fMRI Activation in the Human Frontal Eye Field Is Correlated With Saccadic Reaction Time

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Variation in response latency to identical sensory stimuli has been attributed to variation in neural activity mediating preparatory set. Here we report evidence for a relationship between saccadic reaction time (SRT) and set-related brain activity measured with event-related functional magnetic resonance imaging. We measured hemodynamic activation time-courses during a preparatory “gap” period, during which no visual stimulus was present and no saccades were made. The subjects merely anticipated appearance of the target. Saccade direction and latency were recorded during scanning, and trials were sorted according to SRT. Both the frontal (FEF) and supplementary eye fields showed pretarget preparatory activity, but only in the FEF was this activity correlated with SRT. Activation in the intraparietal sulcus did not show any preparatory activity. These data provide evidence that the human FEF plays a central role in saccadic initiation; pretarget activity in this region predicts both the type of eye movement (whether the subject will look toward or away from the target) and when a future saccade will occur.

INTRODUCTION

More than 150 yr ago, Helmholtz noted that reaction times in sensorimotor tasks varied from trial to trial even though the eliciting stimulus and required response did not change (Helmholtz 1853). Since then, it has been argued that reaction time must therefore reflect factors other than simple summation of neural transduction times between receptors and effectors (Carpenter 1981). Variability in SRT has been shown to be correlated with both pretarget (Dorris et al. 1997, 1998; Everling and Munoz 2000) and post-target (Hanes and Schall 1996; Pare and Hanes 2003) neural activity in the superior colliculus and FEF of monkeys: the greater the buildup of activity, the shorter the saccadic reaction time (SRT).

To induce pretarget activity, previous studies have inserted a variable “gap” interval of darkness prior to target appearance (Connolly et al. 2002; Dorris et al. 1997; Everling and Munoz 2000; Saslow 1967). Activation during the gap interval is not sensory or motor because no eccentric visual stimulus has yet appeared on screen, and no movement has been made. Activation during the gap has therefore been argued to represent a neural correlate of motor preparation (Connolly et al. 2002; Munoz et al. 2000). We further manipulated whether the subject would look toward (pro-saccade) or away from (anti-saccade) the target. This manipulation allowed us to determine whether or not the preparatory functional magnetic resonance imaging (fMRI) signals carry information about the future type of movement to be made. Areas with pretarget activity that is correlated with reaction time and is modulated by the motor plan (a pro- or an anti-saccade) code motor preparation (Evarts et al. 1984).

METHODS

Behavioral tasks

Five subjects participated in this study and each was scanned across two sessions. Visual stimuli were presented using a goggle-based video system (Avotec, Stuart, FL). The peripheral target and central fixation subtended 0.25° of visual angle. Each imaging session began with the saccade localizer task, which consisted of alternating time blocks (30 s) of pro-saccades and central fixation. Periperal targets appeared at a frequency of 2 Hz and stepped to the right or left randomly between 4 and 15° but never >15° from center. During the fixation blocks, subjects fixated on a central cross, and no peripheral targets were flashed. The localizer experiment was 720 s in duration, consisting of 12 blocks of pro-saccades and 12 blocks of central fixation. This allowed us to identify the SEF, the FEF and the IPS (Fig. 1A). The images were analyzed in the control room using the Stimulate software package, to select a functional volume that included the FEF, the SEF, and the IPS.

We measured SRT so that we could sort the event-related fMRI trials off-line according to SRT. Importantly, this enabled us to remove all error trials from the BOLD analysis to increase the sensitivity and accuracy of the design. Each event-related trial began...
by3so ffixation of either the green (pro-saccade trial) or red with3so ffixation of a central white fixation cue. This was followed intraparietal sulcus (IPS).
saccade areas: frontal and supplementary eye fields (FEF and SEF) and tentative subject for our pro-saccade localizer task, illustrating the 3 primary subject). Subjects viewed a white fixation point (FP) presented at the center of white peripheral cue was then flashed for 100 ms along the horizontal pro-saccade trials, the subject made a saccade toward the target (blue position meridian (4° eccentricity) to the right or left of center. Subjects were instructed to look to this location on a pro-saccade trial (green fixation cue) or look to the mirror position on an anti-saccade trial (red fixation cue) and then to hold their gaze at this eccentric location for 2 s until a white central fixation cross appeared that remained on the screen for 12 s. Subjects were required to return gaze to center immediately after reappearance of the central cross and maintain fixation. The 12-s intertrial interval provided time for the fMRI signal to return to baseline after each saccade trial (Connolly et al. 2002). Each experimental run was 528 s in duration, consisting of 8 pro-saccade and 16 anti-saccade trials. Trial types (anti- vs. pro-) were pseudorandomly interleaved, and gap durations were balanced for right- and leftward movements. There were two trial orders (experimental runs) and two or three replications of each were collected for an average total of five experimental runs of the experiment per subject per day. Each subject completed two sessions.

Eye movement recording

Eye movements were recorded from the right eye using a CCD-based infrared video system (iView, SMI, Berlin) that was integrated into the goggle-based fiber optic projection system installed in the fMRI scanner (Avotec Silent Vision SV-4021, Stuart, FL). This system samples horizontal and vertical eye position at 60 Hz and has a resolution of ~0.1° and an accuracy of ~0.5°. The linear range is ~30° horizontally and 20° vertically. Prior to the onset of the experiment, each subject’s eye movements were calibrated in display screen coordinates by fixating five targets with known displacements. The timing of the display and the onset of eye-movement recording were accomplished by a trigger sent by the scanner computer to the stimulus- and eye-tracking computers simultaneously. Computer software determined the beginning and end of each saccade off-line using velocity and acceleration threshold and template-matching criteria. Each trial was examined by the experimenter to ensure that the software was extracting the correct measurements. Saccades made in the wrong direction (direction errors) and trials in which there was some problem with the eye-movement signal or subjects broke central fixation during the intertrial interval were not included in the output analysis for a particular file. Trials with reaction time <800 or >1200 ms were also excluded. In total, <5% of trials were rejected. SRTs with relatively long latencies (500–1,200 ms) were included in the current analysis because earlier work had shown that such long latencies were not uncommon with this protocol (Connolly et al. 2002).

Imaging and data analysis

Experiments were carried out using a 4.0 Tesla Varian Siemens (Palo Alto, CA; Siemens, Erlangen, Germany) UNITY INOVA whole-body imaging system equipped with whole-body shielded gradients. Parietal and frontal cortices were imaged using a head coil. As described in the preceding text, 13 functional slices were collected for our saccade localizer task to first determine the location of the FEF, the SEF, and the IPS. These data were collected using BOLD signal changes related to brain activation (navigator echo corrected T2*-weighted segmented gradient echoplanar imaging (90 images, 64 × 64 resolution, 19.2 cm in-plane FOV, TE = 28 ms, TR = 2 s, FA = 60° with 3 × 3 × 6-mm resolution) (Ogawa et al. 1992). Once the FEF were identified in the control room, an axial slice volume was selected centered on the FEF and SEF but including the entire parietal lobe (6 slices, 64 × 64 resolution, 19.2 cm in-plane FOV, TE = 28 ms, TR = 0.5 s, FA = 30°, 3 × 3 × 6-mm resolution). Functional images were superimposed on anatomical images that were obtained using a T1-weighted (3-dimensional 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 4.9 software package (Brain Innovation, Maastricht, The Netherlands) using a
conventional procedure. After co-registering successive fMRI images to reduce motion artifacts, we corrected for linear drift. All data sets were transformed to Talairach space (Talairach and Tournoux 1988). Activated voxels were identified using a \( t \)-test shifted for the hemodynamic delay and corrected for multiple comparisons (\( t > 4.00 \), cluster of \( \geq 10 \) mm\(^3\) of activation) (Forman et al. 1995). This \( t \)-test was a comparison of saccade with fixation blocks based on our saccade localizer data sets.

The time courses were extracted from the single-subject saccade localizer activation maps rather than the group average map. Using a group average map would have partially washed out the effect because some active portions of the group average map would actually be inactive in a particular single subject map (Connolly et al. 2002). These individual subject activation maps were then superimposed onto the event-related gap saccade data sets for each subject. This allowed us to define the FEF, the SEF, and the IPS independent of the event-related experiment (Fig. 1A). In other words, we did not use a multiple regression analysis to identify cortex that was active during the event-related experiments because we would only “pull out” voxels with a particular temporal waveform shape, i.e., voxels with an event-related activity profile that resembled and thus correlated with the reference waveform. By using the localizer maps instead of a regression analysis, we did not have to make any a priori assumptions about the shape of the event-related BOLD responses.

The event-related time courses for each subject corresponding to the FEF, the SEF, and the IPS were extracted. Event-related averaged files were generated using the Brain Voyager software package with each line representing an average of all trials of a particular trial type and latency range in each subject. The integral value under each of these curves for the entire early or late interval was calculated and submitted to statistical analyses. The time courses were then averaged across subjects (see Fig. 2). The event-related files were base-lined during the last 4 s of the intertrial interval. The signal time courses were shifted by 3 s to account for the estimated hemodynamic lag (Kolias et al. 2000; Schacter et al. 1997).

Because there appeared to be differences in the way that the time courses unfolded for anti- and pro-saccades as a function of SRT, we divided our analysis of the time courses into two equal halves. We defined a late phase as the level of activation integrated under the curve of the time course from the signal peak back to 4 s earlier, and an early phase as the integrated signal 4 s immediately prior to that (i.e., 8–4 s before the peak). Roughly, this meant that we divided the fMRI-BOLD signal rises into two equal halves. Division was based on the observed difference in the way the time courses unfolded. In other words, we did not use a multiple regression analysis to identify cortex that was active during the event-related experiments because we would only “pull out” voxels with a particular temporal waveform shape, i.e., voxels with an event-related activity profile that resembled and thus correlated with the reference waveform. By using the localizer maps instead of a regression analysis, we did not have to make any a priori assumptions about the shape of the event-related BOLD responses.

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words, as we did not attempt any other method of division, this approach was not selected simply to maximize statistical significance. Whereas the anti-saccade FEF 2-s gap time courses appeared to show variation according to SRT for the entire signal rise, the pro-saccade differences appeared to evolve only later on. This type of analysis then allowed us to separate the two portions of the signal rise, to quantitatively argue that the pro-saccade SRT difference developed later on as compared with anti-saccade trials.

It was thus the case that the early phase for 2-s gap trials included only pretarget activity, i.e., changes in activity occurring prior to the onset of the visuomotor signal rise during 0-s gap trials. The late phase included activity changes right up until the signal peak and thus would have also included some visuomotor-related activity changes, in addition to “carry-over” of preparatory changes. This type of analysis provided a suitable approach with which to show that the 2-s gap FEF preparatory changes that correlated with SRT evolved relatively early for anti-saccade trials. An initial analysis in which activations were combined across left and right hemisphere oculomotor areas (bilateral analysis) revealed no systematic relationship with SRT. Therefore subsequent analyses were limited to activation in single hemispheres that were related to contraversive saccades (whether they were pro- or anti-). All the data reported here are from these analyses (with the notable exception of Fig. 2, C and D, which show the ipsilateral FEF responses).

RESULTS

We first ran a block-design localizer task (Connolly et al. 2002) to identify the three cortical eye fields: the FEF, the SEF, and the IPS (Fig. 1A). The mean Talairach coordinates for the areas identified as the right and left FEF were 24.0, −9.0, 45.0, and −27.0, −14.0, 45.0, respectively (where +x is right, +y is anterior, and +z is superior). For the SEF, they were 3.0, −9.0, and 53, and for the right and left IPS, they were 33.0, −48.0, 42 and −26, −53, 37, respectively. The Talairach coordinates for the FEF (Connolly et al. 2000, 2002; Paus 1996), SEF (Shulman et al. 2002; Toni et al. 1999) and IPS (Connolly et al. 2000, 2002; Sereno et al. 2001) were consistent with those reported in previous imaging studies.

The introduction of a gap prior to target appearance led to a reduction in SRT [433 ms for the 2-s gap vs. 500 ms for the 0-s gap, F(1,9) = 56.68, P < 0.001; Fig. 1D] for both pro- and anti-saccade trials (Fischer et al. 1993; Munoz et al. 1998). This straightforward behavioral observation reveals that advance motor preparation took place during the gap interval. The SRTs, on average, were considerably longer than those reported in the literature (e.g., Fischer et al. 1993). In contrast to previous studies that reported shorter reaction times, our longer mean reaction times were presumably the result of having such long inter-trial intervals (ITI) (∼20 s between consecutive saccades), and long gap intervals (2 s). Indeed, we have previously reported SRTs in this exact range when normal subjects were tested outside the scanner using this paradigm with such long intertrial and gap duration intervals (Connolly et al. 2002). Anti-saccades had longer latencies relative to pro-saccades, consistent with previous studies (Fischer and Weber 1996; Munoz et al. 1998) [SRT for anti-saccades was 480 ms; for pro-saccades, 454 ms, F(1,9) = 7.99, P < 0.05]. Correct anti- and pro-saccade trials were sorted into four quartiles: long, mid-long, mid-short, and short SRTs (Fig. 1D).

We tracked the event-related time-courses of activity in the voxels we identified in the contralateral FEF, the ipsilateral FEF, the SEF, and the IPS in the gap saccade task. Figure 2 shows the event-related time courses averaged across the five subjects. Figure 3 shows the time courses for the single subjects for the contralateral FEF only. We then calculated a 4 (SRT quartile) × 2 (gap duration) × 2 (anti- or pro-saccade) × 2 (hemisphere) × 2 (scanning session) ANOVA for the early and late portions of the BOLD signal rise (see METHODS). A parallel examination of ipsiversive saccades revealed no relationship between activation and SRT (Fig. 2). In other words, the ipsiversive BOLD responses for the different SRT quartiles overlapped. This is highly consistent with single-cell recordings for the gap task in monkeys (Dorris and Munoz 1998; Dorris et al. 1997; Everling and Munoz 2000); these recordings also report a correlation only in the hemisphere contralateral to the movement.

In the contralateral FEF (FEFcon; Figs. 2 and 3), the ipsilateral FEF (FEFipsi; Fig. 2, C and D), and the SEF (Fig. 2, E and F), but not the IPS (Fig. 2, G and H), there was an early pretarget activation that occurred in advance of the peak response on 2-s gap trials. This preparatory activity could not have reflected visual or motor processing, because there was no eccentric visual stimulus on the screen and the subjects had not yet moved their eyes (Connolly et al. 2002). The early phase of the rise in activation for 2-s gap trials preceded the rise in activation for 0-s gap trials in the FEF [all statistics are for FEFcon; F(1,4) = 5.04, P = 0.09] and SEF [F(1,3) = 16.25, P = 0.03] but not the IPS [F(1,3) = 1.67, NS]. Although this was only a trend in the FEF, P = 0.09, we are making note of this because of the very limited degrees of freedom in our analysis (F-value error term = 4). The late phase of the BOLD response also increased with gap duration in the FEF [F(1,4) = 17.33, P = 0.01] but not in the IPS [F(1,3) = 1.82, NS]. In the SEF, there was an interaction between gap duration and anti- or pro-saccade for the late phase, [F(1,3) = 11.01, P = 0.05]. Post hoc tests revealed that whereas the pro-saccade 2-s gap saccades in the SEF were higher than the pro-saccade 0-s gap saccades, t(7) = 2.06, P = 0.08, there was no difference for anti-saccades, t(7) = 1.36, P = 0.21. The differences in the late phase of the BOLD response in FEF and SEF almost certainly represented a “carry-over” of the differences in pretarget activation onto a constant posttarget activation that did not vary with gap duration (Connolly et al. 2002). In short, there was evidence for preparatory activity in frontal areas, i.e., the FEF and the SEF, but not in parietal area IPS.

Importantly, for 2-s gap trials there was a significant effect of SRT on pretarget activity for both the early and late phases of the response rise in the FEFcon [early phase: F(3,12) = 8.02, P = 0.003; late phase: F(3,12) = 10.81, P = 0.001]. The activity during the early and late phases of the BOLD signal increased progressively from long- to short-latency saccades. For the early phase, but not the late phase, there was a further interaction between anti- or pro-saccade and latency, F(3,12) = 14.53, P < 0.001. A post hoc comparison revealed that short latency anti-saccades showed higher activity in the early phase than did long latency anti-saccades t(9) = 5.28, P = 0.001; no such difference, however, was evident for pro-saccades, t(9) = 0.30, NS. In contrast, the relationship between the BOLD response and SRT for the pro-saccades emerged only in the late phase, suggesting that for these more automatic responses, only preparation activity immediately before the presentation of the target had any real effect on latency. Taken together, these results suggest that the differences in the
build-up phase for anti-and pro-saccades reflect the activity of separate neural mechanisms within the FEF. In addition to the average FEFcon time courses (Fig. 2, A and B), we also show the single-subject FEFcon time courses for both pro- and anti-saccade trials (Fig. 3). The effect was very robust and apparent at the single-subject level, with the majority of subjects exhibiting the same pattern, i.e., an inverse correlation between the level of fMRI-BOLD preparatory activity and saccadic reaction time.

There was also a significant interaction between gap duration and SRT in the FEF, [early phase $F(3,12)= 7.35$, $P = 0.005$; late phase $F(3,12)= 12.91$, $P < 0.001$]. In other words, whereas FEF activation decreased with increasing SRT latency over the 2-s gap [$t(9)= 5.43$, $P < 0.001$ and $t(9)= 7.33$, $P < 0.001$], there was no correlation between FEF activation and SRT for the 0-s gap trials [$t(9)= 0.02$, NS, and $t(9)= 0.11$, NS] for either pro- or anti-saccades. This observation confirms earlier suggestions that it is pretarget preparatory build-up in the FEF during the gap period that co-varies with SRT (Connolly et al. 2002; Dias and Bruce 1994; Everling and Munoz 2000).

In contrast to the clear relationship between the BOLD response and SRT in the FEF, there was no relationship between these variables in either the SEF [early phase, $F(3,9)= 1.02$, NS; late phase $F(3,9)= 1.65$, NS] or the IPS [early phase $F(3,9)= 1.96$, NS; late phase $F(3,9)= 1.35$, NS]. Taken together, these results suggest that it is variability in the pretarget activity in the FEF that most strongly influences the trial-to-trial variability in saccade latency in a preparatory set task. The FEF is therefore intimately involved in coding motor preparatory set.

We also searched for laterality differences in the correlation between BOLD and SRT. There was a three-way interaction among saccade type, hemisphere, and SRT for the late phase in the FEF, $F(3,12)= 3.51$, $P = 0.05$. SRT was correlated with the BOLD response in both the right and left hemispheres for anti-saccades [right hemisphere $t(9)= 3.59$, $P = 0.006$; left $t(9)= 2.70$, $P = 0.024$]. But for pro-saccades, this correlation was evident only in the right hemisphere [right hemisphere $t(9)= 3.46$, $P = 0.007$, left $t(9)= 0.12$, NS].

Generation of eye movements during the gap period cannot explain the pretarget activation in the FEF (Connolly et al. 2002) or the SEF. Any trial with saccades during the gap interval was removed from the analysis (see METHODS). In addition, this pretarget activation was limited only to the FEF and SEF; it was not observed in the IPS. As is the case with
saccades generated after target appearance, we would expect activation in all of the oculomotor areas if saccades were generated prior to target appearance.

**DISCUSSION**

We have shown that preparatory set activity in FEF predicts the latency of a subsequent saccade to an upcoming target. It is worth emphasizing that this relationship was evident only for contraversive saccades. That is, variation in preparatory activity in a single hemisphere was correlated with variability in the latency of contraversive but not ipsiversive saccades. With the notable recent exception of Koyoma et al. (2004), previous neuroimaging studies have failed to find a relationship between the level of activation in a single FEF and whether the saccades were contraversive or ipsiversive (Connolly et al. 2000, 2002) despite overwhelming evidence of such coding in the monkey (Munoz et al. 2000; Schall 2004). But, as the present experiment shows, it is only when the relationship between FEF activation and the latency of saccades on individual trials is examined that the close coupling between the pretarget amplitude of the FEF signal and contraversive, but not ipsiversive, saccades is revealed. This underscores the importance of examining the relationship between response characteristics on individual trials and the various parameters of the BOLD signal in event-related designs. Build-up activity in the monkey is also only correlated to SRT for contraversive saccades, both in the superior colliculus (Dorris and Munoz 1998; Dorris et al. 1997) and FEF (Everling and Munoz 2000). As with the humans, the monkeys did not know which side the target would appear. Individual cells can only influence contraversive movements; the more prepared the subject is in one direction, the faster the reaction time. The fact that we did not observe a relationship in our original bilateral analysis suggests that the two FEF build toward different target SRTs on a given trial. Another possibility is that the gain is different across the two FEF for the same planned SRT.

Consistent with our previous imaging study (Connolly et al. 2002), we did not observe preparatory activity in the IPS. However, recent evidence in the monkey suggests that parietal cortex does exhibit set-related activity (Calton et al. 2002; Dickinson et al. 2003). The fMRI data suggest that any parietal increases must be many orders of magnitude smaller than the frontal signals. Yet there is also an important methodological difference between the monkey and human work. The monkey studies had very short preparatory periods (≤500 ms) as compared with the human gap durations of 2 s (present study) and ≤4 s (Connolly et al. 2002). If the monkeys were tested over such long durations, the parietal signals might asymptote or decay and thus would not be detectable in the fMRI-BOLD response. Also, fMRI is indicative of an area’s average ensemble activity. The active proportion reported in the monkey studies might not be large enough or exhibit large enough activity changes to be detectable in the average. Yet there is also an important similarity. In a previous study, we showed that parietal IPS exhibits robust memory delay but no preparatory activity (Connolly et al. 2002). The monkey studies show the same overall pattern, i.e., less preparatory than memory delay activity (Calton et al. 2002; Dickinson et al. 2003). In the human frontal cortex, however, the fMRI-BOLD responses were equivocal for preparation and memory delay (Connolly et al. 2002). It would therefore be interesting to see a direct comparison of gap and memory delay in the monkey FEF. Because the human frontal fMRI signals are more robust relative to the parietal lobe, we speculate that the signals in monkey parietal cortex may be due to feedback from frontal cortex. Indeed, frontal areas are richly interconnected with these parietal areas (Tanne et al. 1995; Shipp et al. 1998). Because the frontal cortex is close to the motor output, i.e., M1 (hand) and the FEF (eye), this is the most straightforward argument. What is important then is the relative difference across the two lobes rather than whether the parietal cortex does or does not exhibit some preparatory activity.

The fact that SRT was correlated with pretarget FEF activity on 2-s gap trials but not on 0-s gap trials suggests that the variability in the observed activation during the 2-s gap trials does indeed represent differences in early preparatory signals that are coded within this area. On 0-s gap trials, variability in SRT may have arisen because of differences in posttarget processing (Hanes and Schall 1996; Pare and Hanes 2003) rather than differences in pretarget processing, such as those reported in the monkey. If this was in fact the case, the observed variability in SRT on 0-s gap trials would not have been reflected in any differences in the BOLD signal. Such differences in posttarget activity would evolve quickly (i.e., within a few hundred milliseconds or less) (Hanes and Schall 1996; Pare and Hanes 2003) and thus would not be detectable with our fMRI protocol, which used a sampling rate of 500 ms. Single-unit work in monkey, however, has demonstrated a correlation between SRT and the posttarget neural activity in the FEF (Hanes and Schall 1996) and superior colliculus (Pare and Hanes 2003). In other words, SRT variability on gap trials may reflect differences in pretarget activity in the FEF, whereas SRT variability on no-gap trials may reflect differences in posttarget activity in this same region. Thus variability in the timing of voluntary saccades is a consequence of different kinds of stochastic variability in the activity of neurons in the FEF.

The evidence we have presented here shows that excitability of the neurons distributed throughout the contralateral FEF in humans predicts when a saccade will occur. Both the present study and our previous work have shown that human FEF preparatory signals also predict the type of future saccade (Connolly et al. 2002). Such activity may reflect processes commonly referred to as preparatory set (Evarts et al. 1984). Such an interpretation is consistent with neurophysiological observations in monkey FEF (Everling and Munoz 2000; Hanes and Schall 1996). Importantly, this relationship between saccadic latency and build-up of neural activity is not present in the other cortical eye fields but is evident in the FEF, suggesting that this area is the key player preparing the oculomotor system for action.

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**GRANTS**

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