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The Critical Relationship between the Timing of Stimulus Presentation and Data Acquisition in Blocked Designs with fMRI

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This paper concerns the experimental design and statistical models employed by fMRI activation studies which block presentation of linguistic stimuli. In particular, we note that the relationship between the timing of stimulus presentation and data acquisition can have a substantial impact on the ability to detect activations in critical language areas, even when the stimuli are presented in blocks. Using a blocked word rhyming paradigm and repeated investigations on a single subject, activation was observed in Broca's area (left inferior frontal cortex) and Wernicke's area (left posterior temporoparietal cortex) when (i) the timing of data acquisition was distributed throughout the peristimulus time and (ii) an event-related analysis was used to model the phasic nature of the hemodynamic response within each block of repeated word stimuli. In contrast, when the timing of data acquisition relative to stimulus presentation was fixed, activation was detected in Broca's area but not consistently in Wernicke's area. Our results indicate that phasic responses to stimuli occur even in a blocked design and that the sampling and proper modeling of these responses can have profound effects on their detection. Specifically, distributed sampling over peristimulus time is essential in order to detect small activations particularly when they are transient. These findings are likely to generalize to the detection of transient signals in any cognitive paradigm. © 1999 Academic Press

INTRODUCTION

The aim of this paper is to highlight the disadvantage of using a fixed relationship between stimulus presentation and data acquisition in blocked design fMRI studies, in particular, those involving the recurrent presentation of discrete stimuli. We first address the theoretical considerations that underlie the detection of evoked phasic responses and then illustrate the issues that ensue using an fMRI study of word processing.

The majority of fMRI studies rely upon block designs where changes in brain state are elicited by trains of recurrent stimuli presented in blocks or epochs that last anything between 20 s to several minutes. The resulting differences in neurophysiology are, almost universally, modeled with some form of boxcar regressor, usually convolved with a hemodynamic response function. Implicit in this model is the assumption that steady-state dynamics are attained within each block. In this way fMRI has been used to emulate experimental designs employed by PET that rely explicitly on steady-state dynamics. In PET the half-life of the radiotracers used ensures that steady-state assumptions are valid. However, in fMRI the evoked BOLD response may be short lived in relation to the interstimulus interval (ISI). Under these conditions the steady-state assumption may no longer be appropriate. This paper addresses the problems that are encountered when the steady-state assumption does not hold.

For any given sequence of repeated stimuli, if the ISI is long compared to the evoked and measurable hemodynamic response, then the response to any stimulus will have died away before the presentation of a subsequent stimulus. This will result in a periodic and dynamically modulated measurable response which is a function of peristimulus time. The estimated activation, in steady-state terms, will therefore depend critically on when, in the ISI, the responses are sampled. If the responses are sampled at discrete points in the ISI (i.e., if the temporal relationship between data acquisition and stimulus presentation is fixed), there may be a bias in the estimated activation. Sampling the peaks will lead to an overestimate of steady-state activation and sampling the troughs will lead to a biased underestimate and a potential loss of sensitivity for small and transient signals. In short, even in the context of block designs, the estimated average activation in fMRI experiments may become a function of when the response was measured relative to stimulus onset. The main point of this paper is that sampling the data in a distributed way over the ISI eschews this bias and is essential for a proper characterization of evoked re-

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sponses. Distributed sampling is in turn ensured if the ISI and scan repetition time (TR) are not integer multiples of each other.

Figure 1 illustrates the differences between fixed and distributed sampling when the underlying responses constituting the block are transient. The estimated average activation under fixed sampling (thick gray lines in the top pair of panels) is higher for slice 2 than slice 1 even though the actual hemodynamic response is the same (thin gray lines). The bias is simply due to sampling the data slightly later in slice 2. For multislice acquisition, this means that some slices will be less sensitive to activations than others, resulting in swathes or bands of reduced or increased sensitivity.

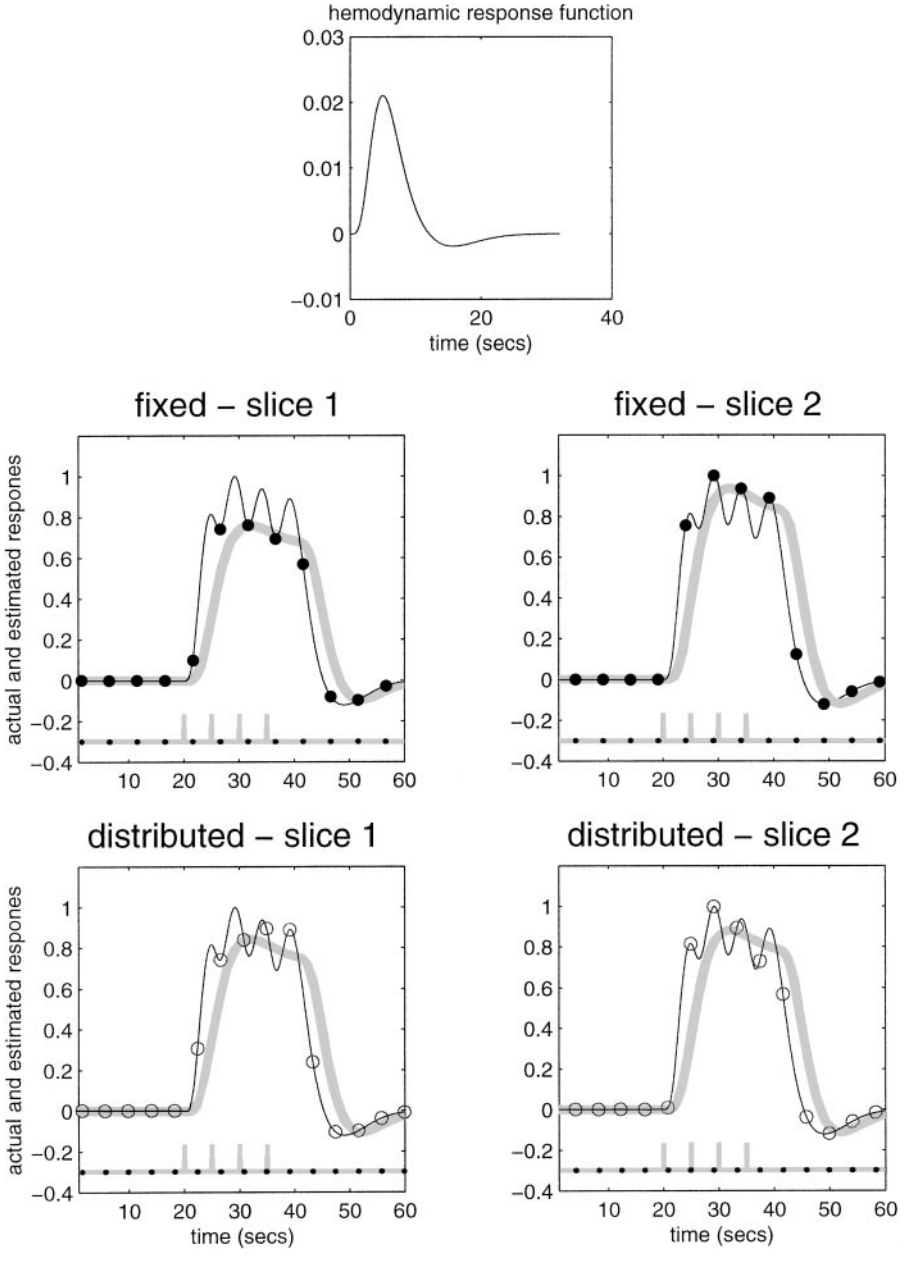

FIG. 1. This figure shows a simulated hemodynamic response to a train of stimuli that constitute a block in an fMRI scanning session. The thin black line (actual hemodynamic response) was obtained by convolving an underlying stick function that represents the transient presentation of, say, word stimuli with a synthetic hemodynamic response function. In this instance the ISI was 4 s and there is quite a profound periodicity in the response profile during the block. The thick gray line represents the estimated hemodynamic response using a conventional boxcar regressor (convolved with the response function). When acquisition and stimulus presentation are fixed, the fit to data from slice 1 underestimates the actual hemodynamic response while estimates based on data from slice 2 overestimate the response. A less biased estimate is obtained when the sampling is distributed over the ISI or acquisition and presentation are uncoupled.

When slice acquisition is not fixed to a particular point in the ISI, but distributed across all peristimulus times, this problem is resolved (see best fitting responses in the bottom panels).

Distributed sampling avoids bias by properly sampling the periodic modulation of evoked responses. To maximize sensitivity further, periodic variation needs to be modeled in the statistical analysis. Analyses of fMRI data conventionally use boxcar regressors for blocked designs but this approach assumes a constant plateau of activity within the block rather than modeling the periodic variation in transient responses. Recently, temporal basis functions have been used to analyze data from single events. The event-related approach has substantially increased our understanding of the nature and form of the hemodynamic response function and it has become clear that blocked-design fMRI experiments can be analyzed from an event-related perspective. In Josephs *et al.* (1997), a simple approach for modeling event-related responses was described in terms of a small number of basis functions of peristimulus time. In the simplest case, one basis function (a synthetic hemodynamic response function) is convolved with the presentations of the stimuli to create a suitable response function. This approach will fit the data better than one using a conventional boxcar formulation. When the periodic variation of transient responses is modeled by event-related analysis, the error variance is reduced thereby increasing the sensitivity of the analysis.

In what follows we use an event-related statistical model to analyze five fMRI studies of word processing that were identical apart from manipulations of the TR. In three of the five studies, data from 32 slices were sampled at one time point in the ISI. This was achieved by making the TR the same as the ISI or twice the ISI. In the other two studies, we chose TR:ISI relationships such that the ISI was sampled as evenly as possible at every slice in the brain (see Fig. 2 for a schematic illustration of how ISI and TR interact in multislice acquisition). Clearly, any bias due to selective (fixed) sampling during the ISI will only be expressed when the underlying responses are transient. In many sensory and motor regions, responses to single word stimuli may endure for several seconds but in language regions, responses can be phasic or transient (Price and Friston, 1997; Cannestra *et al.*, 1998). For instance, the temporal response in Broca's area is more protracted than that in Wernicke's area (Cannestra *et al.*, 1998). This observation suggests that Wernicke's area should be more susceptible to bias with fixed sampling than Broca's area. In fact this is exactly what we found, and report below, using a word rhyming paradigm with, and without, distributed sampling.

MATERIALS AND METHODS

The data were acquired from a normal right-handed female volunteer, on 5 different days. In each of these studies, the activation task involved presenting pairs of words at a rate of one pair per 3.2 s (presentation duration was 1 s). The subject was instructed to press a response key with the right index finger if the words in the pair rhymed (e.g., "YACHT–slot" but not "YACHT–youth"). The presentation of the stimuli was blocked, with eight word pairs in activation blocks (block duration, 25.6 s) alternating with 25–30 s of no stimuli (i.e., the baseline condition was rest).

Predicted Activations

According to cognitive models of word processing, the difference between the rhyming and resting conditions includes visual, orthographic, lexicosemantic, phonological, and motor processing in addition to attention and decision making. Several studies have described the neural correlates of these processes using both PET and fMRI (Price *et al.*, 1994; Bookheimer *et al.*, 1995; Pugh *et al.*, 1996; Price, 1997). In the rhyming paradigm used in this study, we were particularly interested in activation of the left inferior frontal and the left temporoparietal regions because these areas have been specifically associated with phonological processing (Paulesu *et al.*, 1993; Demonet *et al.*, 1994; Bookheimer *et al.*, 1995; Mummery *et al.*, 1996; Pugh *et al.*, 1996; Price and Friston, 1997; Price, 1997).

The Stimuli

The stimuli were constructed from a set of 128 target words printed in uppercase. A second set of 128 words was then generated from the target set and printed in lowercase. The lowercase words were generated such that each target word had 1 word that rhymed but looked dissimilar (e.g., "YACHT–slot," "MOAT–vote") and 1 word that did not rhyme but looked visually similar (e.g., "YACHT–youth," "MOAT–meat"). Each target word was then paired with 4 lowercase words, 1 that rhymed and 3 that did not (e.g., "YACHT–slot," "YACHT–vote," "YACHT–youth," "YACHT–meat"). Similarly, each lowercase word was presented in four different pairs (e.g., "YACHT–slot," "MOAT–slot," "QUEEN–slot," "STEAK–slot"). The number of word-pair stimuli therefore totaled 512 (128×4). These were divided into eight different sets (64 word pairs per set) to rotate over the five different studies comprising the experiment.

Procedure

There were four different scanning sessions per study. Each session lasted approximately 7 min 15 s

A

Block:		1				2				3			
Scan:		1	2	3	4	1	2	3	4	1	2	3	4
Slice:	1:	*	*	*	*	*	*	*	*	*	*	*	*
	2:	.1	.1	.1	.1	.1	.1	.1	.1	.1	.1	.1	.1
	3:	.2	.2	.2	.2	.2	.2	.2	.2	.2	.2	.2	.2
	4:	.3	.3	.3	.3	.3	.3	.3	.3	.3	.3	.3	.3
	5:	.4	.4	.4	.4	.4	.4	.4	.4	.4	.4	.4	.4
	6:	.5	.5	.5	.5	.5	.5	.5	.5	.5	.5	.5	.5
	7:	.6	.6	.6	.6	.6	.6	.6	.6	.6	.6	.6	.6
	8:	.7	.7	.7	.7	.7	.7	.7	.7	.7	.7	.7	.7
	9:	.8	.8	.8	.8	.8	.8	.8	.8	.8	.8	.8	.8

B

Block:		1				2				3			
Scan:		1	2	3	4	1	2	3	4	1	2	3	4
Slice:	1:	*	.8	.6	.4	.2	*	.8	.6	.4	.2	*	.8
	2:	.2	*	.8	.6	.4	.2	*	.8	.6	.4	.2	*
	3:	.4	.2	*	.8	.6	.4	.2	*	.8	.6	.4	.2
	4:	.6	.4	.2	*	.8	.6	.4	.2	*	.8	.6	.4
	5:	.8	.6	.4	.2	*	.8	.6	.4	.2	*	.8	.6
	6:	*	.8	.6	.4	.2	*	.8	.6	.4	.2	*	.8
	7:	.2	*	.8	.6	.4	.2	*	.8	.6	.4	.2	*
	8:	.4	.2	*	.8	.6	.4	.2	*	.8	.6	.4	.2
	9:	.6	.4	.2	*	.8	.6	.4	.2	*	.8	.6	.4

FIG. 2. Examples of data acquisition which is either (A) fixed to the one point in the interstimulus interval (ISI) or (B) distributed throughout the ISI. In both cases, an asterisk indicates the slice where the stimulus is presented. .1 through .9 indicate the time relative to onset as a proportion of the ISI. When the stimulus onset is fixed to slice 1 (A), data acquisition from each slice always coincides with the same point in the ISI. When the stimulus onset is distributed (B), data acquisition from each slice coincides with each point in the ISI (.2 through .8). The difference between A and B is generated simply by manipulating the ISI or the TR.

during which one set of words was presented (see above), with eight pairs of words alternating with rest. Over the five studies there were 20 different scanning sessions (4 per study) but only eight sets of words. Thus four word sets were seen twice and four word sets were seen three times over a 1-year interval. The replication was counterbalanced across the sessions and no word set was repeated within the same study. The large number of stimuli and the inconsistency between words of a pair ensured that the subject could only make a correct response by reading and rhyming the words. Responses could not be learned over the experiment or made on the basis of an orthographic (visual) decision.

The Variables

Two further experimental/acquisition factors were manipulated (1) the scan TR and (2) the direction of sequential multislice data acquisition (ascending versus descending).

1. The choice of TR was determined by its relationship with the ISI. For instance, the TR was an integer value of the ISI (3.2 s) for TR = 6.4 s or TR = 3.2 s, but not for TR = 4.1 s and TR = 5.4 s. The order of TR (in seconds) over the five different studies was 6.4, 4.1, 3.2, 5.4, and 6.4. The replication of the TR = 6.4 s study was included to balance for time effects.

Altering the TR had two effects. First, it allowed us to vary the points in the ISI that data were acquired while

keeping all the details of the experimental paradigm constant across studies. For TRs of 6.4 s and 3.2 s, there was a fixed relationship between the ISI (3.2 s) and data acquisition, so that each slice sampled data from only one particular point in the ISI (see Fig. 2A). For TRs of 4.1 and 5.4 s, there was a variable relationship between stimulus onset and data acquisition so that each slice sampled data evenly throughout the ISI (see Fig. 2B).

The second effect of altering the TR, while keeping the stimulus presentation constant, was that for the same number of stimuli, different numbers of scans were acquired. For instance, twice as many scans were acquired when the TR was 3.2 s than when the TR was 6.4 s. The effect of this difference was evaluated by equating the number of scans before comparing the results from different studies. As it turned out, the difference in the number of scans had virtually no effect on the observed results. Therefore, the primary effect of altering the TR related to whether there was a fixed relationship between stimulus onset time and slice acquisition.

2. *The direction of sequential multislice acquisition* was either ascending (A) or descending (D). For each study, the acquisition order was counterbalanced over session (i.e., A, D, D, A). The purpose of varying the direction of the data acquisition sequence was to manipulate the time point that data were acquired in the ISI while keeping all other parameters constant. For instance, with a TR of 3.2 s, slice 1 samples data early in the ISI and slice 32 samples data late in the ISI. When the acquisition order is reversed slice 1 samples late in the ISI and slice 32 samples early in the ISI. If a signal is transient, the likelihood of detecting an activation will vary for ascending or descending acquisition when the stimulus onset slice is locked (i.e., TRs of 3.2 and 6.4 s) but not when data for each slice have been evenly sampled throughout the ISI (i.e., TRs of 4.1 and 5.4 s).

In summary, we reasoned that if hemodynamic responses during a block of stimuli are transient, the likelihood of detecting an activation would be more consistent when data from each slice were acquired throughout the ISI. When data are always acquired at the same point in the ISI, the chance of detecting activity would be more *ad hoc*. For instance it might be detected during ascending but not descending data acquisition.

Technical Details

The data were acquired at 2 T using a Magnetom Vision whole-body MRI system (Siemens, Erlangen), equipped with a head volume coil. Contiguous multislice T2*-weighted fMRI images were obtained with a gradient-echo planar sequence using an axial slice orientation (TE = 40 ms). The brain volume sampled in all five studies covered 9.6 cm and was represented by 32 slices (3 mm slice thickness). Only the most dorsal regions of the cerebral cortex (such as the supplementary motor area) were out of the field of view. Structural images were obtained at the same orientation, using a T1-weighted sequence with $1 \times 1 \times 1.5$-mm voxels.

Data were analyzed with SPM97 (Wellcome Department of Cognitive Neurology, http://www.fil.ion.ucl.ac.uk/spm). After discarding the initial eight scans (to allow for magnetic saturation effects), the time series of images was realigned, corrected for movement-related effects, and spatially normalized into the standard space of Talairach and Tournoux (1988) using each subject's coregistered structural T1 image. The data were smoothed spatially with a 6-mm isotropic Gaussian kernel and temporally with a 2.8-s Gaussian kernel to ensure stationality.

Statistical Analysis

Differences between conditions (words − rest) were estimated using the general linear model (i.e., multiple regression) and an event-related analysis in the context of statistical parametric mapping. The regressor of interest was constructed by taking a "stick function" representing each stimulus occurrence and convolving it with a synthetic hemodynamic response function (Fig. 1, top). Activations were assessed with linear contrasts. Global effects and low-frequency components up to a frequency of one cycle per 240 s were removed as confounds. Each study was analyzed independently and the effect of the word task was assessed for (i) each session independently, (ii) pooled over the ascending or descending sessions, and (iii) pooled over all four sessions. In regions of interest (left inferior frontal, left posterior temporoparietal), statistical inferences about the activation effects were made on the basis of the SPMs thresholded at $P = 0.001$ (uncorrected). In all other regions, the SPMs were corrected for multiple comparisons ($P < 0.05$) using the theory of random Gaussian fields.

RESULTS

Table 1 summarizes the areas of activation for each of the five studies that were common for both ascending and descending image acquisition. Remarkably consistent activations were detected for each study in occipital, parietal, prefrontal, and motor areas. The consistency was obtained in spite of the differences in TR and stimulus timing. In particular, despite there being twice the number of scans in the TR = 3.2 s study relative to the TR = 6.4 s studies, the TR = 3.2 s study did not yield more significant results. Empirically, it appears that above a certain level, the number of acquisitions ceases to be a critical factor.

TABLE 1

Regional Activation Associated with the Rhyming Task for Each of the Five Studies

	TR = 6.4 s (a)	TR = 4.1 s	TR = 3.2 s	TR = 5.1 s	TR = 6.4 s (b)
Occipital fusiform and lingual gyri	−22 −74, −14 (8.0) −46 −74 −18 (7.6) −4 −90 −12 (7.2)	−22, −74, −10 (9.0) −44 −72 −16 (8.7) −6 −80 2 (8.8)	−18 −76 −8 (8.4) −40 −76 −14 (7.6) −2 −78 4 (8.0)	−22 −76, −12 (8.6) −50 −76 −16 (8.0) −4 −84 8 (7.7)	−22 −76 −10 (7.8) −38 −74 −10 (7.4) 4, −80 −4 (5.6)
	30 −76, −16 (7.6) 46 −72 −16 (6.8) 6 −76 6 (7.1)	20, −74 −6 (8.8) 42 −70 −10 (8.5) 6 −66, 12 (8.9)	28 −74 −8 (8.7) 48 −70 −10 (7.7) 10 −66 10 (8.2)	20 −78, −8 (8.6) 42 −74, −10 (7.8) 4 −68 10 (8.4)	16 −72 −10 (7.6) 40 −72 −12 (4.4) 6 −66 8 (7.1)
Fusiform/basal temporal	−44, −62, −20 (6.5) 54, −66 −2 (5.6)	−46 −58 −18 (8.0) 48 −56 −16 (4.7)	−40 −64 −16 (7.4) 40 −54 −22 (5.6)	−50 −62 −14 (6.7) 44 −60 −10 (5.9)	−52, −62, −14 (6.8) 40 −72 −12 (4.4)
Parietal	−20 −78 36 (7.0) 26 −68, 42 (7.5) 28 −80 26 (4.9)	−24 −76 34 (8.5) 26 −70 44 (8.3) 30 −76 28 (8.1)	−18 −74 36 (7.9) 32 −62 44 (7.9) 36 −76 26 (6.9)	−24 −78 34 (8.1) 24 −70 44 (8.2) 30 −80 26 (7.8)	−24 −74 34 (7.1) 26 −68 42 (5.7)
Frontal	−44 6 34 (7.1) −58 4 14 (4.6) NS 38 2 38 (4.5)	−44 8 36 (8.4) −58 8 18 (8.1) −40 28, 18 (7.6) 42 8 34 (8.5)	−42 8 28 (7.3) −56 6, 14 (6.2) −40 28 10 (5.2) 40 10 38 (5.9)	−44 4 36 (8.1) −54 6, 16 (7.6) −40 30, 16 (5.9) 42 6 36 (7.3)	−44 8 32 (7.3) −60 6 20 (5.7) −40 28 16 (5.5) 72 6 32 (6.0)
Temporal	NS NS	−44 −64 0 (5.9) −62 −40 20 (7.1)	NS NS	−44, −64, 0 (6.5) −62 −42, 20 (6.1)	NS NS
Motor	−56 −6 38 (7.0) NS	−58, 0 40 (7.8) −58 −18 30 (6.0)	NS NS	−60 −4 38 (8.1) −60 −20 28 (6.0)	−58 0 40 (6.7) NS
Subcortical	NS	−18 −14 10 (5.3)	NS	−14 −20, 12 (5.0)	NS

Note. The coordinates for the anatomically described regions are according to the stereotaxic atlas of Talairach and Tournoux (1988) and reported in the order x (− is left, + is right), y (− is posterior to the anterior commissure line, + is anterior to the anterior commissure line), and z (− is inferior to the intercommisural line (AC–PC line), + is superior to the AC–PC line). NS, not significant at $P < 0.001$. The order of studies from left to right corresponds to the order in which they were performed in time.

It can be seen in Table 1, however, that the predicted activations in the left temporoparietal regions were found only in those studies that acquired data throughout the ISI (i.e., with TR = 4.1 and 5.4 s, respectively) (Fig. 3). To pursue this further, we report activations in these three regions for ascending and descending image acquisition separately (Table 2). Table 2 confirms that, in the two studies that acquired data throughout the ISI for every slice, activations in left temporoparietal regions occur with both scanning orders. In contrast, for the TR = 3.2 s study, activation in the left supramarginal gyrus was registered during ascending but not descending image acquisition and activation in the left posterior middle temporal cortex was captured during descending acquisition only. Likewise, for the TR = 6.4 s studies no activation was detected in either temporal region with the exception of the left posterior temporal cortex that was registered during ascending image acquisition for one session only. Finally, we did not find evidence of thalamic activation in any of the ascending or descending sessions in the three studies with a fixed relationship between stimulus presentation and data acquisition.

A formal analysis of differential activations in the five different sessions (i.e., session by task interactions) is not presented because the TRs were different, leading to differences in the serial (i.e., temporal) correlations among the sessions. These differences in the autocorrelation structure preclude the use of a single statistical model that subsumes all five studies.

The differences between distributed vs fixed sampling have been replicated, without exception, in individual analyses of eleven other subjects. In four subjects, the stimulus onset was evenly distributed across the ISI and activation was observed in all regions reported in Table 1 including the temporal and parietal word processing areas. In the other seven subjects, stimuli occurred at fixed points in the data acquisition interval and no activation in temporal and parietal regions was detected.

DISCUSSION

Our results show that for some regional responses, detecting activation depends critically on the point in the ISI that data are acquired. In occipital, parietal, frontal, and motor cortices, the activations observed across different TRs, scanning orders, and stimulus presentations were almost identical, but activations were far less consistent in the left temporal and inferior

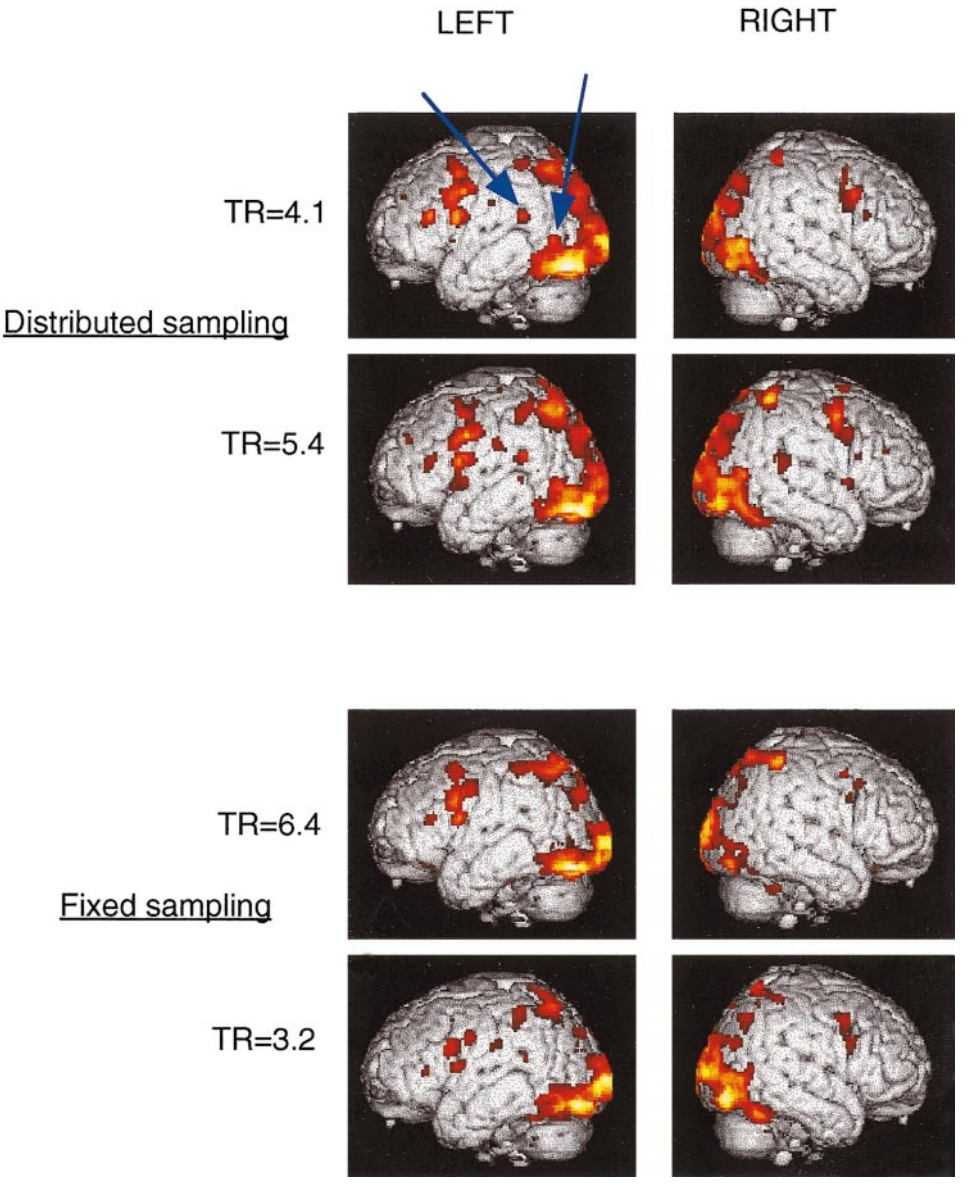

FIG. 3. Activation observed for the rhyming paradigm when there was distributed sampling throughout peristimulus time (TR = 4.1 s or TR = 5.4 s) and when the sampling was fixed to one or two points in the peristimulus time. The red and yellow areas highlighted on the left and right views of the brain indicate those regions that were activated above a threshold of $P < 0.001$ uncorrected. The blue arrows indicate the regions that were consistently detected only when sampling was distributed.

parietal word processing areas. In these areas, activation was only observed reliably, when data were acquired throughout the ISI, but not when sampling occurred at only one or two points, i.e., when the TR was the same as the ISI (3.2 s) or twice the ISI (6.4 s).

The finding that activation in the temporal and parietal word processing areas was only detected when data was acquired throughout the ISI indicates that a steady-state BOLD signal did not develop even when stimuli were repeated every 3 s. If a steady-state BOLD signal had developed then the timing of data acquisition would not have been important (as was the case in the visual, superior parietal, and frontal cortices). We are therefore inferring that the signals from the temporal and parietal word processing areas, during our particular paradigm, must have been transient. By acquiring data throughout the ISI we ensured that the peak of the signal, irrespective of which point in time it occurred, was detected in at least some scans. Obviously, detection of transient signals could be enhanced further if data acquisition during a blocked design coincided precisely with the peak of the signal during every scan. However, this approach is probably not feasible for a number of reasons. First, in multislice

TABLE 2

Regional Activation Dependent upon the Relationship between TR and ISI

		TR = 6.4 s (a)	TR = 4.1 s	TR = 3.2 s	TR = 5.1 s	TR = 6.4 s (b)
Midtemporal	A	NS	−44, −64, 0 (3.7)	NS	−42, −66, 2 (5.2)	−42, −62, −2 (4.3)
	D	NS	−44, −64, 0 (4.7)	−40, −66, 0 (6.9)	−48, −64, −4 (4.9)	NS
T-P	A	NS	−64, −40, 20 (6.3)	−54, −42, 20 (3.7)	−60, −42, 20 (4.2)	NS
	D	NS	−64, −42, 20 (4.6)	NS	−62, −42, 20 (4.6)	NS
Motor	A	NS	−56, −18, 30 (5.0)	−56, −18, 28 (5.4)	−60, −20, 30 (4.8)	NS
	D	NS	−58, −18, 30 (3.7)	NS	−60, −22, 28 (4.1)	NS
Subcortical	A	NS	−16, −14, 10 (3.9)	NS	−14, −22, 10 (3.7)	NS
	D	NS	−20, −14, 10 (4.5)	NS	−14, −20, 12 (3.8)	NS

Note. When there is distributed sampling of the ISI (i.e., when TR = 4.1 or 5.4 s), activation is detected in the left posterior middle temporal cortex (midtemporal), the left temporoparietal junction (T-P), the left precentral gyrus (motor), and the left thalamus (subcortical) irrespective of scanning order (A, ascending; D, descending). When data sampling is fixed to a particular point in the ISI, the observed activation is not consistently detected. NS, not significant at $P < 0.001$. For further details see legend to Table 1.

acquisition, it would not be possible to time data acquisition to coincide with the peak of activation in two anatomically segregated regions (e.g., the posterior temporal and inferior parietal regions in this study) which may each have there own distinct time course. A region of interest analysis would therefore be required focussing on detecting signals in one region at a time. Second, timing data acquisition to the point in the ISI where the signal was greatest would require *a priori* knowledge of the time course of activation in response to each stimulus. Furthermore, this might vary from subject to subject. Third, the peak is not necessarily the best metric of an activation; the area under the response curve represents another. In conclusion, in the context of blocked designs, detecting activation of transient as well as small more prolonged signals can be effectively achieved using the simple approach we used above, i.e., sequentially sampling all points in the ISI.

In terms of selecting the appropriate stimulus parameters, there is an equivalence between "when" one samples in the ISI and "where" in the brain the stimulus coincides with image acquisition. In the five studies reported here we wanted to keep the ISI constant across sessions. With multislice acquisition, this meant that a distributed sampling of the ISI was achieved by timing the presentation of the stimuli to coincide with the acquisition of data from successive slices. In other cases, it may be more appropriate to vary the ISI. A proper sampling includes as many points in the ISI as possible because biased sampling may occur even if a small number of time points in the ISI are repetitively sampled. For example, an ISI of 3 or 2 s and a TR of 4.5 s would only sample the stimulus at two or four points, respectively, in relation to its onset. This may not be sufficient for unbiased estimates.

Our findings have two implications. First, although the phenomenon of biased sampling may seem obvious, it is surprising how many study designs limit sampling to some fixed or regular relationship between the ISI and the TR. In some cases, this choice of design may be necessary. For example, with stimuli triggered by image acquisition, or in acoustic paradigms when stimulus presentation is constrained to the interscan interval to avoid interference from the noise of the scanner. In these experimental designs it is important to be aware that transient event-related effects may not be detected.

Second, it could be argued that the risk of incorrect inference due to biased estimates of activation is greater in single-session experiments relative to experiments when independent sessions are pooled from the same subject or from different subjects. This is because variability in acquisition parameters (e.g., different positioning in the scanner) will contribute to distributed sampling of the ISI over sessions. The mean response in a pooled analysis will therefore be a less biased estimate of the true response.

To spell this out, take the example of the position of the subject in the scanner. Even when care is taken to initiate the data acquisition sequence from the same point in each subject, there will be subtle intersubject differences in where in the brain the peak response is expressed and these differences will be magnified as the peristimulus time progresses because of variability in the size and shape of individual brains as well as differences between subjects in the time course of activation. When the activation is transient and data acquisition only occurs at one point in the ISI, a signal may be detected in one subject but not another. Pooling data from different subjects together should therefore introduce some variability in the peristimulus time that data is acquired. A preferable solution, however, is when data acquisition is distributed throughout the ISI. In this case, activation should be detected irrespective of the precise positioning of the subject, the timing of activation, or the the size and shape of the brain.

This issue is particularly important in relation to evaluating individual differences in functional anatomy. Individual variation between subject activation profiles may be exaggerated when the onset of stimulus and scan is fixed, not because of differences in functional anatomy, but because of differences in the position of the subject in the scanner, the size and shape of his/her brain, and the time course of activation. Differences in the position of the subject in the scanner will also influence studies where activation profiles from the same subject are contrasted from one day to another, e.g., monitoring the recovery of a cognitive process in a neurologically damaged patient. Hence, the differences detected may relate to factors other than those introduced by time (e.g., recovery) and these artifacts will be substantially reduced when care is taken to sample data throughout the ISI.

Apart from highlighting the importance of choosing a suitable TR/ISI ratio, our results demonstrate the utility of properly modeling event-related responses even in the context of blocked designs where events of one type are presented sequentially in trains. An event-related analysis models responses to individual stimuli without assuming constant within block responses, as in a conventional boxcar analysis. When there is a periodic variation in activation (i.e., when a steady-state hemodynamic signal does not develop), an event-related analysis is better suited for modeling the response and reducing the error variance. A related issue that we have not addressed above is that differences in the timing of the stimulus train relative to acquisition may introduce differences in sensitivity across the brain, not due to the bias discussed above, but because the stimulus function used to model the hemodynamic responses only fits in some parts of the brain but not others. This is a much less severe problem and is generally dealt with by including the temporal derivative or slope of the stimulus functions as an extra regressor or covariate in the analytical model.

In conclusion, we have described a fMRI language experiment in which activations in temporoparietal areas were consistently observed only when care was taken to acquire data throughout the ISI by varying stimulus onset with respect to slice acquisition. Our results suggest that the temporoparietal activations, in contrast to those in primary sensory and motor areas, are of a brief duration because their estimation appears to be a strong function of when in the ISI the responses are measured. A TR/ISI ratio that allows for sampling at as many different points in the ISI as possible will avoid missing similarly small and transient activations in any cognitive paradigm. Such a design also accommodates the use of an event-related analysis even when the stimuli are presented in blocks.

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