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The Importance of Distributed Sampling in Blocked Functional Magnetic Resonance Imaging Designs

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In this study we demonstrate the importance of distributed sampling of peristimulus time in blocked design fMRI studies. Distributed sampling ensures all the components of an event-related hemodynamic response are sampled and avoids the bias incurred when stimulus presentation is time-locked to data acquisition. We found that differences in the temporal offset between stimulus presentation and data acquisition had a significant effect on some language-related activations. These effects, induced by simply shifting stimulus presentation by a fraction of the interscan interval, suggest that fixed sampling does indeed bias estimated responses, even in blocked designs.

Key Words: **distributed sampling; blocked design; BOLD signal.**

INTRODUCTION

In functional magnetic resonance imaging (MRI), whole-brain images are constructed by combining data from slices of the brain that have been sampled sequentially over time, rather than simultaneously (as for positron emission tomography (PET)). Although each slice can be acquired in less than 100 ms, the hemodynamic response in each slice is sampled at a frequency of only 1/TR Hz (or even 1/2 TR Hz for segmented echo-planar imaging sequences), resulting in a "sparse" temporal sampling of the hemodynamic response. This is a potential problem because the true hemodynamic response may have high-frequency components (particularly when the design is event related) that may not be fully characterized if they occur within the TR (e.g., 2–4 s). However, the hemodynamic response can still be estimated if, over the scanning session, the stimuli are systematically presented at several different time points within the TR. This results in sampling that is distributed throughout peristimulus time. Distributed sampling can be achieved in two ways. First, the presentation of the stimuli can be jittered by varying the interstimulus interval (Josephs *et al.*, 1997; Henson and Josephs, 1999; Miezin *et al.*, 2000). For example, the stimulus onset asynchrony (SOA) might be 2 s ± 150 ms. Alternatively, distributed sampling can be achieved by fixing the SOA (e.g., stimuli always occur at 2-s intervals) and selecting a TR that is not an integer multiple of the SOA (Price *et al.*, 1999). For example, in Mechelli *et al.* (2000), a TR of 3.15 s with an fixed SOA of 3.0 s allowed data to be acquired every 90 ms of peristimulus time.

In blocked designs, we assume that the accumulated hemodynamic response has predominantly lower frequencies and may be more robust to sparse sampling; therefore, condition-specific effects should be more adequately assessed without distributed sampling. However, distributed sampling may still be necessary if high-frequency components persist. This could occur during high-level cognitive processing if the synaptic response was biphasic or if the response included complex waveforms with several components. Complex waveforms could arise endogenously due to "top-down" processing that is not evoked directly by exogenous stimuli. In these circumstances, to estimate high-frequency components efficiently, it is necessary to ensure that data are acquired throughout peristimulus time (distributed sampling).

In a previous study (Price *et al.*, 1999), we found that, even in blocked designs, signal detection in two key language areas was reliably detected only when sampling was distributed throughout peristimulus time but not when sampling was locked to one peristimulus time point. Our results indicated that the timing of data acquisition can be critical even in blocked designs. However, in this previous study, we were unable to make direct statistical comparisons between data sampled from one or multiple peristimulus time points, because the TRs for the different data sets were not the same. The aim of the present study was to demonstrate the effect of distributed sampling in a

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blocked design language study and evaluate the effects statistically. To do this, we kept both the TR and the SOA constant across all conditions so that differences in sampling time could not be attributed to either of these factors. Sampling time was then manipulated by varying the stimulus onset relative to onset of the TR. There were four different conditions with stimulus onset within a block occurring either (1) in synchrony with the onset of the TR or with a delay of (2) 0.25 of the TR, (3) 0.5 of the TR, or (4) 0.75 of the TR. Sampling was therefore "fixed" within block but "distributed" over blocks. The stimuli were written words, and subjects performed a reading task alternating with blocks of fixation. We hypothesized that, if there are high-frequency signal changes within a reading block, then the effect of reading relative to fixation would vary with the timing of data acquisition. In other words, we would observe an effect of block type.[2] Conversely, if fixed sampling (within a block) does not induce any bias, the estimated responses to each block type should be the same.

METHODS

Subjects

Six right-handed healthy volunteers (four females and two males, mean age 28.5, range 20–38 years) were studied. All subjects had English as their first language. Written informed consent was obtained from each subject prior to scanning. Scanning protocols were approved by the local ethics committee.

Task Paradigm and Stimuli

All subjects participated in two scanning sessions. During each scanning session, the subjects silently read 8 blocks of 8 (W)ords and 8 blocks of 8 (P)seudowords (i.e., a total of 128 stimuli). The baseline condition involved blocks of (F)ixation alternating with the reading blocks (e.g., WFPFWFPF etc. or PFWFPFWF etc.). Each stimulus was presented for 600 *ms* in lowercase (Courier font), with a SOA of 3 s. Thus, the duration of reading blocks was invariably 24 s (8 stimuli $\times$ 3 s SOA). Critically, the TR was also 3 s so that, within block, data acquistion was fixed to one point in peristimulus time. The timing of data acquisition within block was manipulated by varying the duration of fixation between reading blocks: Fixation occurred for either (A) 6 TRs (18 s), (B) 6.25 TRs, (C) 6.5 TRs, or (D) 5.75 TRs. This resulted in four stimulus/TR onset relationships, with stimulus presentation occurring at (A) TR onset, (B) 0.25TR, (C) 0.50TR, or (D) 0.75TR. This was our only variable of interest and allowed us to assess whether the timing of data acquisition (A, B, C, or D) had an effect on reading-related activation.

Stimulus type (words or pseudowords) was not a variable of interest in this analysis. The words were matched between block for frequency (Kucera and Francis, 1967), length (4, 5, or 6 letters), and number of syllables (1–3). A total of 128 words were selected; all had regular/consistent spelling-to-sound mappings which allowed them to be converted to 128 pseudowords (matched to the words for length and number of syllables) by changing the onset, internal consonants or coda. For example the word *golf* became the pseudoword *ponf*, and the word *lemon* became the pseudoword *lenos*. Bigram frequency was also matched between all word and pseudoword blocks. Over the two scanning sessions, each subject saw 4 blocks of words and 4 blocks of pseudowords at each data acquisition time (A, B, C, and D). This resulted in 32 reading blocks with no stimulus repetition within block and each experimental condition repeated four times in a counterbalanced order across subjects. An eye movement tracker was used to ensure the subjects were looking at the words.

Data Acquisition

A 2-T Siemens VISION system (Siemens, Erlangen, Germany) was used to acquire both T1 anatomical volume images (MP-RAGE, 1 $\times$ 1 $\times$ 1-mm voxels) and T2*-weighted echo planar images (64 $\times$ 64 matrix, TR = 3 s, TE = 40 ms). We used a 32-slice sinusoidal EPI sequence, axial ascending with the first slice positioned near the temporal poles using a parasagittal scout, slice thickness 1.8 mm with a 1.2-mm interslice gap, resulting in 3 $\times$ 3 $\times$ 3-mm voxels. A total of 232 volumes images were acquired in two runs, the first 6 (dummy) volumes in each session being discarded to allow for T1 equilibration effects.

Data Analysis

Data were analyzed using SPM99 software (Wellcome Department of Cognitive Neurology, http://www.fil.ion.ucl.ac.uk). All scans from each subject were realigned to the first image of the first session and each subject's structural T1 was coregistered to the mean EPI image. The images were transformed to a standard stereotactic space (Talairach and Tournoux, 1988) and spatially smoothed with an 8-mm Gaussian filter. Next, the data were bandpass filtered and analyzed in an event-related fashion, with each stimulus presentation modeled with a canonical hemodynamic response function and its temporal derivative. Four different event types were modeled (pooling over words and pseudowords). We then looked at effects of reading relative to fixation that were:

[2] Confounds from any latency difference between real and modeled responses were excluded by including temporal derivatives in the statistical model that accommodate these latency effects (to first order).

TABLE 1

Common Effects (Irrespective of Timing)

	Coordinates			Brodmann area	*T* score
Left temporo-occipital cortex	−40	−50	−22	20/36	21.5
Fusiform gyrus	−44	−70	−14	37	18.7
Posterior fusiform gyrus	−36	−88	−12	18/19	15.9
Middle and inferior occipital	−10	−92	−10	17	18.3
	−16	−100	−2	17	14.7
	−24	−94	0	18	14.3
Right temporo-occipital cortex	40	−50	−24	20/36	11.9
Fusiform gyrus	24	−82	−14	18	15.8
	36	−78	−14	18	13.3
Middle and inferior occipital	16	−92	−8	17	14.1
	28	−94	8	18	13.4
Right posterior middle temporal	48	−68	−2	19	11.3
Left parietal	−30	−68	50	7	12.6
	−28	−52	44	40	10.6
Right parietal	30	−64	38	40	10.4
Left precentral	−54	−8	36	6	11.1

TABLE 2

Timing-Specific Effects (Conjunction of $A + B > 2F$ and $A > B$)

	Coordinates			Brodmann area	*Z* score	Minimal *T* score
Left frontal	−52	10	32	44	4.96	3.25
	−48	38	16	45/46	4.93	3.23
	−48	32	18	46	5.18	3.41
	−48	40	4	46	6.59	4.45
Right frontal	54	30	24	46	5.35	3.57
	50	34	14	45/46	5.84	3.90
	50	48	−4	47	6.02	4.03
Left temporal	−50	−52	−16	20/37	6.31	4.24
Right temporal	56	−36	2	21	5.41	3.58
Left parietal	−38	−48	50	40	5.31	3.51
Motor	−46	−10	50	4	5.79	3.87
	60	0	38	6	5.24	3.45
Cerebellum	42	−50	−34	—	6.02	4.04
	−38	−42	−32	—	5.2	3.42

a. Common to all sampling conditions (i.e., not affected by sampling time): These were identified by looking at the main effect of reading (A + B + C + D − 4F) masked inclusively with the simple main effects of A − F, B − F, C − F, and D − F, each thresholded at $P < 0.05$ (after correction for multiple comparisons). The inclusive masking technique in SPM includes voxels in the statistical map only if they were present in each of the masks specified.

b. Specific to one sampling condition (i.e., affected by sampling time). The criterion for specific effects was a conjunction of (i) the difference between two conditions (e.g., A–B) where there was (ii) a main effect of reading over these conditions relative to fixation (A + B − 2F).

RESULTS

Common main effects for task (across A–D and masked inclusively with A, B, C, and D) are reported in Table 1.

Reading versus fixation resulted in activations in bilateral occipital, posterior inferior temporal, and parietal cortices as well as left motor cortex. In other words, these areas were identified irrespective of the timing condition.

To make our point simply, the results focus only on differences between conditions A and B. The only difference between these two conditions was a relative shift in stimulus presentation by 0.25TR. Comparing main effects for conditions A and B, we found that A gave apparently larger activations in bilateral prefrontal cortex, left inferior temporal cortex, and cerebellum (Table 2, Figs. 1 and 2). In other words, identification of reading-related activation in these areas was dependent on the timing of data acquisition even though the stimuli, task, and, presumably, hemodynamic response were identical.

These differences persisted when (i) the temporal derivative of the hemodynamical response function was included as an additional regressor and (ii) movement parameters were added as nuisance covariates. We did not find regions where B gave larger activations than A.

DISCUSSION

In the present study we investigated the effect of distributed sampling in a blocked design reading paradigm. Consistent with our previous findings (Price *et al.*, 1999), we demonstrated that when the relationship between TR and SOA is phase-locked, the biased sampling of peristimulus time-induces biased estimates of task-related activation. Although activation was reliably detected in some reading regions (e.g., bilateral occipital and parietal cortices) irrespective of the timing of data acquisition, other areas (e.g., bilateral prefrontal cortex, left inferior temporal cortex, and

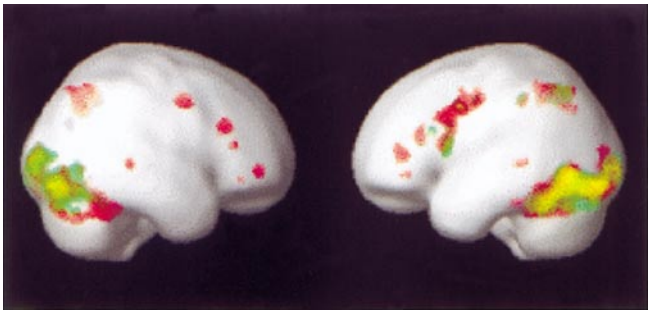

FIG. 1. Main effects of $A - F$ (red), $B - F$ (green). Yellow shows overlap of red and green.

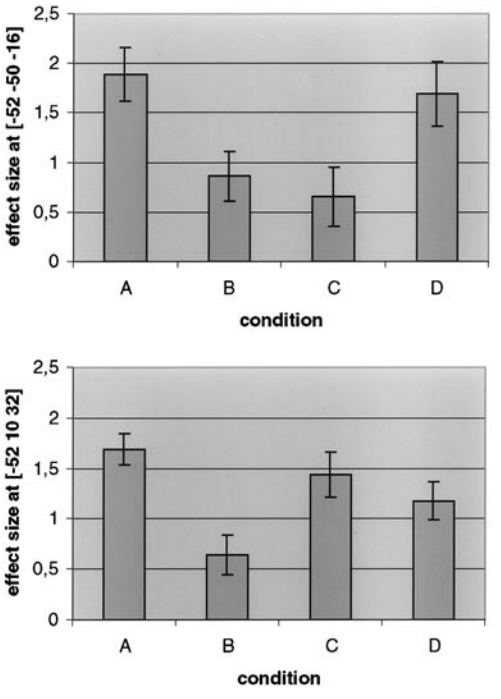

FIG. 2. Effect sizes (in % of global signal ± SE) for scanning conditions A–D in left posterior inferior temporal cortex (top) and left inferior prefrontal cortex (bottom).

cerebellum) were detected only at specific timing conditions.

These findings indicate that signals in some reading-related areas do not reach a steady state within each block, implying there are high-frequency components in the signal. One possibility is that the hemodynamic response to language stimuli in some areas is atypical; e.g., the BOLD signal may be less dispersed than in other areas. This might occur if complex waveforms were induced by endogenous ("top-down") effects rather than exogenous ("stimulus-driven") effects. Although the source of these high-frequency components is not yet clear, we can exclude explanations that relate to the type of analysis used. For example, we found that our results are not altered by (i) including the temporal derivative of the canonical hemodynamic response function as an additional regressor; or (ii) including between-scan movement parameters as covariates of no interest. Thus our results can not be explained by latency differences between real and modeled responses or condition-related subject movement (although we cannot rule out confounds from within-scan subject movement). Furthermore, it should be noted that sampling bias (that occurs "within slice") cannot be remedied by slice timing interpolation, which corrects for *between*-slice acquisition time differences.

In conclusion, we found that even in blocked designs, phase-locked SOA/TR relationships may lead to biased sampling, reducing sensitivity to responses in some of the areas associated with word reading. These effects apparently do not result from inadequate timing of the analytical model or scan-to-scan subject movement, but may be due to high-frequency components in the BOLD response in language areas. Although the importance of distributed sampling is fully appreciated in event-related designs, the present results suggest that sampling should also be distributed in blocked designs. Further research is needed to investigate the temporal profile of the hemodynamic responses in the regions identified in this study.

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