subjects first saw the target for the first saccade, then, after a 12-s delay the target for the second saccade and then, after another 12-s delay, they made saccades successively to the remembered stimulus locations (see Fig. 2). The paradigm consisted of four different conditions regarding the cued locations of the two targets: either both were cued in the right visual field or in the left visual field or the first target was flashed in the right and the second target in the left visual field or vice versa. The four conditions are represented as RR, LL, RL, and LR, respectively (Fig. 6A).

Figure 6B shows the mean response of seven subjects in the left parietal region for each of the four conditions. As shown,

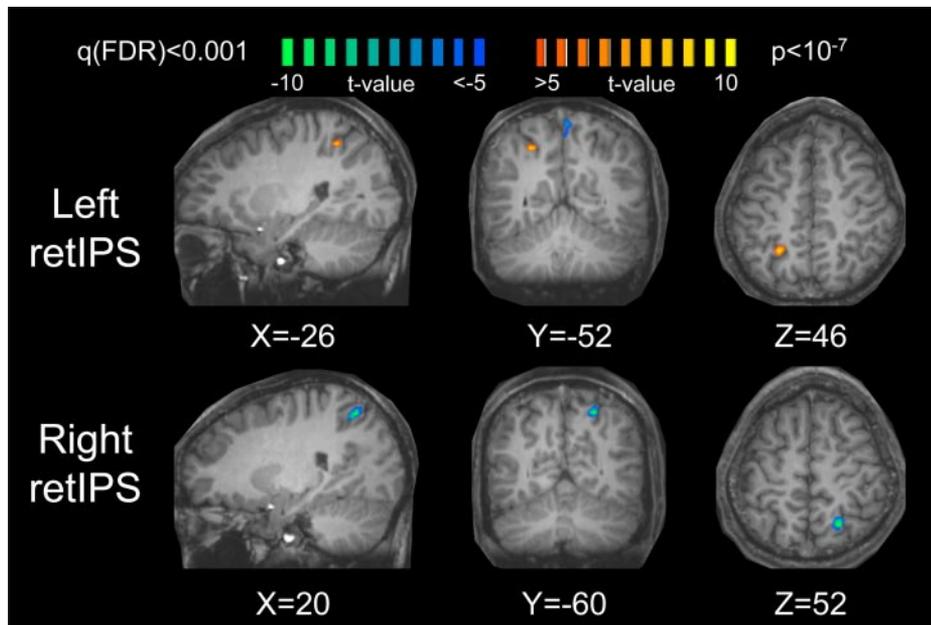


FIG. 4. A bilateral topographic region within the PPC (retIPS) of 1 representative subject (*subject S1*). The region, located along the intraparietal sulcus, shows a contralateral topography for remembered target locations in a single saccade task ( $P < 0.001$ , after FDR correction for multiple comparisons). Yellow voxels show a stronger activation for remembered target locations in the right visual field than in the left; blue voxels represent the opposite.

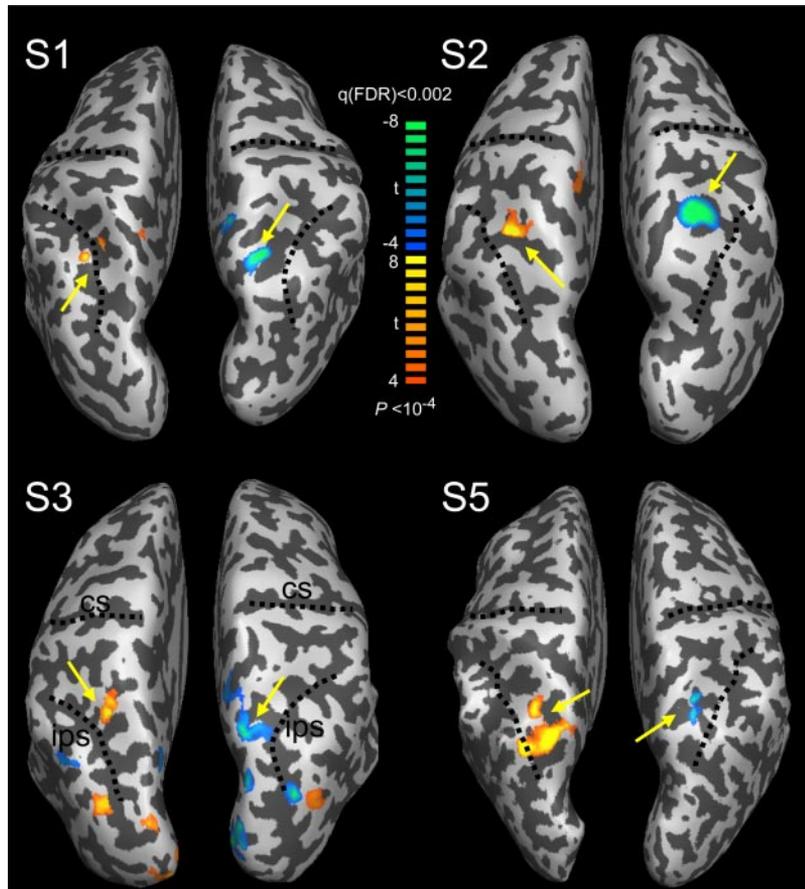


FIG. 5. RetIIPS in 4 subjects (*S1, S2, S3, S5*) shown on their inflated cortical surface (dark gray sulci, light gray gyri). CS, central sulcus; IPS, intraparietal sulcus. LH: Left Hemisphere; RH: Right Hemisphere. Yellow arrows point to retIIPS. The peak of activation within retIIPS is in the posterior superior parietal lobule, within a small sulcus running medially from the intraparietal sulcus. Yellow voxels show a stronger activation for remembered target locations in the right visual field than in the left; blue voxels represent the opposite.

after the brief presentation of the first target, cortical activation during the first delay period shows first a phasic response (time interval: 2–8 s), followed by a tonic response (time: 8–14 s). Both the phasic and tonic activity are higher in the left parietal cortex when the target was flashed in the right (contralateral) hemifield, the RR and RL conditions, compared with when it was cued in the left (ipsilateral) hemifield, the LL and LR conditions. Left and right patterns of activation are reversed for responses to the first target in the right parietal cortex (Fig. 6C).

What happens when the location of a second saccade target must be stored in memory? As shown, in all conditions, there is a phasic response coincident with the appearance of the target (time interval: 14–22 s). Again the amplitude of this response relative to the level of activation prior to target presentation is selective to the spatial location of the target.

More specifically, the amplitude of the phasic response is higher in the hemisphere contralateral to the target. This pattern of activation is thus not different from the observations made for the phasic response of the region to the first target. More interesting is the region's activation in the later part of the second delay period (time interval: 22–26 s), when the phasic response has virtually diminished. Then the region's sustained activation seems to depend on the specific spatial configuration of the two saccade targets. That is, for the left cortex, if the two saccade targets were presented in the contralateral hemifield (the RR condition), a high sustained activation was observed in the second delay period. But if they were cued in the ipsilateral hemifield (the LL condition), the level of activation was low. The right parietal region (Fig. 6C) showed a similar, but mirrored, pattern of activation. Further-

TABLE 1. Talairach coordinates, peak *t*-values, and corresponding *P* values (FDR-corrected) of retIIPS in seven subjects

Subject	Left Hemisphere					Right Hemisphere				
	<i>x</i>	<i>y</i>	<i>z</i>	Peak <i>t</i> -values	<i>q</i> (FDR)	<i>x</i>	<i>y</i>	<i>z</i>	Peak <i>t</i> -values	<i>q</i> (FDR)
<i>S1</i>	-26	-53	45	10.3	<0.001	22	-62	51	11.4	<0.001
<i>S2</i>	-30	-48	51	14.4	<0.001	26	-54	54	11.3	<0.001
<i>S3</i>	-25	-63	46	10.2	<0.001	11	-57	48	4.6	<0.002
<i>S4</i>	-25	-63	39	11.2	<0.001	29	-72	35	7.5	<0.001
<i>S5</i>	-22	-69	48	15.6	<0.001	23	-57	47	8.5	<0.001
<i>S6</i>	-22	-65	39	7.9	<0.001	17	-60	37	4.9	<0.002
<i>S7</i>	-22	-69	45	8.0	<0.001	23	-54	51	8.3	<0.001
Mean	24.6 ± 1.1	61.4 ± 3.0	44.7 ± 1.6	11.1 ± 1.1		21.6 ± 2.2	59.4 ± 2.4	46.1 ± 2.8	8.1 ± 1.0	

Values are means ± SE. Coordinates (in mm): *x* (lateral/medial), *y* (anterior/posterior), and *z* (superior/inferior) according to Talairach and Tournoux (1988).

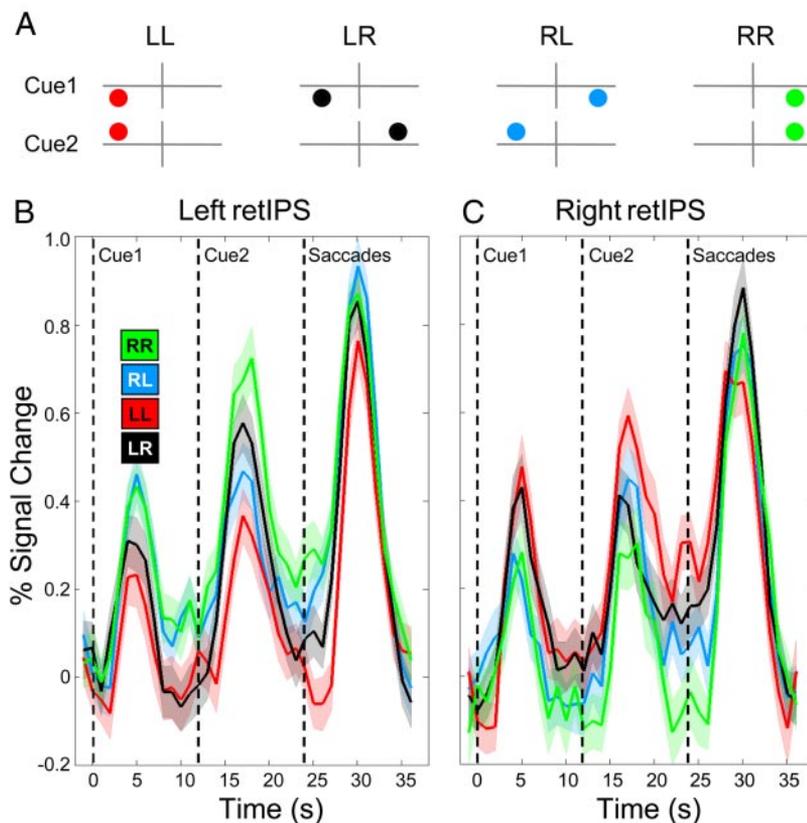


FIG. 6. RetIPI stores the locations of both stimuli in a delayed double saccade task. A: RR, LL, RL, LR signify the 4 possible conditions of the paradigm; 1st letter signifies the location of the 1st target (R, right hemifield; L, left hemifield), 2nd letter refers to the location of the 2nd cue. B and C: left (B) and right (C) retIPI activation [mean  $\pm$  SE across trials and subjects (shaded regions)] for each of the 4 conditions. Dotted lines indicate presentation of the 1st stimulus, presentation of the 2nd cue, and the cue to initiate a double saccade (i.e., offset fixation point), respectively.

more, when the two saccade targets were in opposite hemifields, the RL and LR conditions, the region's sustained activation reached a value in between these two levels, close to the level of activation that was reached in the first delay for just one memorized contralateral target. This suggests that the activation of each unilateral retIPI depends on the number of targets that need to be memorized from its contralateral hemifield. Closer scrutiny of RL and LR conditions, however, also suggests a difference between these activation levels. Even though the visuospatial stimulation is identical in these conditions—a target in either hemifield—the region seems more responsive to the first target memorized, which is the first target for action. In other words, this would suggest that retIPI is not only involved in storing the memory of subsequent targets but is also selectively tuned to the target that drives the first saccade.

The observations made in Fig. 6 were also seen in individual subjects. More specifically, we observed this pattern of activity in six of seven subjects for the left retIPI region and in five subjects for the retIPI region in the right hemisphere. In the following, we will further quantify the observations made in Fig. 6 and test the significance of the effects across subjects. We will first focus our analysis on the phasic response to the two cues, the peak of which can be observed at  $\sim 6$  s after presentation of the cue, consistent with the hemodynamics of the BOLD response (Boynton et al. 1996). Figure 7 illustrates the results for the four different conditions, presenting the amplitude of the phasic response, which was computed as the difference between the mean activation at 5–6 s after presentation and 1–2 s before presentation of the cue, averaged across subjects. As the *top panels* show, the amplitude of the phasic activity related to first cue was significantly higher in the

hemisphere contralateral to its location [ $F(1,6) = 9.1$ ,  $P = 0.02$ ]. This is in correspondence with the topographic nature of the region. The same was found for the BOLD response to the presentation of the second cue, irrespective of the remembered location of the first stimulus. Again there was significantly more activation in the hemisphere contralateral to the cue [ $F(1,6) = 245.6$ ,  $P \ll 0.001$ ], irrespective of whether the first

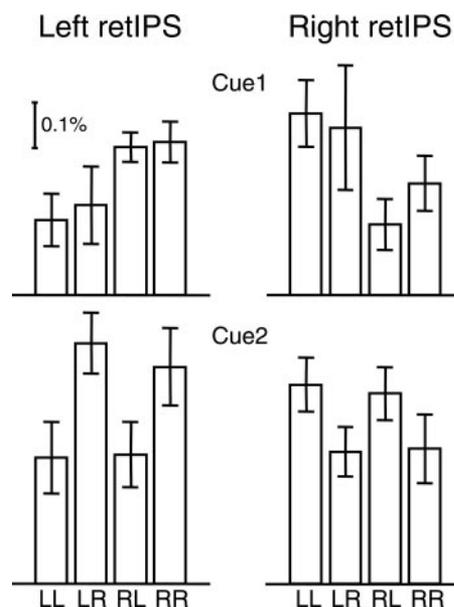


FIG. 7. The phasic response to the 1st and 2nd cue in the event-related paradigm. A: Response to the 1st cue, averaged across subjects. B: same for 2nd cue. Conditions are described in Fig. 6. A clear contralateral bias can be seen. Error bars, SE.

cue was presented in the contralateral or ipsilateral hemifield [ $F(1,6) = 0.4, P = 0.55$ ].

Figure 8 presents an analysis of the tonic, sustained activation in response to either of the two stimuli averaged across subjects. We determined these levels of activation for the different conditions as the mean response of the fMRI signal at time period 10 to 12 s (just before the 2nd cue) and at time period 22–24 s (just before initiation of the saccades), respectively, relative to the precueing intervals, with no targets in memory. The *top panels* compare the percentage signal changes for a single target in either the left or right visual hemifield. These data show a clear contralateral bias, as was also observed for the phasic response (see Fig. 7). A repeated-measures multivariate ANOVA (MANOVA), with hemisphere (left/right) and stimulus location (left/right) as factors, revealed a significant two-way interaction [ $F(1,6) = 10.6, P = 0.017$ ]. This confirms again the topography of the sustained activity in the region as well as our earlier results (Medendorp et al. 2003, 2005a,b). The *bottom panels* in Fig. 8 quantify the sustained responses in the second delay period. Recall that during this time interval our subjects had to remember two stimulus locations for later successive saccades. As shown, the activation in either hemisphere is significantly higher when two contralateral targets are to be memorized compared with just one [ $F(1,6) = 11.6, P = 0.01$ ]. This shows that the region retains storage capacity for multiple (at least 2) target locations, with the neural activation distributed across the two hemispheres. The region in the left hemisphere stores the locations of targets cued in the right visual field; the region in the right hemisphere encodes target representations from the left hemifield. The activation during the second delay, with two target locations in memory, differs for all four stimuli conditions. A repeated-measures MANOVA, with hemisphere (left/right) and test condition (LL, LR, RL, RR) as factors, revealed a significant two-way interaction  $F(3,18) = 16.4, P \ll 0.001$ . This interaction effect confirmed that the sustained activations prior to the saccades clearly depend on the respective locations of the two stimuli and that the activity in the right and left retIPS was dissociable.

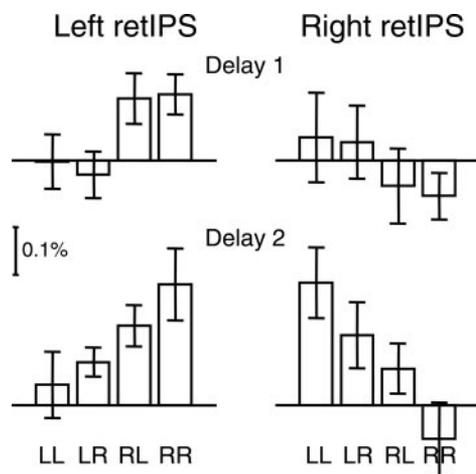


FIG. 8. Activation level of the tonic, sustained response in the 1st and 2nd delay period. *A*: response to the 1st cue, averaged across subjects. *B*: same for 2nd cue. Conditions are described in Fig. 6. Note a clear contralateral memory load effect as well as a predominant contralateral bias toward the 1st target. Error bars, SE.

To further examine the contralateral memory-load effect in the second delay interval, we averaged the activity of the RL and LR conditions, and tested the hypothesis that  $RR > (RL + LR)/2 > LL$  in the left hemisphere and vice versa for the right hemisphere. Using this approach, we confirmed the memory load effect with a significant interaction effect in a two-way MANOVA with the two hemispheres and three memory loads as factors [ $F(2,12) = 21.7, P < 0.001$ ]. To test our hypothesis that there is more activation in the hemisphere contralateral to the target of the first saccade, we applied a one-tailed MANOVA with hemisphere (left/right) and condition (LR/RL) as factors. This analysis revealed a significant interaction [ $F(1,6) = 4.1, P < 0.05$ ], confirming the observation in Fig. 4 that the activation in the region is not only dependent on the respective visual locations of both targets but also on the temporal order in which they were presented.

Finally, we performed a control experiment to test whether the tonic activation as described in Figs. 6 and 8 is truly related to the processing of target locations for saccades. The results of four subjects are shown in Fig. 9. As the figure shows, there is a clear response after presentation of each of the two stimuli suggesting that the phasic component of the activation is sensory in nature. In the left hemisphere (Fig. 9*B*), the phasic response to the first stimulus appears to be more spatially selective than the response to the second stimulus.

Figure 10*A* quantifies this observation by showing the amplitude of the phasic response to the two cues. For the left hemisphere, the mean difference in activation between a contralateral target and an ipsilateral target was 0.23% for the first cue and 0.09% for the second cue, respectively. For the right hemisphere, this difference did not diminish over the course of the trial, showing values of 0.13% for the first cue and 0.11% for the second cue, respectively. More importantly, unlike the results for the double saccade task (Fig. 6), the time courses of Fig. 9 provide no clear indication of a sustained response during the delay periods let alone a modulation of this activation by the stimulus direction or memory load. Figure 10*B* presents an analysis of the tonic response which confirms this observation, by showing the activation close to a value of zero in all conditions (compare with Fig. 8). This suggests that the tonic activation observed in the double saccade paradigm (see Figs. 6 and 8) is a marker of neural processing for a visuomotor transformation.

## DISCUSSION

We have investigated the characteristics of a working memory representation within retIPS—a bilateral topographic region in the human posterior parietal cortex. More specifically, we examined how this region, the putative human homologue of monkey area LIP, processes and stores spatial information for a delayed double-step saccadic sequence. To do so, we applied an event-related fMRI paradigm, which employed substantial time intervals between occurrence of the first target, the second target, and the saccadic responses. In this way, we could dissociate the metabolic demands related to the first target (1-target memory load) and the first and second target together (2-targets memory load). Our results are consistent with previous fMRI studies by showing a load dependency in spatial working memory in retIPS (Linden et al. 2003; Todd and Marois 2004). In addition, the results have revealed several

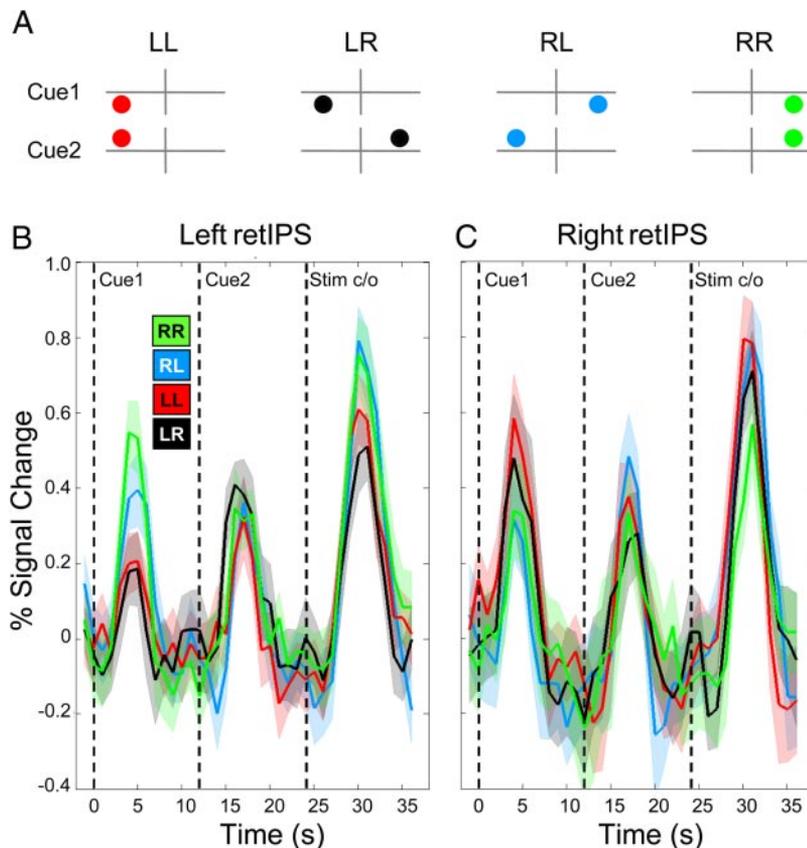


FIG. 9. The response of retIPS during the control task, in which subjects viewed the same stimuli as in Fig. 4 but maintained fixation at all times. *A*: RR, RL, LR, LL as in Fig. 6. *B* and *C*: left (*B*) and right (*C*) retIPS activation [mean  $\pm$  SE across trials and subjects (shaded)] for each of the 4 conditions. Dotted lines indicate presentation of the 1st cue, presentation of the 2nd cue, and time of flashing of the irrelevant letters, respectively.

new properties of the memory representation in this retinotopic region along the IPS. First, memory activity in retIPS is spatially selective with each hemisphere predominantly representing contralateral targets. Second, activity for two memorized targets, one from either hemifield, depends on the sequence in which they were presented with more activation in the hemisphere contralateral to the first target—the target for the first action. This suggests that the role of retIPS cannot be limited to visuospatial memory alone, but extends to participating in action (saccade) planning (Andersen and Buneo 2002; Mazzoni et al. 1996; Medendorp et al. 2005a). One could also argue that storing target locations and selecting the goal for first action should reflect separate and successive stages in sensorimotor processing (Tian et al. 2000). However, here we have shown that in humans they occur in the same brain region: retIPS.

Our results have shown that in response to the visual cue, either the first or the second cue, the phasic component of the activation was higher in the hemisphere contralateral to the cue (Figs. 6 and 7). What type of processing does this response reflect? Because the same response was found in our control experiment (Fig. 9), in which subjects viewed the stimuli but did not have to memorize them for later saccades, it can be argued that the phasic component merely characterizes a sensory response. In the monkey, Bisley et al. (2004) have shown that the initial burst in LIP in response to a visual stimulus has an extremely short and precise latency ( $\sim 45$  ms), also suggesting it reflects an uncontaminated sensory signal. In due course, the later sustained activity would reflect more cognitive functions that transcend simple visual analysis. Let us now further

discuss this component and its relation to previous studies and interpretations.

In a recent study, monkey area LIP was shown to reflect the outcome of the process of target selection for saccades rather than a storage center for multiple targets (Mazzoni et al. 1996). The same was found for the parietal reach region, located on the medial bank of the intraparietal sulcus, which specifies the target for the impending reach (Batista and Andersen 2001). In the experiment by Mazzoni et al., monkeys were presented with two brief visual stimuli and, after a delay, looked to them in sequence. Most neurons sampled in monkey LIP showed persistent activity during the delay period only when the first target (the target for the 1st saccade) was in the neuron's receptive field and not when the second target (for the subsequent saccade) appeared in the receptive field. The fact that retIPS shows a predominant contralateral bias toward the first presented cue is consistent with the notion of a selection mechanism that selects the first cue as target for first planned action. This mechanism may operate by means of a top-down control signal that enhances the internal representation of the first cue, as relevant for the first action. This could be tested by changing the sequence of saccades to the targets and examining whether the activity in retIPS is specific to the location of the second cue, which is then the target for the first intended action.

However, the virtual absence of memory activity related to the target for the subsequent, second saccade (2nd target) in monkey LIP, as found by Mazzoni and colleagues, disagrees with the present demonstration that the massed activation in retIPS does represent the goals of both saccades. Thus although retIPS shares the property of target selection with monkey LIP

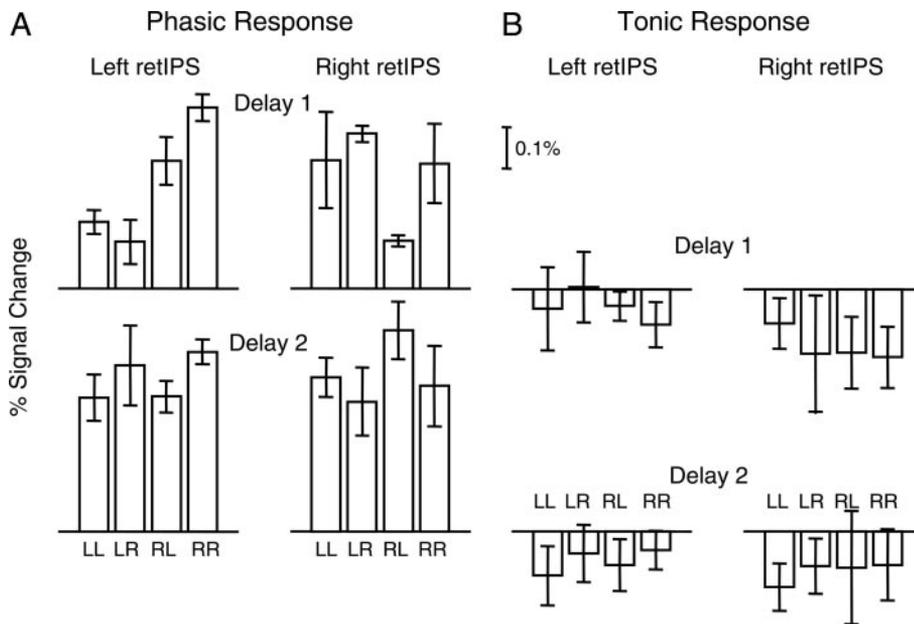


FIG. 10. Quantification of the phasic and tonic response of retIPS during the control task. *A*: phasic response. *B*: tonic response. Data in same format as in Figs. 7 and 8.

on the basis of its predominant bias toward the first target, it is, in contrast to its monkey equivalent, also involved in the storage of the location of the second target. If it is assumed that human retIPS is homologous to monkey LIP, what could account for this difference? Two explanations can be advanced. First, the discrepancy may be explained by the fact that, in monkeys, usually a selected population of neurons is studied, while BOLD activation may reflect activity of any group of neurons in a given area. Second, the spiking activity recorded in monkeys likely represents the output of LIP because microelectrode recordings are biased toward large pyramidal cells. On the other hand, fMRI signals appear to be correlated to a large degree with synaptic potentials (Logothetis et al. 2001). Thus they represent local processing and inputs as well as outputs. The storage for the second target may reflect top-down or intracortical processes that do not appear in the output spiking activity.

One could also argue that the bias for the first saccade as observed in the present study may support the view that the region stores multiple saccadic amplitudes rather than multiple locations of targets relative to a current reference point (say, gaze direction). More specifically, in that scheme, the bias for the first saccade follows from the fact that the metrics of the second saccade are such that it does not activate the voxels that comprise retIPS as defined by our localizer because this was identified using smaller saccades in mainly horizontal directions. In other words, the second saccade in the present RL or LR conditions is about double in amplitude compared with the localizer saccades. Likewise, the direction of the second saccade is mainly in vertical direction in the RR and LL conditions—a direction not incorporated in the localizer saccades. If the tonic activation of the region would indeed code saccadic amplitudes, including the amplitude and direction of the second saccade, one could thus argue that retIPS should not tonically respond to the second stimulus. The data show that this is not the case: there is a clear tonic activation to either of the two targets. Other evidence against this view has been provided by our previous fMRI study showing that retIPS codes target location relative to the current gaze direction and

updates this information after the eyes have moved (Medendorp et al. 2003). As an example, consider the RL case. At time zero, a target, say  $9^\circ$  to the right, is displayed briefly and its location stored in the left parietal cortex. At time 12 s, a target of  $9^\circ$  to the left is displayed and stored in the right parietal cortex. At time 24 s, a saccade is made to the first target and then after  $\sim 300$  ms to the second target. There is no doubt regarding remapping of target locations after the first saccade so that the second target location is now coded as  $18^\circ$  to the left in the right parietal cortex. However, it is important to note that this would occur at a time after the first saccade and thus after our measurement period, beyond 24 s. At the time of our measurements (Fig. 8), the target locations would be coded as  $9^\circ$  to the left and  $9^\circ$  to the right. Thus on the basis of our previous and current data, we can rule out that retIPS stores multiple saccade amplitudes but rather the targets that define these saccades relative to the current gaze direction. It should also be emphasized that this is most consistent with monkey physiological evidence (Schall et al. 1995; Sparks 2002).

Recently, two reports have suggested that the human PPC contains several topographically organized regions for delayed saccades and visual spatial attention (Schluppeck et al. 2005; Silver et al. 2005). It remains to be established whether one of these exhibits the functional properties we have defined for retIPS here, and in our previous studies (Medendorp et al. 2003, 2005a,b).

What are the limits of the storage capacity of the map in retIPS? Here we have shown that a hemisphere can encode at least two contralateral target locations. Other evidence has shown that activity in bilateral posterior parietal cortex increases with the number of objects to be stored, to a maximum of about four objects (Linden et al. 2003; Todd and Marois 2004). These studies, however, did not examine lateralized effects on working memory capacity constraints. Therefore in the context of our findings, it remains to be elucidated whether this number of four objects relates to a capacity limit per hemisphere or if it reflects the total resource available to store target locations, to be shared across hemispheres. In this vein, Vogel and Machizawa

(2004) reported ERP evidence for lateralized activity over posterior parietal sites in human spatial memory. They found the amplitude of this activity to be strongly modulated by the number of objects being held in memory but approaching a limit of about three to four targets. Thus a crucial experiment to be performed in retIPS should go along the lines of Vogel and Machizawa's experiment, testing the configuration of target locations that can be reliably encoded by this region.

Could the persistent activity in retIPS simply reflect visual attention? A popular account of visual attention is the spotlight theory, which states that attention can only be directed to one region of space at a time (Brefczynski and DeYoe 1999; Posner et al. 1980; Tootell et al. 1998). The spotlight can be switched rapidly between locations and can be adjusted, like a zoom lens, to expand or contract the size of the attended region. Our data are incompatible with this single resource model of attention. For example, when subjects have to memorize two targets, one in either hemifield (the RL and LR conditions), the focus of attention should span both fields, and thus be more diffuse. Accordingly, we might expect the activation after the second cue to become reduced, which is not what the data show. That is, the tonic activation during the RL and LR conditions after the second cue is greater than that after the first cue (Figs. 6 and 8).

Recent studies however have challenged the unitary spotlight theory of visual attention, and suggested that the spotlight can be divided flexibly to disparate locations (McMains and Somers 2004; Muller et al. 2003). As of yet, the cost of this division is unknown as are the factors that determine it (Tong 2004). Nevertheless, accepting this idea of divided attention, it could be argued that spatial working memories or maintained attention to particular regions in space (indicated by our targets) are two sides of a single coin. In other words, in the context of the present study, multiple attentional spotlights and spatial memories are concepts that may reflect the same neural mechanism.

Finally, in our study we did not record from the frontal cortex; the surface coil limited us to only the parietal ROIs, with the necessary high sensitivity. Thus the patterns of activation in the frontal areas during our saccade task remain to be discovered. Several studies have emphasized the importance of the frontal lobe in visual working memory for saccades (Linden et al. 2003; Tian et al. 2000). However, other recent studies have argued against the intimate involvement of these regions in spatial storage but instead have proposed that they perform top-down executive functions, such as response selection, movement initiation, and rehearsal, on representations stored in more posterior cortical regions, including PPC (Curtis and D'Esposito 2003). Although our results cannot differentiate between these interpretations, it is obvious that future research is required to determine more precisely the nature of the code in the networks of the brain that store and transform spatial information into motor action.

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