Human fMRI evidence for the neural correlates of preparatory set

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We used functional magnetic resonance imaging (fMRI) to study readiness and intention signals in frontal and parietal areas that have been implicated in planning saccadic eye movements—the frontal eye fields (FEF) and intraparietal sulcus (IPS). To track fMRI signal changes correlated with readiness to act, we used an event-related design with variable gap periods between disappearance of the fixation point and appearance of the target. To track changes associated with intention, subjects were instructed before the gap period to make either a pro-saccade (look at target) or an anti-saccade (look away from target). FEF activation increased during the gap period and was higher for anti- than for pro-saccade trials. No signal increases were observed during the gap period in the IPS. Our findings suggest that within the frontoparietal networks that control saccade generation, the human FEF, but not the IPS, is critically involved in preparatory set, coding both the readiness and intention to perform a particular movement.

Knowing that a particular sensory event is about to occur allows one to prepare for that event and to respond to it more quickly than a naïve responder would. Moreover, humans are capable of deciding in advance to perform one action and not another when a sensory event occurs. Both the readiness to respond and the intention to perform a particular act are commonly referred to as 'preparatory set', and are arguably hallmarks of the most cognitively complex organisms—those that can plan ahead and choose between alternative courses of action[1,2].

In the oculomotor system, intention can be investigated by manipulating the type of eye movement response required when a stimulus is presented. Although the common response is to look toward a suddenly flashed visual stimulus (pro-saccade), humans can be instructed in advance not to look to the stimulus but instead to look in the opposite direction (anti-saccade)[3,4]. In the pro-saccade condition, an automatic visuomotor response is required, whereas in the anti-saccade condition, this automatic response must be inhibited and replaced by a voluntary response. Thus, by combining pro- and anti-saccade trials in the same experiment, we were able to study the effects of intention on motor programming. We also investigated how readiness to make the planned response influences motor programming by varying the time between the instruction and stimulus onset.

Studies of human patients with focal frontal lobe lesions suggest that specific regions, including the FEF and the dorsolateral prefrontal cortex (DLPC), are critically involved in the anti-saccade task[5–7]. Although lesions of parietal cortex often slow saccadic reaction times, they do not produce specific deficits in the anti-saccade task[6]. These results are supported by recent imaging studies that identify similar areas of frontal cortex that are selectively activated or whose activity is augmented in the anti-saccade task when compared to the pro-saccade task[8–12]. Most of these neuroimaging studies also identify differences in activation between pro- and anti-saccade tasks in the intraparietal sulcus (IPS), a region of the parietal cortex[8,9,11]. Although these pro- versus anti- differences may be related to intention, a major limitation in the design of imaging studies to date has been the inability to determine whether these differences are due to changes in brain activation before or after target appearance on a given trial.

Recent neurophysiological studies contrasting the pro- and anti-saccade tasks in awake monkeys show intention-related neuronal activity in the FEF[13], DLPC[14] and supplementary eye fields[15]. In addition, the magnitude of these preparatory signals is enhanced when a gap is inserted between fixation point disappearance and peripheral target appearance. This gap serves as a warning to remove active visual fixation and leads to disinhibition of pre-saccadic activity in cortical and subcortical structures and to a reduction in saccadic reaction time[16–19]. The pre-target discharges in FEF also differ for pro- and anti-saccade tasks[13]. In a memory task in which the target is presented before the go signal, activity in the posterior parietal cortex does not reliably encode saccade direction when probed using the anti-saccade task; instead it signals the location of the visual cue[20]. Thus, there seems to be a division of labor between frontal and parietal oculomotor areas, with the frontal cortex involved in motor planning and the parietal areas in encoding the location of the relevant sensory stimuli for transformation into the appropriate motor coordinates for action.

The goal of the present study was to use fMRI to search for neural correlates of preparatory set (both intention and readiness to act) in the oculomotor areas of the frontal and parietal

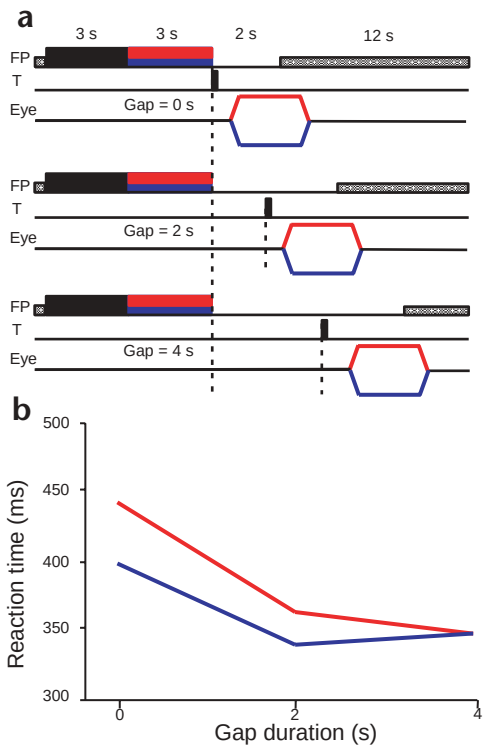

Fig. 1. Gap paradigm for the event-related design, and the effect of gap duration on saccadic reaction time. (**a**) Subjects viewed a white fixation point (FP) presented at the center of the screen for 3 s. The FP turned either green (pro-saccade trial) or red (anti-saccade trial) and remained on screen for 3 s. The change to green is indicated by the blue line; the change to red by the red line. A target (T) was then flashed for 100 ms along the horizontal meridian. For pro-saccade trials, the subject made a saccade toward the target (blue position trace) and for anti-saccade trials, the subject made a saccade away from it (red trace). The target was flashed at 0, 2 or 4 s after the disappearance of the FP. During the gap interval, no visual cue appeared, and no eye movements were executed. Two seconds after target appearance, a central fixation cross appeared, and the subject returned gaze to center and maintained central fixation for 12 s, constituting the intertrial interval. (**b**) Mean saccadic reaction time as a function of gap duration for the behavioral experiment.

cortex of normal human subjects during planning of pro- and anti-saccades. To track the changes in the blood oxygenation level–dependent (BOLD) signal that correlated with readiness to act, we used an event-related design with relatively long gap periods between fixation disappearance and peripheral target appearance. This allowed us to separate activation that occurred before and after target appearance. We found that pre-target activation in the FEF, but not in the IPS, increased during the gap period and depended on the instructional fixation cue. The differences in activation in the FEF between pro- and anti-saccade trials were evident before the peripheral target stimulus appeared. We also examined activity during a memory delay—activity that occurred after target onset but before movement onset—using a variable memory delay interval. Unlike in the gap task, both the FEF and the IPS were active during the memory delay interval. These findings highlight the differential functions of parietal and frontal cortex in sensorimotor tasks. An abstract of some of these data has appeared previously (J.D.C., M.A.G. & D.P.M., *Soc. Neurosci. Abstr.* **27**, 575.7, 2001).

Results

We first carried out a behavioral experiment (**Fig. 1a**) in which we measured saccadic responses using the same protocol that was later used in the scanner. Each trial began with 3 s of fixation on a central white fixation point (FP) followed by 3 s of fixation on an 'instructive' FP (it turned either green, signaling a pro-saccade trial, or red, signaling an anti-saccade trial). This instructive FP was then extinguished, and a 0-, 2- or 4-s gap period of darkness preceded the appearance of the target, which was flashed for 100 ms either 10° right or left of center. Subjects maintained fixation at the remembered location of the central FP until target appearance, and then they looked to the target on a pro-saccade trial or looked to its mirror position on an anti-saccade trial; finally, they held their gaze at this eccentric location for 2 s before returning to center.

Consistent with previous studies[21–23], the introduction of the gap led to a reduction in saccadic reaction time (SRT for 0-s gap, 420 ms; 2-s gap, 352 ms; 4-s gap, 348 ms; $F_{2,14} = 26.71$, $P < 0.001$) in eight subjects (**Fig. 1b**). In addition, pro-saccade SRT was significantly shorter than anti-saccade SRT for the no-gap condition ($t_7 = 2.73$, $P < 0.025$), and the difference gradually diminished across gap duration, resulting in an interaction of task with gap duration ($F_{2,14} = 6.05$, $P < 0.025$). *Post-hoc* tests revealed that the pro-saccade SRT advantage occurred only when the gap duration was zero.

We used the same protocol for the fMRI portion of the experiment, except that in the scanner, the peripheral target stimulus was presented randomly at different positions along the horizontal meridian (9–15° to the right or left of center). At the start of each scan, we ran a short block-design experiment comparing pro-saccades and fixation to localize the FEF and an eye-movement related area in the IPS that may be analogous to an area in the lateral intraparietal sulcus (LIP) in non-human primates (**Fig. 2**). Mean Talairach coordinates (*x, y, z*) for the swath of enhancement in the FEF were 21.0, –10.0, 45.0 and –28.0, –10.0, 46.0 for the right and left FEF, respectively (where $+x$ is right, $+y$ is anterior and $+z$ is superior), which is consistent with

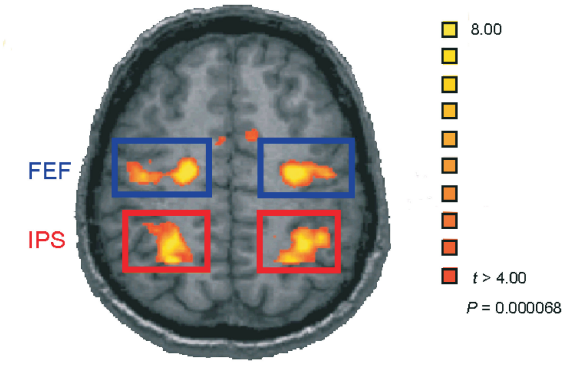

Fig. 2. Pro-saccade localizer map used to identify the frontal eye fields (FEF) and the lateral intraparietal area (IPS). Subjects initially completed a block design saccade task consisting of alternating time blocks of pro-saccades and central fixation to identify the FEF and IPS. This map is the result of contrasting the two types of blocks and shows two bilateral regions of interest: the FEF in frontal cortex and the IPS in parietal cortex. The individual subject localizer maps were superimposed onto the corresponding event-related functional time courses to examine activity levels in each region during the gap, memory-delay and fixation experiments.

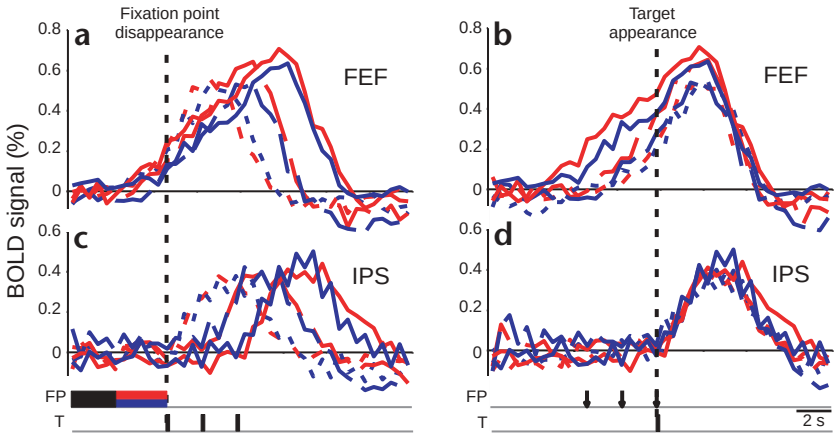

Fig. 3. Event-related time courses for the gap saccade task (solid lines, 4-s gap trials; dashed lines, 2-s gap trials; dotted lines, 0-s gap trials; blue lines, pro-saccades; red lines, anti-saccades). The BOLD fMRI signals are aligned on FP disappearance (**a**, **c**) and target appearance (**b**, **d**) for both the FEF (**a**, **b**) and the IPS (**c**, **d**). Each time bin represents a 500 ms interval (functional TR = 500 ms). In the FEF (but not in IPS), the BOLD signal begins to rise immediately after disappearance of the instructional cue for each gap period.

previous studies that have localized FEF using saccades[24]. Mean Talairach coordinates for the eye-movement area in the parietal cortex were 28.0, –46.0, 42 and –33, –45, 42 for the right and left IPS, respectively, consistent with previous studies[11,25].

We tracked the event-related time courses of activity in the voxels we had identified in the FEF and IPS in the preparatory set task (**Fig. 3**). We made several key observations in these single-event BOLD responses. First, for both the FEF and IPS, the peak in the BOLD response obtained for each gap duration occurred at approximately the same time after target appearance (**Fig. 3b** and **d**), and the recovery of the response to baseline followed a similar time course. We ascribe this peak response to the combined visual and motor activation of FEF and IPS neurons upon target appearance[13,20].

Second, in the FEF but not the IPS, there was an early pre-target activation that occurred in advance of the peak response (**Fig. 3b**). The pre-target rise in FEF activation for 4-s gap trials preceded the rise in activation for 2-s and 0-s gap trials. An analysis of variance (ANOVA) with gap duration (0, 2 or 4 s) and trial type (anti or pro) as factors revealed a significant influence of gap duration on BOLD signals in the FEF ($F_{2,14} = 17.44$, $P < 0.0001$) but not the IPS ($F_{2,14} = 0.814$, n.s.; **Fig. 4a**). In addition, there was a significant difference in the pre-target BOLD response between anti- and pro-saccade tasks in the FEF ($F_{1,7} = 6.935$, $P = 0.034$) but not the IPS ($F_{1,7} = 2.266$, n.s.). There was no interaction between gap duration and trial type in either the FEF ($F_{2,14} = 2.613$, n.s.) or the IPS ($F_{2,14} = 0.719$, n.s.).

Third, the peak of the BOLD response increased with increasing gap duration in the FEF ($F_{2,14} = 6.683$, $P = 0.009$) but not in the IPS ($F_{2,14} = 1.779$, n.s.; **Figs. 3** and **4b**). In addition, within the FEF, the peak BOLD response was significantly greater for anti-saccade trials ($F_{1,7} = 29.288$, $P = 0.001$). This difference was not observed in the IPS ($F_{1,7} = 5.340$, n.s.). There was no significant interaction between gap duration and trial type in either the FEF ($F_{2,14} = 0.246$, n.s.) or the IPS ($F_{2,14} = 0.063$, n.s.). The finding of an enhanced peak with a preceding gap period in the FEF is important because fMRI-BOLD responses that partially overlap in time have been shown to be additive[26]. That is, the greater peak FEF response in trials with longer gap duration (**Figs. 3b** and **4b**) presumably resulted from a superposition of an increasing pre-target response and a constant post-target response.

Fourth, the duration of the BOLD response recorded from the FEF increased with increasing gap duration, but this was not the case for the IPS. To quantify this, we measured the time from when the BOLD signal exceeded 25% of the peak value to when it fell below 25% of the peak value. An ANOVA with gap duration (0, 2 or 4 s) and trial type (anti or pro) as factors revealed a significant influence of gap duration on BOLD duration in the FEF ($F_{2,14} = 18.433$, $P < 0.0001$), but not the IPS ($F_{2,14} = 2.102$, n.s.; **Fig. 4c**).

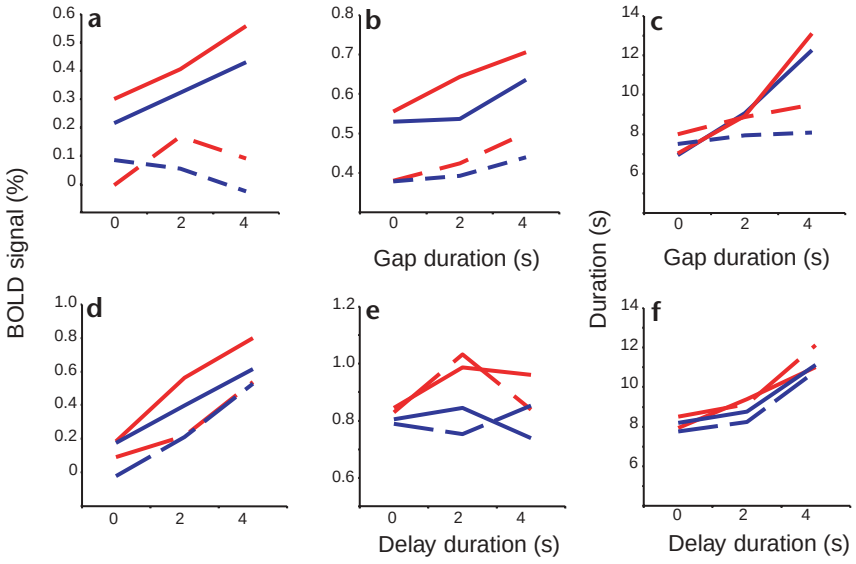

Fig. 4. Quantification of BOLD responses as a function of gap duration (**a**–**c**) and memory delay duration (**d**–**f**) (solid lines, FEF; dashed lines, IPS; blue lines, pro-saccades; red lines, anti-saccades). (**a**) The pre-target responses increased with gap duration in the FEF but not in IPS. (**b**) The peak post-target responses increased with gap duration in the FEF but not in IPS. (**c**) The duration of the BOLD responses increased with gap duration in the FEF but not in IPS. (**d**) The delay responses increased with memory delay duration in both the FEF and IPS. (**e**) There was no difference in the peak response after fixation disappearance with memory delay duration in the FEF or in IPS. (**f**) The duration of the BOLD response increased with memory delay duration in both the FEF and IPS.

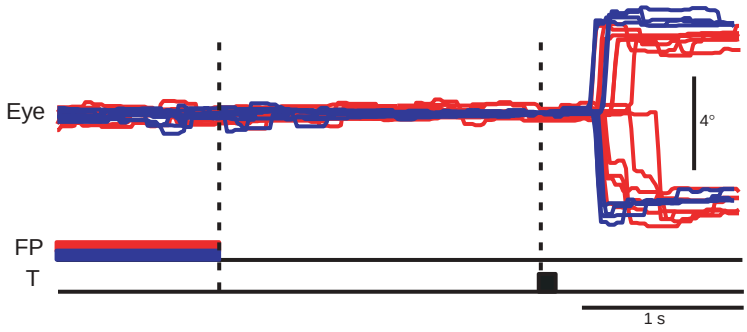

Fig. 5. Eye position traces recorded in the scanner during the combined anti-/pro-saccade task. Note that there were no shifts in fixation during the gap period and that the anti-saccades (red traces) show a slower reaction time than the pro-saccades (blue traces).

There was no significant difference in the duration of the BOLD response between anti- and pro-saccade tasks in the FEF ($F_{1,7}$ = 1.912, n.s.) or the IPS ($F_{1,7}$ = 3.458, n.s.). In addition, there was no interaction between gap duration and trial type in the FEF ($F_{2,14}$ = 0.989, n.s.) or the IPS ($F_{2,14}$ = 0.937, n.s.).

Generation of eye movements during the gap period did not account for pre-target activation in the FEF. First, this pre-target activation was limited to the FEF; it was not observed in the IPS (**Fig. 3**). Saccades, whether generated before or after target appearance, should show activation in both the FEF and IPS. Second, we measured eye movements from subjects outside the scanner with an infrared eye tracker and did not find any detectable eye movements (other than microsaccades) during the gap periods. Third, in the behavioral task before scanning, we never observed saccadic eye movements during the gap period. And finally, in subsequent follow-up experiments using three of the same subjects, we measured eye movements on-line in the scanner and found that subjects did not generate saccades during the gap period (**Fig. 5**), but still showed the same pre-target BOLD responses in the FEF—and not the IPS—during the gap period.

Two additional experiments verified that this FEF-specific, pre-target BOLD increase was related to preparatory set. We first showed that it was possible to see an increase in activation in the IPS during a variable delay, but only in the context of a memory task in which subjects were asked to keep the location of a previously flashed target in mind during the delay period. Eight subjects were scanned as they performed a memory saccade task in which the target for the saccade was flashed for 100 ms, but the FP remained illuminated for 0, 2 or 4 s. The subjects were instructed to fixate on the central FP until it disappeared and to look toward the remembered location of the target flash if the FP had been green; away from the location of the target flash if the FP had been red. Activation in both the FEF and the IPS began to increase immediately after the appearance of the eccentric target stimulus (**Fig. 6a** and **c**) and continued to increase during the 2- and 4-s delay periods. When the signal time courses were aligned to FP disappearance (**Fig. 6b** and **d**), the early pre-movement memory delay activation could be seen to increase across the delay interval in both the FEF and the IPS ($F_{2,14}$ = 11.84, $P < 0.001$; $F_{2,14}$ = 18.43, $P < 0.001$, respectively; **Fig. 4d**). In the FEF, there was also a difference in pre-movement activation for anti- and pro-saccade trials ($F_{1,7}$ = 5.69, $P < 0.05$), a difference that was not present in the IPS ($F_{1,7}$ = 1.86, n.s.). A peak in activation was also observed in both regions after the FP disappeared—when the memory-guided saccade was generated—but there were no differences in peak activation as a function of delay interval (**Fig. 4e**). Peak activation was greater for anti- versus pro-saccades in both the FEF ($F_{1,7}$ = 10.95, $P < 0.01$) and the IPS ($F_{1,7}$ = 13.99, $P < 0.007$). In neither case was there an interaction between trial type and delay interval. Finally, the duration of the BOLD response recorded from the FEF and IPS increased with increasing memory delay ($F_{2,14}$ = 29.56, $P < 0.001$ and $F_{2,14}$ = 28.64, $P < 0.001$, respectively; **Fig. 4f**). There was no significant difference in the duration of the BOLD response between anti- and pro-saccade tasks in the FEF ($F_{1,7}$ = 0.037, n.s.) or the IPS ($F_{1,7}$ = 2.270, n.s.). Neither was there an interaction between memory delay duration and trial type in the FEF ($F_{2,14}$ = 0.647, n.s.) or the IPS ($F_{2,14}$ = 0.138, n.s.). Thus, pre-saccadic BOLD activation in the IPS required presentation of the target before the delay period; it was present in the memory-delay task (**Fig. 6c** and **d**) but absent in the gap saccade task (**Fig. 3c** and **d**).

In a final experiment, we showed that removal of the fixation spot alone was not sufficient to produce pre-target activation in the FEF or the IPS. Eight subjects were scanned in a task in which

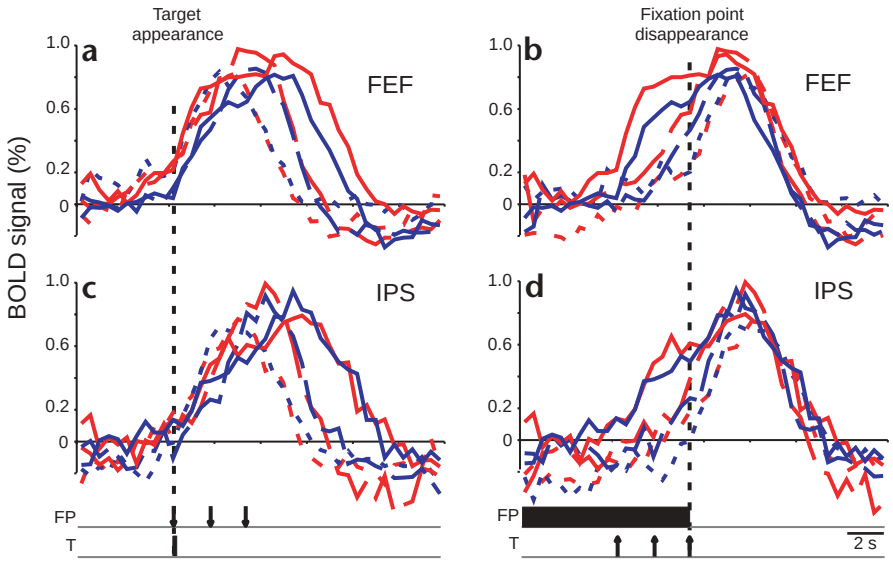

Fig. 6. Event-related time courses for the memory-guided delay saccade task (solid lines, 4-s delay trials; dashed lines, 2-s delay trials; dotted lines, 0-s delay trials; blue lines, pro-saccades; red lines, anti-saccades). The BOLD fMRI signals are aligned on onset of the target flash (**a**, **c**) and disappearance of the FP (**b**, **d**) for both the FEF (**a**, **b**) and IPS (**c**, **d**). Subjects initially fixated a central white spot for 3 s, which then changed to either green or red for 3 s to signify a pro- or anti-saccade trial. The target was then flashed for 100 ms randomly on either the right or left side at 9–15° eccentricity. Simultaneously, the FP changed back to white and disappeared either 0, 2 or 4 s after the target flash.

the fixation disappeared and there was a gap period of 0, 2 or 4 s before the fixation cue reappeared. No peripheral target was ever presented. Subjects were simply required to maintain central gaze throughout the entire experiment. In this task, the averaged event-related signals were stable throughout the sample period, and there was no rise in activity during the gap intervals (**Fig. 7**), unlike that observed during either the gap saccade task (**Fig. 3**) or the memory-guided delay task (**Fig. 6**). It therefore cannot be argued that the gap-related increases in FEF activity that we observed during the gap saccade task (**Fig. 3a** and **b**) were simply due to the removal of the FP.

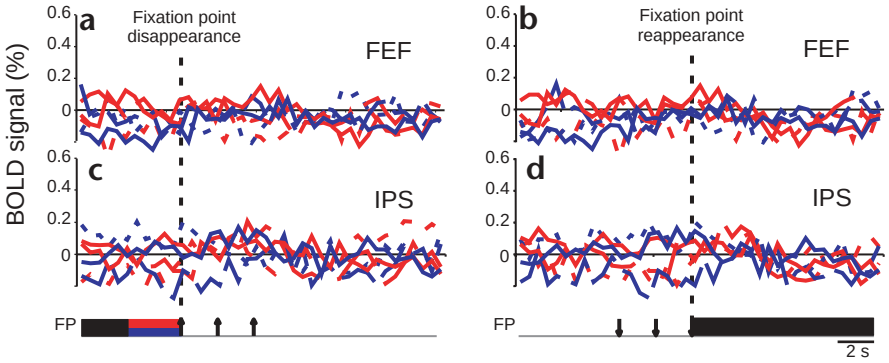

Fig. 7. Event-related time courses for the fixation control experiment (solid lines, 4-s gap trials; dashed lines, 2-s gap trials; dotted lines, 0-s gap trials; blue lines, pro-saccades; red lines, anti-saccades). The BOLD fMRI signals are aligned on FP disappearance (**a**, **c**) and reappearance (**b**, **d**) for both the FEF (**a**, **b**) and IPS (**c**, **d**).

Discussion

This study is the first to use an event-related design to reveal preparatory set activity in the human FEF that is related to both intention and readiness to respond. Comparable activation was not observed in the IPS. In contrast, memory-related activation was observed in both the FEF and the IPS in the delay task in which target location was specified at the beginning rather than the end of the delay interval. These data are discussed first in relation to other human imaging studies and then within the context of neurophysiological results obtained from studies using non-human primates.

Recent brain imaging studies have identified similar areas of frontal cortex that are selectively activated or whose activity is augmented in the anti-saccade task, as compared to the pro-saccade task[8–12]. Most of these neuroimaging studies have identified differences in activation between pro- and anti-saccade tasks in frontal and parietal brain regions[8,9,11]. For example, FEF activation is greater in the anti-saccade task, and it includes activation of an area immediately rostral to the part of the FEF activated in the pro-saccade task[9,11]. Because they used a block design, these studies could not differentiate between activation related to preparatory set, which occurs before target appearance, and activation related to sensory or motor processing, which occurs after target appearance.

In the FEF, not only was there greater activation during the gap period on anti-saccade versus pro-saccade trials, but this difference was also evident in the peak activation associated with the appearance of the target and the occurrence of the motor response. Although it is quite likely that this difference in the peak response was due to a carry-over of the hemodynamic response associated with the preparatory stage of the gap task, at least a portion of it could also have reflected post-target differences. We found the same difference in peak activation for anti- and pro-saccades in the memory delay task in FEF, where there were also differences in anti- versus pro-saccade activation during the delay period.

Notably, there was indication in the gap task that in the IPS, the post-target differences in activation were higher for anti- versus pro-saccades; peak activation was definitely higher for anti-saccade activity in the memory delay task. Because there was no pre-movement difference in anti- versus pro-saccade activation in IPS in either task, the difference in peak activation in IPS may have reflected differences in motor activity associated with these two very different kinds of movement.

Correct performance on the anti-saccade task requires the successful suppression of an automatic saccade to the peripheral target stimulus, then a volitional saccade to a location void of any sensory stimuli[4]. Patients with DLPC lesions have difficulty suppressing the pro-saccades in this task[5,6,27], and these pro-saccades are often triggered at very short latencies, in the range of express saccades[28]. Thus it is likely that the DLPC is involved in generating a suppression signal to block the automatic pro-saccade. This suppression signal is presumably passed on to the FEF. Patients with lesions localized to the FEF have difficulty generating volitional saccades, but their ability to suppress pro-saccades is unimpaired[27].

The role of the FEF in preparing for eye movements, as shown here, is paralleled by other work that implicates another region in the frontal cortex—the dorsal premotor area—in preparatory set for finger and limb movements. Removal or temporary inactivation of the dorsal premotor cortex impairs selection between movements[29,30]. Results from neurophysiological[31], event-related fMRI[32] and transcranial magnetic stimulation (TMS)[33] studies have shown set-related activity in the dorsal premotor cortex. Moreover, set-related premotor single-unit activity is reported to persist for as much as 7.5 s after an instruction stimulus[34], suggesting that frontal preparatory activity should be amenable to investigation with event-related fMRI. Neuroimaging studies using event-related fMRI also suggest that the pre-supplementary motor area is involved in preparatory set for finger movements[35,36].

The general pattern of activation we observed in the human FEF is similar to the pattern of activity recorded from individual corticotectal neurons in layer V of the FEF in macaque monkeys performing pro- and anti-saccade tasks[13]. Although saccade-related corticotectal neurons in the FEF of the monkey had discharges that varied between pro- and anti-saccade tasks, and many also changed their discharge rate during the gap period, they were more active for pro- as compared to anti-saccades[13]. The fact that we and others[8,9,11] have observed a larger hemodynamic response in the human FEF on anti- as compared to pro-saccade trials may indicate that the BOLD response is more highly correlated with local field potentials than with action potentials[37]. The local field potentials would reflect the activity of afferent input and interneuron activity within a cortical area rather than the spiking of neurons that form the output from that area. In any case, it is likely that the FEF efferent neurons convey both

intention and readiness signals to the superior colliculus, as the activity of collicular saccade-related neurons is also correlated with preparatory set[38,39]. The reduction in pre-target activity of saccade-related neurons in the FEF and superior colliculus is thought to be essential for suppressing saccades to the stimulus on anti-saccade trials[13,38,39].

A long history of work in monkeys has shown that the FEF and LIP, which are closely and reciprocally interconnected[40,41], are key components in the frontoparietal network mediating the generation of saccades[42,43]. Our imaging data provide compelling evidence that, in humans, the FEF has a pivotal role in preparatory set, coding both the intention to perform a particular kind of saccadic movement and the readiness to perform that movement. In contrast, activity in the IPS, which is closer to the sensory side of the frontoparietal axis mediating the generation of saccades, does not seem to code preparatory set, although it does code for the location of a target in a memory delay task. Indeed, the region of the IPS in which we saw this activation may well be homologous with monkey LIP. Although it is true that some LIP neurons in the monkey show a gradual buildup of activity before a saccade is initiated, this has been shown to occur only in delay or memory saccade tasks in which the location of the target is already specified[44].

It is important to acknowledge that in some neurophysiological experiments in which the target is not specified at the beginning of the trial, a gradual buildup of activity in LIP occurs before appearance of the peripheral target[45]. Because the trials showing this effect were done in blocks, it is possible that the pre-target increase in spiking activity in LIP reflected the memory of the target from the previous trial[46]. But the increase could also be construed as evidence of preparatory set. We did not see this signal in our gap task, however, which suggests that the increase in LIP activity in the monkey in the blocked-trial task reflected memory rather than preparatory set. Nevertheless, one must be cautious in drawing this conclusion, because the effect in the monkey is limited to a few spikes, a level of activity that could be below the threshold of fMRI.

Because only one parietal region of interest (ROI) was identified with the saccade localizer task, we could not examine the possibility that other regions of the parietal cortex might show a preparatory response. The ROI we identified in the IPS, however, showed a robust and reliable activation with saccadic activity, and is a prime candidate for the human homolog of LIP. In fact, as reviewed above, both the monkey work and our observations are consistent with a large body of data implicating LIP in transforming the retinal coordinates of a particular sensory target into the motor coordinates required for a saccadic eye movement[20,42,47].

Our results support the argument that one of the main functions of frontal cortex is to decide how and when to act in the world[5,48]. The collectively sampled FEF signals described here reflect the formation of early motor plans. Thus, our results suggest that mechanisms in the frontal cortex are critically involved in the human and primate ability to resist the impulsive demands of the sensory environment. If such frontal instruction-based preparatory signals could be sampled and interpreted in real time, it would be possible to know a human's behavior in advance of sensory stimulation.

METHODS

Subjects. Sixteen subjects (ten male, six female) provided informed consent and participated in this study (eight subjects for the gap experiment and eight for the delay and fixation experiments). Two subjects, J.C. and D.P.M., were coauthors. Each subject had two training sessions that lasted until they performed the tasks without error (one day before testing and just before entering the scanner). The experiments were approved by the University of Western Ontario Review Board for Health Sciences Research Involving Human Subjects.

Stimuli and tasks. Visual stimuli were generated using Director 5.0 software (Macromedia, San Francisco, California) and presented using a computer connected to a video projector (NEC, Japan). The image was projected off a mirror onto a screen secured to the ceiling of the magnet bore.

The peripheral and central fixation cues subtended 0.25° of visual angle. A green FP was used to signal a pro-saccade trial and a red FP an anti-saccade trial. Each event-related trial began with 3 s of fixation on a white central cue. This was followed by 3 s of fixation of either the green (pro-saccade trial) or red (anti-saccade trial) instructional cue. The instructional cue was then extinguished and a gap period—0, 2 or 4 s of darkness—occurred during which the subject's gaze remained stationary at the remembered location of the central instructional cue. A white peripheral cue then flashed along the horizontal meridian (9–15° eccentricity) to the right or left of center for 100 ms. Subjects were instructed to look to this location on a pro-saccade trial (green fixation cue) or to look to the mirror position on an anti-saccade trial (red fixation cue) and then to hold their gaze at this location until a white central fixation cross appeared (an additional 2 s interval). Subjects returned gaze to center immediately upon reappearance of the central cross, which remained on screen for 12 s; this constituted an intertrial interval (ITI). The ITI provided time for the fMRI signal to return to baseline after each saccade trial. Each experimental run was 528 s in duration, consisting of 12 pro-saccade and 12 anti-saccade trials. Trial types (anti- versus pro-) were pseudorandomly interleaved, and the amplitudes and gap durations were balanced for right and leftward movements. There were three trial orders (experimental runs), and two replications of each were collected for a total of 6 experimental runs of the gap experiment per subject.

The memory-delay experiment followed the same time course as the gap experiment. In this experiment, however, the visual cue was presented prior to the 0, 2 or 4 s delay interval. In contrast to the gap experiment, the white central fixation cue remained visible during the memory delay. This was then extinguished after the memory delay interval, and this represented the instruction for the subject to move either toward (pro-saccade) or away from (anti-saccade) the remembered location of the visual cue. The fixation experiment was identical to the gap saccade experiment, with the exception that no peripheral targets were presented. Subjects were instructed to look straight ahead during the entire functional run, including the gap interval.

Each imaging session began with the saccade localizer task, which consisted of alternating time blocks (30 s) of pro-saccades and central fixation. Peripheral targets appeared at a frequency of 2 Hz and stepped to the right or left randomly between 4° and 15°, but never more than 15° from center. During the fixation blocks, subjects fixated on a central cross, and no peripheral targets were flashed. The localizer experiment was 360 s in duration, consisting of six blocks of pro-saccades and six blocks of central fixation. Only one run of the localizer experiment was collected, and the images were analyzed in the control room using the Stimulate software package (Center for Magnetic Resonance Research, University of Minnesota Medical School, Minneapolis, Minnesota) in order to select a functional volume that included the FEF and an oculomotor region in the IPS.

Behavioral experiments and eye tracking. An initial behavioral experiment was conducted with eight subjects (two male, six female) to verify the protocol for the single-event design. Horizontal eye movements were measured with DC electrooculography, and stimuli were presented using procedures described previously[23]. Eye position data were digitized at 500 Hz for data analysis. The timing of stimulus presentation was identical to that described above for the imaging experiment, except that the peripheral targets were presented only at 10° right or left of center. In three subjects, eye movements were measured on-line in the scanner with a video-based eye tracker (Avotec Inc., Stuart, Florida) during imaging,

and these subjects maintained steady fixation during the instructional fixation and gap periods and generated no eye movements during this time. All targeting saccades occurred after target appearance.

Imaging and data analysis. Experiments were carried out using a 4.0-tesla Varian (Palo Alto, California) and Siemens (Erlangen, Germany) Unity Inova whole-body imaging system equipped with whole body shielded gradients. Occipital, parietal and frontal cortices were imaged using a full head coil. As described above, 13 functional slices were collected for our saccade localizer task to first determine the location of the FEF. These data were collected using BOLD signal changes related to brain activation[49] (navigator echo corrected T2*-weighted segmented gradient echoplanar imaging: 90 images, 64 × 64 resolution, 19.2 cm in-plane f.o.v., TE = 28 ms, TR = 2 s, FA = 60° with 3 × 3 × 6 mm resolution). Once the FEF were identified in the control room, an axial slice volume was selected that centered on the FEF but also included the entire parietal lobe (6 slices, 64 × 64 resolution, 19.2 cm in-plane f.o.v., TE = 28 ms, TR = 0.5 s, FA = 30°). Functional images were superimposed on anatomical images that were obtained using a T1-weighted (3D magnetization-prepared turbo FLASH acquisition, 128 slices, TI = 700 ms, TE = 5.2 ms, TR = 10 ms, FA = 15°) image set acquired in the same scan session with the same slice orientation and in-plane field of view.

Analyses were conducted using the Brain Voyager 4.3 software package (Brain Innovation, Maastricht, Netherlands). After co-registering successive fMRI images to reduce motion artifacts, we corrected for linear drift. All data sets were transformed to Talairach space. Activated voxels were identified using a smoothed *t*-test shifted for the hemodynamic delay and corrected for multiple comparisons ($t > 4.20$, cluster of 10 mm^3 or greater of activation). This *t*-test was a comparison of saccade with fixation blocks based on our saccade localizer data sets. The time courses were derived from the single-subject saccade localizer activation maps, rather than from the group average map, as a group map would create a 'blurring' of the effects by having inactive regions of cortex in each subject contribute to a time course. These individual subject activation maps were then superimposed onto the gap pro- and anti-saccade, the memory delay, or the fixation data sets for each subject, to define the FEF independent of the event-related experiment. We extracted the event-related time courses for each subject corresponding to the FEF and the IPS, and generated event-related averaged files using Brain Voyager software. Each line represented an average of all trials of a particular trial type in each subject. These time courses were then averaged across subjects (see **Figs. 3**, **6** and **7**). For each condition, we collapsed the data across saccade direction, and the signal time courses were then shifted by 3 s to account for the estimated hemodynamic lag[50].

We used the saccade localizer task to independently identify the FEF and the IPS in each subject so that the full spectrum of all possible waveform types would be included in the event-related analysis. A multiple regression analysis was not used because it would only identify voxels with a particular waveform shape.

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Competing interests statement

The authors declare that they have no competing financial interests.

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