NeuroImage **11,** 735–759 (2000)
doi:10.1006/nimg.2000.0568, available online at http://www.idealibrary.com on IDEAL®

Characterizing the Hemodynamic Response: Effects of Presentation Rate, Sampling Procedure, and the Possibility of Ordering Brain Activity Based on Relative Timing

F. M. Miezin, L. Maccotta, J. M. Ollinger, S. E. Petersen, and R. L. Buckner

Department of Psychology, Department of Radiology, Department of Anatomy and Neurobiology, and Department of Neurology and Neurological Surgery, Washington University, St. Louis, Missouri 63130

Received October 1, 1999

Rapid-presentation event-related functional MRI (ER-fMRI) allows neuroimaging methods based on hemodynamics to employ behavioral task paradigms typical of cognitive settings. However, the sluggishness of the hemodynamic response and its variance provide constraints on how ER-fMRI can be applied. In a series of two studies, estimates of the hemodynamic response in or near the primary visual and motor cortices were compared across various paradigms and sampling procedures to determine the limits of ER-fMRI procedures and, more generally, to describe the behavior of the hemodynamic response. The temporal profile of the hemodynamic response was estimated across overlapping events by solving a set of linear equations within the general linear model. No assumptions about the shape were made in solving the equations. Following estimation of the temporal profile, the amplitude and timing were modeled using a γ function. Results indicated that (1) within a region, for a given subject, estimation of the hemodynamic response is extremely stable for both amplitude (r^2 = 0.98) and time to peak (r^2 = 0.95), from one series of measurements to the next, and slightly less stable for estimation of time to onset (r^2 = 0.60). (2) As the trial presentation rate changed (from those spaced 20 s apart to temporally overlapping trials), the hemodynamic response amplitude showed a small, but significant, decrease. Trial onsets spaced (on average) 5 s apart showed a 17–25% reduction in amplitude compared to those spaced 20 s apart. Power analysis indicated that the increased number of trials at fast rates outweighs this decrease in amplitude if statistically reliable response detection is the goal. (3) Knowledge of the amplitude and timing of the hemodynamic response in one region failed to predict those properties in another region, even for within-subject comparisons. (4) Across subjects, the amplitude of the response showed no significant correlation with timing of the response, for either time-to-onset or time-to-peak estimates. (5) The within-region stability of the response was sufficient to allow offsets in the timing of the response to be detected that were under a second, placing event-related fMRI methods in a position to answer questions about the change in *relative* timing between regions. © 2000 Academic Press

INTRODUCTION

Rapid-presentation event-related functional MRI (ER-fMRI) allows neuroimaging methods based on hemodynamics to employ behavioral task paradigms typical of cognitive settings (Dale and Buckner, 1997; Burock *et al.*, 1998; Clark *et al.*, 1998). Different trial types spaced a few seconds apart can be randomly intermixed and/or sorted post hoc based on subject performance (e.g., Buckner *et al.*, 1998a; Clark *et al.*, 1998; Wagner *et al.*, 1998). However, several limitations and methodological issues place constraints on how rapid-presentation ER-fMRI paradigm can be applied including: (1) how the hemodynamic response summates over separate neuronal events, (2) the variance associated with the hemodynamic response, (3) how the response is sampled in relation to trial presentation, and (4) the analytic procedure by which the hemodynamic response is extracted. In the following article, a review of these issues is followed by presentation of two new empirical studies. The two studies provide constraints on the design, analysis, and interpretation of rapid-presentation ER-fMRI. Results suggest that robust estimates of the hemodynamic response can be obtained for stimulus trials spaced as closely as 2.5 s apart (mean spacing = 5 s). These responses are roughly comparable to responses to trials spaced widely apart. Furthermore, the timing and shape of the hemodynamic response can be estimated with accuracy sufficient to indicate temporal offsets within a region of less than 1 s, even for procedures using whole-brain imaging with a repetition time (TR) of greater than 2.5 s.

1053-8119/00 $35.00

Hemodynamic Response Summation

The robust positive component of the blood-oxygenation level-dependent (BOLD) hemodynamic response evolves over a 10- to 12-s period even for brief stimulus events of a few seconds or less (Blamire *et al.*, 1992; Bandettini, 1993; Savoy *et al.*, 1995; Boynton *et al.*, 1996; Buckner *et al.*, 1996; Konishi *et al.*, 1996). Subtle residual components may persist for up to a minute (Fransson *et al.*, 1998a, 1999). On first appearance, these findings would seem to negate the possibility of separating rapidly presented events. However, the actual situation provides more hope than this first impression. While the temporally extended nature of the hemodynamic response presents challenges to data analysis, rapidly presented trials that overlap in time can be separated. The basis of separation is the finding that sequential (or continuous) events summate in a *roughly* linear fashion (Boynton *et al.*, 1996; Dale and Buckner, 1997). That is, the effect of a neuronal event will be to further increase the existing hemodynamic responses even if the hemodynamic responses from preceding events have not completely decayed. This summation occurs in a nearly linear fashion under many circumstances: as a new distinct event is presented, the BOLD response increases by a *similar* amount regardless of the prior history of events. Demonstrating an extreme application of this principle, rapid presentation of sequential events separated by 750 ms has been shown to be sufficient for producing activation maps (Burock *et al.*, 1998). However, subtle nonlinearities in the summation of the hemodynamic response have also been noted and present potential challenges to paradigm design and interpretation of data.

Boynton *et al.* (1996), in their seminal work describing the roughly linear summation of the hemodynamic response to stimuli of varied duration, noted that the shortest stimulus durations overestimated the response to longer durations—a form of nonlinear summation. Their interpretation was that adaptation occurred for the longer sustained stimuli. A similar form of nonlinearity as a function of visual stimulus duration has also been noted by Vazquez and Noll (1998) and also by Robson *et al.* (1998) in response to auditory stimuli. Dale and Buckner (1997) showed that the hemodynamic responses to temporally separated stimulus events summate in a nearly linear fashion, but subsequent events show a steeper decay and undershoot of the baseline compared to isolated events. This latter paradigm presumably was not influenced by adaptation when between-event summation was considered, suggesting that subtle nonlinearities exist in a range of paradigm types.

The aforementioned studies suggest that caution should be exhibited in assuming a precisely linear model of hemodynamic response summation. Nonetheless, the major component of the BOLD response does appear to summate in a nearly linear fashion, and the nonlinear components appear to be either subtle or exhibited under extreme paradigm situations with very high presentation rates (e.g., Friston *et al.*, 1997). These findings collectively suggest that estimates of responses in the context of rapidly presented trial events should be similar, but not necessarily identical to, estimates obtained in the context of trials widely spaced in time. A goal of the present series of studies was to address whether different estimates of the hemodynamic response would be obtained at different trial presentation rates, at which varied degrees of hemodynamic response overlap occur.

Variance of the Hemodynamic Response

The hemodynamic response has been shown to vary in timing, amplitude, and shape across brain regions and cognitive task paradigms, and variance across subjects has been observed for nominally similar regions and tasks (Lee *et al.*, 1995; Buckner *et al.*, 1996, 1998b; Kim *et al.*, 1997; Schacter *et al.*, 1997; Aguirre *et al.*, 1998; Robson *et al.*, 1998; D'Esposito *et al.*, 1999). Such variation is expected given that estimation of the hemodynamic response occurs in a real-world system that has many sources of measurement and biological noise. Several possible sources of variation can be considered including (1) variation across data sets within an individual for a given region, (2) variation across individuals for the same region, and (3) variation across regions. The latter two sources may be particularly relevant in between-group comparisons involving abnormal populations in which there is evidence for baseline differences in hemodynamic response properties (Ross *et al.*, 1997; Taoka *et al.*, 1998). We consider each of the three possible sources of variation separately.

Within an individual subject, for a fixed region, the hemodynamic response is highly reproducible in timing, shape, and amplitude within the same experimental session (if many events are considered). Given two separate sets of data from the same subject, Dale and Buckner (1997) showed that the hemodynamic response was nearly identical across separate measurements within visual cortex. Any individual event, of course, can show considerable variation much in the same manner in which a behavioral estimate of performance may vary from one trial to the next (Kim *et al.*, 1997). The extremely high level of stability within a subject for a given region's response places event-related fMRI in a strong position to contrast within-subject conditions, even those that require detection of timing offsets that are as brief as a few hundred milliseconds (e.g., Savoy *et al.*, 1995; Menon *et al.*, 1998).

When one moves beyond this reliability for a given region within a subject, the situation becomes more

complicated. Across subjects, for nominally the same region, the hemodynamic response varies on the order of a few seconds (in timing range) and the amplitude can be doubled from one subject to the next (Kim *et al.*, 1997; Buckner *et al.*, 1998b). Across patient populations, there is some evidence of systematic differences in the timing of the response (Taoka *et al.*, 1998). However, when averaged groups of subjects from the same population are considered, the central tendency of the hemodynamic response for a given region is such that—even for a relatively small group of subjects ($N \sim 6$)—the mean amplitude and time to onset of the response can be specified and reproduced in a separate group of subjects to within tenths of a percent and fractions of a second (Buckner *et al.*, 1998b). Thus, while random variation exists across subjects, and systematic variation may further exist across different populations, strong central tendencies are present that allow for meaningful averaging of the hemodynamic response across subjects and for modal properties to be characterized.

Finally, separate regions of cortex can exhibit widely disparate hemodynamic response shapes and amplitudes even within individual subjects (Lee *et al.*, 1995; Buckner *et al.*, 1996, 1998b; Schacter *et al.*, 1997; Robson *et al.*, 1998; Bandettini, 1999). Two levels of regional analysis have been examined. At the most local level, adjacent or nearby voxels have been shown to vary widely in their timing onset (up to 2 s) and amplitude (1–5% in range). Because these differences seem highly unlikely to be due to neuronal activity (Lee *et al.*, 1995; Robson *et al.*, 1998; Bandettini, 1999), the results suggest separate influences of micro- and macrovasculature. At the level of larger regions, encompassing 500–1000 cc, variations in amplitude and timing that may reflect differential vascular sampling or perhaps differences in neural activity across regions have also been noted (Schacter *et al.*, 1997; Buckner *et al.*, 1998b). The presence of regional variation presents a serious challenge to using ER-fMRI as a means of resolving the temporal cascade of neural activity across the cortex. It seems possible, if not likely, that across certain regions the absolute hemodynamic response lags will go in a direction opposite to the underlying neuronal activity, making possible a serious interpretational error.

Another goal of the present series of studies was to explore the variation of response magnitude and timing within and between regions. Two separate issues are considered. The first is the variation and predictability across regions. We ask this question: If one knows the properties of the hemodynamic response in one region (e.g., the amplitude), can the behavior of another spatially separate region be predicted? In other words, are there global response properties in an individual subject that span regions? The second issue relates to the stability of the hemodynamic response within a region for a given subject, revisiting a question addressed by Menon *et al.* (1998; see also Savoy *et al.*, 1995; Rosen *et al.*, 1998; Dymond *et al.*, 1999; Bandettini, 1999). Namely, can the stability of the response within a region be used to detect temporal offsets of fractions of a second? These two issues are explored in concert to ask if ER-fMRI is capable of resolving *relative* offsets in timing across separate regions of cortex (see also Friston *et al.*, 1998).

Hemodynamic Response Sampling

A potentially tricky issue surrounding estimation of the hemodynamic response is how the response is sampled. In many studies, there is a fixed relation between the image acquisition and the presentation of a trial event resulting in discrete sampling. If the TR is 3 s, the hemodynamic response will be sampled with a resolution of 3 s. It is easy to imagine that certain components of the response, such as the peak, will be missed and the response estimated inaccurately. A clever alternative has been offered by Josephs *et al.* (1997), whereby the relation between the image acquisition and the trial presentation is systematically varied so that, across trials, numerous time points along the response are sampled. In many situations in which behavioral and analytical methods can handle the added dimension of a varied sampling time, this approach provides a useful means of oversampling the hemodynamic response in time. In situations in which a fixed relation is desired between the image acquisition and the hemodynamic response, there remains open the question of how much statistical power is lost and the degree to which parameter estimates of the response (e.g., the amplitude) are misestimated. Analysis of blocked-design fMRI data suggests that errors may be significant (Price *et al.*, 1999). A third goal of the present studies was to compare two effective rates of sampling, in which the relation between the image acquisition and the trial presentation is either held constant or systematically varied.

Hemodynamic Response Estimation

An issue related to sampling is the method by which the estimate of a hemodynamic response is derived. In the most extreme case, in which long intertrial intervals are present (>16 s apart), the estimate is the mean signal change over time for an activated region, for which the mean is found by simply averaging across trials. The assumption is that the robust positive deflection from one trial has decayed before the occurrence of another event (Blamire *et al.*, 1992; Boynton *et al.*, 1996; Buckner *et al.*, 1996; Konishi *et al.*, 1996; McCarthy *et al.*, 1997; Bandettini, 1999; see also Fransson *et al.*, 1998 for discussion of potentially longer components of the hemodynamic response).

As multiple trials in a sequence are moved closer together in time, the underlying physiology and appropriate analysis become more complicated. The evoked hemodynamic response to each trial may overlap with the next, yielding a complex waveform that represents the accumulated hemodynamic response to many events. A secondary procedure to solve for the estimated contribution of trial events becomes necessary. Several approaches to this problem have been effectively applied, including a straightforward, linear method based on subtracting away prior and future histories of overlapping trials (Dale and Buckner, 1997) and modeling the temporally overlapping responses based on multiple regression within the general linear model (Clark *et al.*, 1998). Glover and colleagues (1999) have recently suggested a promising method for response estimation based on linear deconvolution.

Most directly relevant to the present article, the approach of Clark *et al.* (1998), which shares similarities to the approach applied by Josephs *et al.* (1997), potentially allows for an estimate of the hemodynamic response to each kind of trial type within a rapidly presented, randomly intermixed series. Critically, the estimate could be obtained with no assumption about the specific shape of the response other than the assumption that responses, whatever their temporal profile, summate in a linear fashion. To the degree that they do linearly summate, the same response estimate should be obtained whether the trial events are isolated and widely separated in time or whether they are rapidly presented, yielding overlapping hemodynamic responses. A fourth goal of the present studies was to estimate the hemodynamic response for rapidly presented trials using the general linear model while making no assumptions about the specific shape of the response.

The final issue addressed in the present paper is how the hemodynamic response is described once its temporal evolution is estimated. That is, how does one answer the question of what the magnitude or timing of the response is? This issue is important for using fMRI-based measurements as quantitative (in terms of percentage MR signal intensity) rather than just qualitative measurements. Questions are beginning to be asked that require comparison of response levels within a region across conditions or populations, rather than simply the presence or absence of a response. Currently, while it is often difficult to equate a meaningful biological unit of measure to the level of the BOLD-contrast response, one can nonetheless describe the response in terms of percentage signal change. To the degree this estimate is reliable and valid, the relative amplitude of the hemodynamic response can be compared across conditions and across different subject populations.

However, there are tradeoffs in adopting a descriptive estimate (or series of descriptive estimates) of the hemodynamic response. For one, the temporal evolution of the response is reduced to a set of values that may lose components of the response that are informative. Furthermore, the estimate of the response is, in practice, dependent on a model of the shape of the hemodynamic response, which may only partially accommodate the structure of the real response and may also have nonlinear components.

A final goal of the present studies was to employ a model estimate of the hemodynamic response based on a γ function (Boynton *et al.*, 1996) to determine how stable quantitative estimates of the hemodynamic response are in terms of estimates of the peak percentage signal change (*amplitude*), the time lag to response onset (*time to onset*), and the time lag to response peak (*time to peak*). These quantities are offered as possible measurements that can be used to make comparisons across conditions, studies, patient populations, and laboratories to assess relative hemodynamic signal change.

METHODS

Overview

The present series of studies provides an empirical characterization of the hemodynamic response across paradigms that vary the intertrial interval (experiment 1) and sampling procedure (experiment 2). Two features of the paradigms employed allowed questions about within- and between-region variation and the limits of within-region response characterization to be answered. First, both studies used a paradigm involving sensory (visual) and motor response demands. Subjects viewed an 8-Hz large-field flickering checkerboard for approximately 1.5 s and pressed a key upon stimulus onset (experiment 1) or upon its onset and offset (experiment 2). In this manner, separate primary sensory and motor responses could be compared for each subject, allowing analysis of how response properties in one region predict properties in another region. For the motor variant in experiment 2, subjects pressed a key with one hand when the stimulus began (Onset) and with the other hand when the stimulus ended (Offset), allowing us to ask whether event-related fMRI can detect the temporal separation of sequential responses in motor cortex that would occur about 1 s apart. For both experiments, whole-brain imaging using a 1.5-T scanner was employed with a TR of about 2.5 s. General methods pertaining to both studies are discussed first, followed by specific descriptions of the two separate experiments.

Subjects

Eighteen subjects were recruited from the Washington University community in return for payment.

Eight subjects served in experiment 1 (3 males, mean age 22.3, range 18–26 years) and 10 in experiment 2 (4 males, mean age 23.1, range 20–27 years). All had normal or corrected-to-normal vision and reported no history of significant neurological problems. Subjects provided informed consent in accordance with the guidelines set by the Washington University Human Studies Committee.

Imaging Procedures

Scans were conducted on a Siemens 1.5-T Vision System (Erlangen, Germany) with a standard circularly polarized head coil. A pillow and thermoplastic face mask were used to minimize head movement. Headphones dampened scanner noise and enabled communication with subjects.

Structural images were acquired using a high-resolution (1.25 × 1 × 1 mm) sagittal MP-RAGE T1-weighted sequence (TR = 9.7 ms, TE = 4 ms, flip angle 12°, TI = 300 ms, TD = 0 ms). Functional images were collected with an asymmetric spin-echo-planar sequence sensitive to BOLD contrast [volume TR = 2.50 s (experiment 1) or 2.68 s (experiment 2), 3.75 × 3.75-mm in-plane resolution; T2* evolution time 50 ms, $\alpha = 90°$]. In each functional run, 128 sets of 16 contiguous, 8-mm-thick axial images were acquired parallel to the anterior–posterior commissure plane; this procedure offered whole-brain coverage at a high signal-to-noise ratio (Conturo *et al.*, 1996). Thus, each cortical region was sampled 128 times per run, with 1 sample occurring every 2.50 s (experiment 1) or 2.68 s (experiment 2). Throughout the paper, we refer to each 16-slice set of images covering the whole brain as an "image acquisition" and its particular position in time as a "time point." The first 4 image acquisitions in each run were discarded to allow stabilization of longitudinal magnetization. Each run lasted approximately $5\frac{1}{2}$ min. A 2-min delay existed between runs, during which time subjects were permitted to rest.

Behavioral Procedures

A Power Macintosh computer (Apple, Cupertino, CA) controlled by PsyScope software (Cohen *et al.*, 1993) displayed the visual stimuli. Subjects responded by pushing a fiber-optic light-sensitive key press connected to a PsyScope button box (Carnegie Mellon University, Pittsburgh, PA). Stimuli were rear projected (AmPro Model LCD-150) onto a screen placed at the back of the magnet bore. Subjects viewed the screen via a mirror fastened to the head coil.

For both experiments the basic stimulus was a large-field 8-Hz counterphase flickering checkerboard (black to white) subtending approximately 12° of visual angle (6° into each visual field). The Onset of the stimulus was triggered by the scanner in relation to the beginning of the image acquisition via the PsyScope button box. Spatial frequency of the reversing checkerboards within the display decreased with visual angle to be approximately constant in relation to acuity across the visual field. The duration of the stimulus was approximately 1.5 s. Measuring the duration of the stimulus from the monitor revealed that the actual presented duration varied by as much as 166 ms on a given trial, with most trials (~70%) being within 100 ms (mean 1.53 s). For this reason we consider the timing approximately 1.5 s. Calculation of reaction times to the Onset or Offset of the stimulus are in relation to the presentation duration of each trial.

Estimate of the Hemodynamic Response

For each functional MRI run, data were first preprocessed to remove several sources of noise and artifact. All functional image runs were corrected for odd/even slice-intensity differences and motion artifact using a rigid-body rotation and translation correction (Snyder, 1996). Data were then analyzed within the general linear model to estimate effects of stimulus presentation. Each stimulus event was assumed to produce a response lasting 7 time points (~18-s response epoch) after the start of the stimulus. No assumptions were made about the shape of the response at this stage of analysis. Additionally, a mean and a linear drift component for each run were included in the general linear model. In this manner, estimates of the hemodynamic response were made for trial events spaced a few seconds apart (Dale and Buckner, 1997; Burock *et al.*, 1998; Clark *et al.*, 1998). The possibility of separating the BOLD responses to closely spaced stimuli can be counterintuitive and is influenced significantly by exactly how the trials are temporally spaced in relation to one another (Burock *et al.*, 1998; Buckner and Braver, 1999). For this reason, a more detailed account of the analysis is provided.

The solution to estimating responses in a continual series of trials can be thought of in terms of a set of linear equations. A set of linear equations can be uniquely solved if there are as many equations as there are unknowns. At each time point in the MRI time series, the measured value is equal to the sum of BOLD responses plus noise, i.e., the measured value is a linear equation of the BOLD responses. Figure 1 illustrates this point. These equations are repetitive for uniformly (fixed) spaced trials, such as when trials are presented every $7\frac{1}{2}$ s (Fig. 1 left). In this example, there are a total of three independent equations for the seven unknown time points in the BOLD response. If the interval is randomized (jittered) between stimuli as in Fig. 1 (right), the number of unique equations more than doubles. The underlying BOLD response can then be uniquely estimated. In situations in which there is a range of varied intervals between stimuli, the situation further improves.

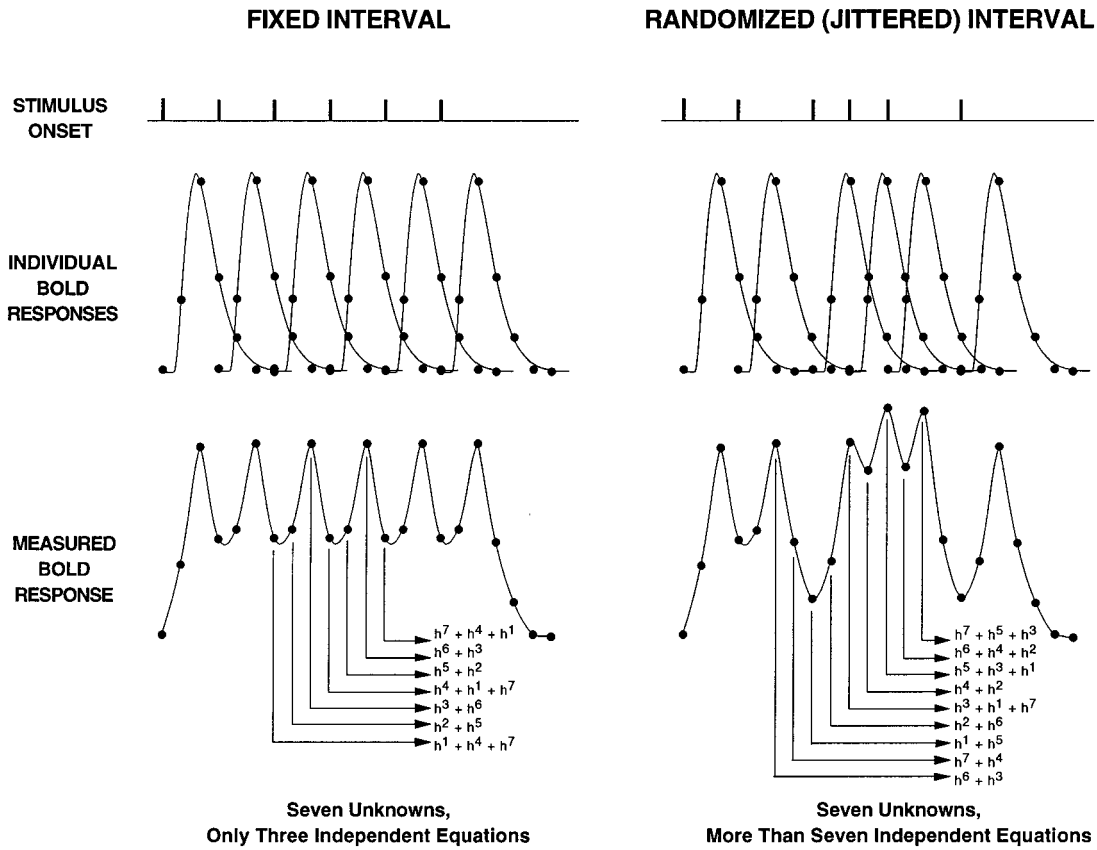


FIG. 1. The use of linear equations to estimate the hemodynamic response for overlapping events is illustrated. The estimated time points for the BOLD response are annotated as h^1–h^7. The first line (labeled Stimulus Onset) plots the relative positions for trials in two separate situations: for when the interval between stimulus events is fixed (Fixed Interval; left column) and for when the interval between stimulus events is jittered in time [Randomized (Jittered) Interval; right column]. Each stimulus has its own signal change as shown by the rows labeled Individual BOLD Responses. The Measured BOLD Response will be a complex waveform that represents the summation over individual BOLD responses. Analytically, when a fixed interval between trials is used (left), a constant MRI signal waveform results, yielding equations that confound the signal contribution by each event type; fewer equations exist than unknowns. The independent signal contributions for each event cannot therefore be estimated. However, by varying the interval around the events (right) a modulating waveform is produced that can be solved to estimate the separate contributions of the events, to the degree the separate events summate linearly. Linear estimation based on the general linear model was used as the basis of response estimation in the present paper.

Thus, in the presence of noise, it is desirable to have considerably more equations than unknowns, so it is desirable to jitter the stimuli by several unique values either explicitly (Buckner and Koutstaal, 1998) or by placing gaps interspersed randomly throughout the run (Buckner *et al.*, 1998a; Wagner *et al.*, 1998). In both experiment 1 and experiment 2, we implemented this procedure using gaps (fixation trials) that were randomly interspersed with equal probability with the trial events of interest (the visual checkerboards). The ordering of trials and gaps was random. On each trial, there was a 50% probability of a visual stimulus and 50% probability of a gap. Sequential ordering was also ensured so that gaps and trials followed each other equally often (Buckner *et al.*, 1998a). The resulting distribution of gaps followed a near exponential distribution with long gaps underrepresented because of the sequential ordering constraint. In this manner, a high degree of temporal jitter between stimulus trials was achieved, providing a robust paradigm for analysis within the general linear model.

A further important aspect of our methods was to use an "interleaved" procedure to increase the effective sampling rate of the hemodynamic response. Extending from Josephs *et al.* (1997), the stimulus Onset was varied in relation to the timing of the image acquisition. On every other run the stimulus was presented either at the beginning of image acquisition or 1.25 s after the onset of image acquisition. The response estimates from the two runs were interleaved to sample the averaged hemodynamic response across trials at a higher temporal resolution than achieved with either run alone. Specifically, each interleaved run pair was constructed to yield a hemodynamic response estimate that effectively sampled the hemodynamic response at twice the TR (approximately one sample every 1.25 s in

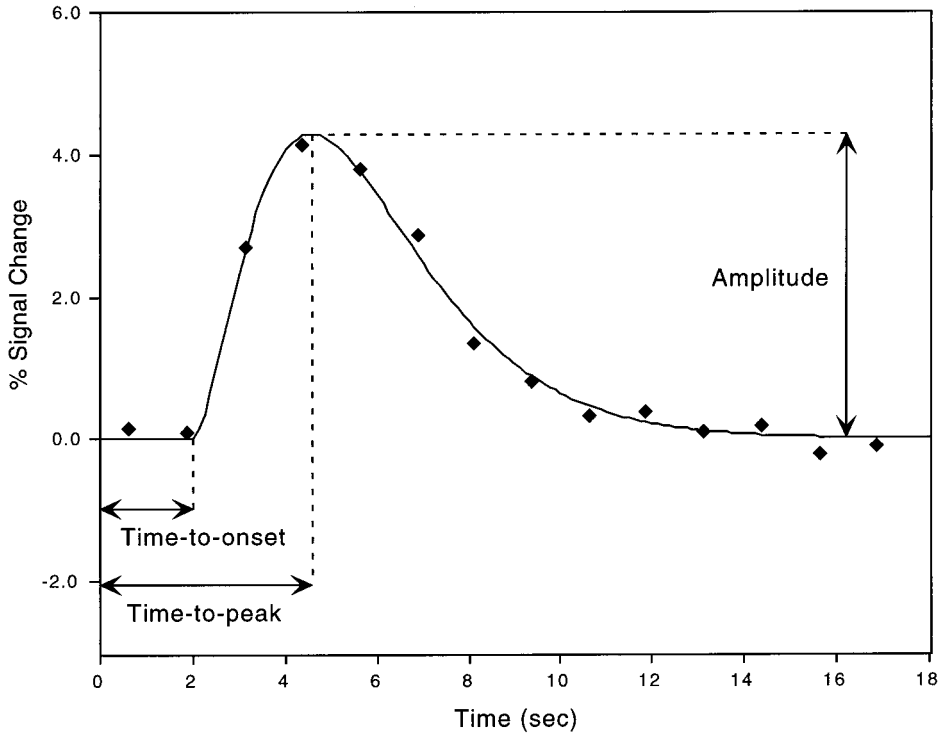


FIG. 2. Illustrated are the three estimated parameters obtained based on the least mean square γ function fit. The hemodynamic response profile for a single region estimated from the general linear model is plotted as filled diamonds. The model fit is superimposed as a solid line. Amplitude, time to onset, and time to peak are estimated, as shown, based on the model fit. This model estimate can be made based on individual subject data or based on all estimates plotted across subjects.

experiment 1 and one sample every 1.34 s in experiment 2). This procedure is a variant of the procedure proposed by Josephs *et al.* (1997) in which the presentation of the trial occurs at multiple, systematically offset time points in relation to the image acquisition. In our procedure, the effective sampling rate is doubled and the run pairs can be systematically combined to estimate the effect of sampling procedure (see description of experiment 2).

In instances in which an interleaved procedure was employed, the two kinds of events (those occurring at the beginning of the image acquisition and those occurring near the middle of the image acquisition) were incorporated into the linear model as separate factors. These factors were recombined following linear response estimation to yield a continuous 14-time-point estimate of the hemodynamic response (all 14 time points still being comprised within an ~18-s epoch). Importantly, estimation of the responses made no assumptions about the specific shape of the response.

The procedures described thus far result in an estimate of the temporally evolving response magnitude for each time point related to the onset of the stimulus. The full estimate could be used in its entirety for certain forms of data analysis procedures (e.g., exploration of an effect of time; Cohen *et al.*, 1997). However, as a simplified description of the response we adopted an additional modeling procedure by which we assume a general shape of the response and then estimate the peak amplitude, time to onset, and time to peak of the response based on this assumed shape. A three-parameter γ function was used as the generalized model (Boynton *et al.*, 1996) with an additional parameter specifying the time delay to response onset (Dale and Buckner, 1997; Schacter *et al.*, 1997; Buckner *et al.*, 1998a). Nonlinear least-squares fitting of the fMRI response using the Levenberg–Marquardt method (Press *et al.*, 1992) provided estimates of the model parameters (see Fig. 2).

Statistical Map Generation

To construct statistical maps, the estimated time curves were cross-correlated with a lagged γ function (Boynton *et al.*, 1996; Dale and Buckner, 1997). *Z*-statistical maps were then computed based on this cross-correlation.

Regional Analyses

Regions were defined on multiple functionally distinct areas across the brain. Two separate regions were defined for experiment 1 (visual cortex and left motor cortex) and three separate regions for experiment 2 (visual cortex, right motor cortex, and left motor cortex). Regions were selected in each subject based on

those voxels most active within the general region of primary visual cortex and primary motor cortex. For the visual region, a threshold value that isolated the region to the medial wall of each hemisphere including striate cortex was selected for each subject. The threshold Z ranged from 7 to 10 except for one subject for whom a threshold of $Z = 19$ was used. These values produced region sizes varying from 10 to 58 voxels across subjects (mean 37.4 voxels). For motor cortex, the threshold Z ranged from 5.6 to 10, producing region sizes varying from 4 to 23 voxels (right motor cortex mean 9.0 voxels, left motor cortex mean 7.3 voxels).

Two additional features were considered in constructing regions. First, regions were confined to a single slice and the time of acquisition of that slice was precisely specified based on when, in the slice acquisition order, the slice was acquired. Such a procedure eliminated artifacts associated with acquisition timing which vary from slice to slice for planar scanning. Estimates derived from the general linear model were time shifted to the precise timing relative to the onset of the visual stimulus of each selected slice for each subject. Second, so as not to bias definition of the region in relation to any of the comparisons of interest, the regions were defined using all conditions (runs) from a given subject. This pooling produced equal contributions from each condition and allowed for significant power in defining the regions. Subsequent effects between conditions would reflect genuine differences and not artifacts of the regional selection method.

Time course estimates were obtained for each region by extracting the mean signal intensity for each time point within the ~18-s epoch.

Experiment 1: Effect of Trial Presentation Rate

Experiment 1 manipulated the mean intertrial interval (ITI) to determine the comparability of hemodynamic response estimates at different rates of trial presentation. The basic trial consisted of a 1.5-s 8-Hz flickering checkerboard. Subjects were instructed to press a key, with their right hand, each time the checkerboard appeared. In this manner, hemodynamic responses in visual and motor cortex would be elicited and would provide a basis for estimation and comparison of the hemodynamic response in two separate brain regions.

During each of eight functional runs (four sets of "interleaved run pairs"), one of four mean trial rates was employed as described in Table 1. The trial rate was varied between a mean rate of one trial every 5 s (minimum 2.5 s) to one trial every 20 s. For the three fastest rates, the spacing between individual trials was jittered in time to allow linear estimation of the hemodynamic response as previously described by Dale and Buckner (1997) and Burock *et al.* (1998) and applied in Buckner *et al.* (1998a) and Wagner *et al.* (1998). From the subject's perspective, this kind of trial spacing appears as one continuous task block in which the onsets of trials are unpredictable. For the most widely spaced trials, the trials were presented at a fixed interval of 20 s apart. Responses during these widely spaced trials served as the "gold standard" and were assumed to be essentially unaffected by response overlap. At the end of the eight functional runs, which were counterbalanced for order of presentation rate across subjects, a final set of widely spaced (20 s) trial runs was conducted. This last set was included for two reasons. The first reason was to boost the number of trials contributing to the gold standard estimate of the response. The second was to explore the effect of run order since the final set of widely spaced runs could be compared to the identical set of runs occurring earlier in the session. For analyses in which effect of rate was the focus, only the counterbalanced interleaved run pairs from the first eight runs were considered. Thus, effects of rate were explored in the context of fully counterbalanced data.

TABLE 1

Trial Presentation Rates and Numbers of Trials for Experiment 1

Cond	Trial spacing	Min interval	Mean interval	No. of trials
TR1	Randomized	2.5 s	5.0 s	120
TR2	Randomized	5.0 s	10.0 s	60
TR3	Randomized	7.5 s	15.0 s	40
TR8	Fixed	20.0 s	20.0 s	30
TR8[a]	Fixed	20.0 s	20.0 s	30

Note. Cond is the condition name based on the minimum number of TR intervals that separate presentation of unique trials (each TR = 2.5 s). No. of trials is the number of trials per subject which were divided across two separate functional runs that constitute an "interleaved" run pair. For analysis of effects of rate, only the initial four counterbalanced conditions were used.

[a] The last TR8 condition (Cond) always occurred in the last two run positions, whereas the order of all other conditions was counterbalanced across subjects.

Experiment 2: Effect of Sampling Rate and Estimating Brief Temporal Offsets

Experiment 2 held constant the mean ITI at a rate near the fastest rate considered in experiment 1 (5.36 s mean, minimum 2.68 s). The basic trial was a slightly modified variant of experiment 1. Subjects were again presented with a 1.5-s 8-Hz flickering checkerboard on each trial. However, subjects now made two separate motor responses. With one hand, they pressed a key when the checkerboard appeared (Onset). With the other hand, they pressed a second key when the checkerboard disappeared (Offset). Across four runs (two interleaved run pairs), subjects alternated which hand (right or left) was used to indicate Onset or Offset. In this manner, comparing trials across runs, the same

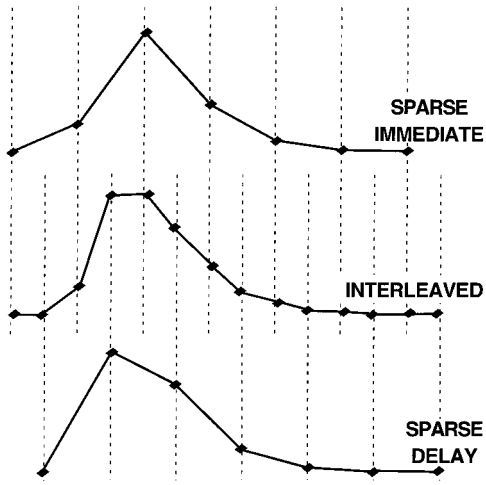


FIG. 3. The interleaved procedure for data acquisition is illustrated. The runs differ as to when the stimulus occurs in relation to the image acquisition (represented by vertical broken lines), either occurring at the onset of the image acquisition (referred to as the Immediate condition; top) or delayed by 1.25 s from the onset (referred to as the Delay condition; bottom). By obtaining separate runs of each of the Immediate and Delay acquisitions, the two runs can be combined (interleaved) to yield a temporal sampling rate twice that of either run alone (middle).

motor region could be examined for a response to the Onset of the checkerboard and separately to the Offset of the checkerboard. The two motor responses would occur approximately 1 s apart. Note that this is not the same as comparing responses *across motor regions* (which was also possible). In the present comparison, the motor region was held constant and the response condition (right hand, then left hand, or left hand, then right hand) varied across runs. Moreover, for each subject, two separate estimates of the Onset and Offset responses could be made (one for left motor cortex and one for right motor cortex) enabling generalization of any within-region findings.

A further modification of experiment 2 was that two separate interleaved run pairs at the same fast rate were acquired (four runs total). Having two run pairs allowed the data to be combined in two separate manners (Fig. 3). Each interleaved pair could be combined to provide an estimate of the hemodynamic response in which the effective sampling rate is twice that of the TR, such as was done for experiment 1. In addition, by combining the runs *across* interleaved pairs, the hemodynamic response could also be estimated for the more conventional case in which the stimulus presentation was fixed in relation to the image acquisition. Moreover, this estimate could be obtained when the fixed relation involved a trial occurring at the onset of the image acquisition (Immediate) or delayed 1.25 s from the onset (Delay). Thus, by combining the runs in two separate manners, hemodynamic response estimates could be obtained for the same amount of data for which: (1) an interleaved sampling procedure was used, providing an effective sampling rate of 1.34 s, and (2) a conventional Sparse sampling procedure (fixed relation between image acquisition and trial presentation) was used yielding a sampling rate of 2.68 s. In this manner, the effect of sampling procedure could be determined. As a gold standard for comparison, the combined data from all four runs were used.

Finally, because the interleaved run pairs were repeated in each subject, the test–retest reliability of hemodynamic response estimates could be explored. That is, one interleaved run pair could be used to determine hemodynamic response estimates and the second interleaved run pair could be used to assess whether those estimates could be reproduced.

RESULTS

Trial Presentation Rate Modestly Affects Response Estimation for Visual Cortex and Motor Cortex

Analyses were conducted on the data from experiment 1 to characterize properties of the hemodynamic response as a function of trial presentation rate. First, several analyses were conducted to check the validity of the procedures. In order to check the validity of the methods for characterizing the hemodynamic response, the fitted γ function was plotted on top of each subject's visual and motor cortex data based on the gold standard responses from the widely spaced trials (all four widely spaced trial runs were included). Qualitatively, the model fit predicted the empirically derived visual cortex data (Fig. 4) yielding a mean estimate of amplitude of 2.30%, a mean estimate of time to onset of 1.82 s, and a mean estimate of time to peak of 4.88 s. Motor cortex data were also modeled well by a γ function, yielding mean estimates of amplitude of 1.93%, of time to onset of 2.54 s, and of time to peak of 4.44 s. It should be noted that although the assumed γ function model described the response for most types of trials well, certain components appeared to be missed such as the presence of a poststimulus undershoot, rendering the fits less optimal than might be achieved with an increased number of model parameters or with a different model function (Fig. 4).

Furthermore, the amplitude, time-to-onset, and time-to-peak estimates in visual cortex did not show an effect of run order as determined by comparing early (beginning of session) and late (end of session) widely spaced trial runs ($P > 0.15$). Qualitatively, the three estimates were quite similar (amplitude 2.35% for early runs and 2.27% for late runs, time to onset 1.68 s for early runs and 1.85 s for late runs, time to peak 4.91 s for early runs and 4.85 s for late runs). Similarly, no effect of run order was observed in time-to-onset and time-to-peak estimates for the motor cortex data (time to onset 2.53 s for early runs and 2.54 s for late runs,

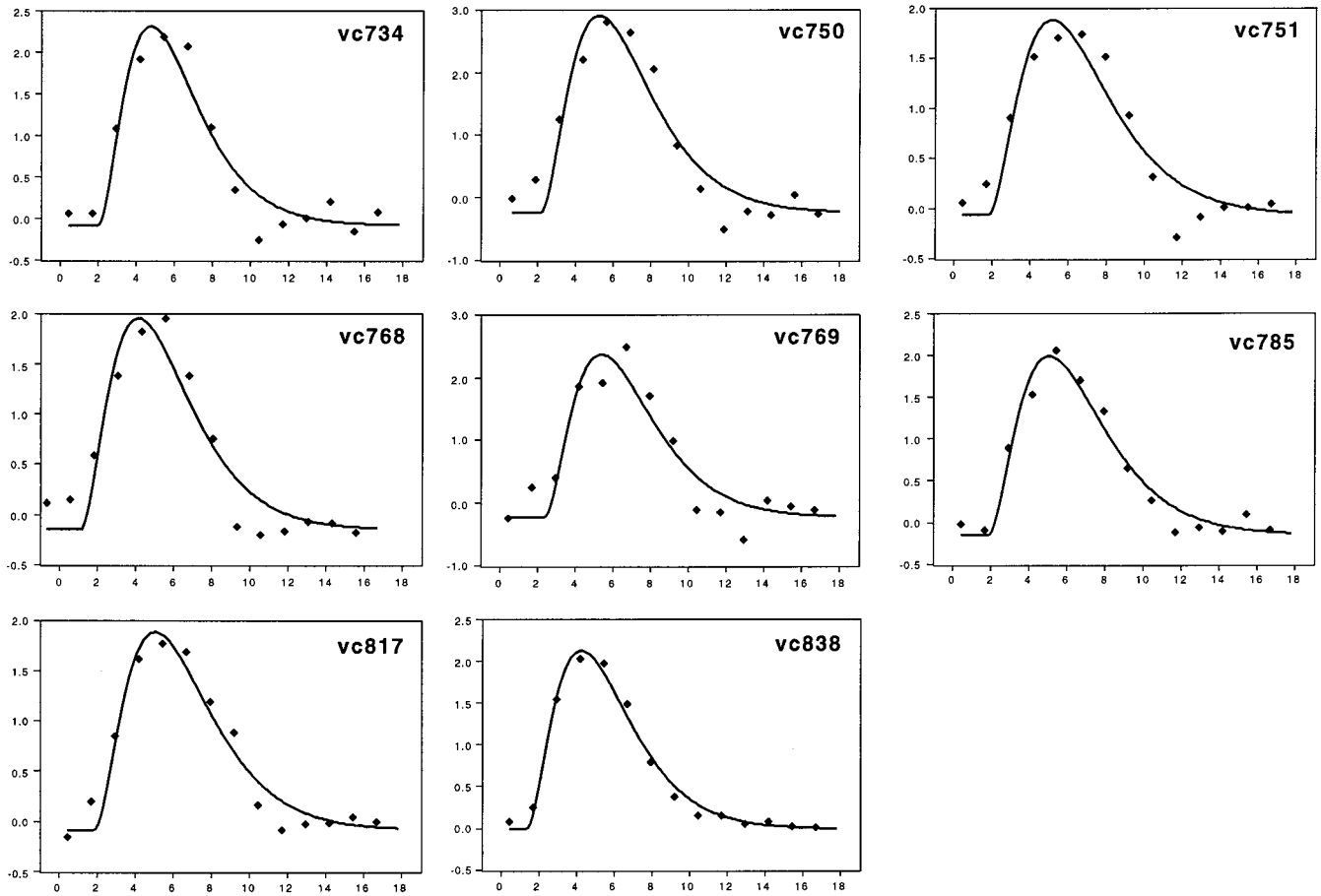


FIG. 4. Visual cortex hemodynamic response estimates are shown for each subject in experiment 1. The *x* axis displays time (in seconds) and the *y* axis amplitude (in percentage signal change). Hemodynamic response estimates from the general linear model are plotted as filled diamonds. The curves represent the best fit model based on a γ function.

time to peak 4.43 s for early runs and 4.42 s for late runs). Run order did significantly affect the motor cortex amplitude estimate [ANOVA $F(7,1) = 9.13$, $P < 0.05$; amplitude 2.09% for early runs and 1.81% for late runs].

The presence of an order effect in motor cortex and not visual cortex may be attributed to the fact that visual cortex ability coincides with a relatively "passive" response (not requiring voluntary control), while motor cortex activity is related to an "active" response (a finger press) which requires voluntary control and thus may become more efficient during the experiment. Thus, order effects can be significant, even in this simple sensory/motor paradigm, reinforcing the need to counterbalance order across conditions.

Turning to the specific question of trial presentation rate, the basic shape of the hemodynamic response was similar, but not identical, across presentation rates. Considering only the first eight runs in which rate was fully counterbalanced, the fastest presentation rate (mean ITI of 5 s) tended to show a smaller amplitude hemodynamic response and the second fastest rate (mean ITI of 10 s) tended to show a larger hemodynamic response for visual cortex. Using the model estimate of amplitude, for visual cortex this effect of presentation rate was found to be significant [ANOVA $F(7,3) = 29.65$, $P < 0.0001$] (Fig. 5A). Consistent with the qualitative observation mentioned above, the fastest rate was associated with the smallest hemodynamic response amplitude, although the quantitative estimate of the amplitude reduction was modest (17% below the estimate for the widely spaced trials). The greatest amplitude was found for the second fastest rate (11% above the estimate for the widely spaced trials). Both of these deviations in amplitude estimate from the widely spaced trials were significant in post hoc statistical tests: the fastest rate was associated with a significantly reduced amplitude [two-tailed *t*-test; $t(7) = 4.03$, $P < 0.005$] and the second fastest rate was associated with a significantly increased amplitude [two-tailed *t*-test; $t(7) = 5.27$, $P < 0.005$]. Thus, the overall effect of rate on response amplitude was significant with a nonlinear pattern (Fig. 5A). Trial presentation rate showed a trend for an effect on am-

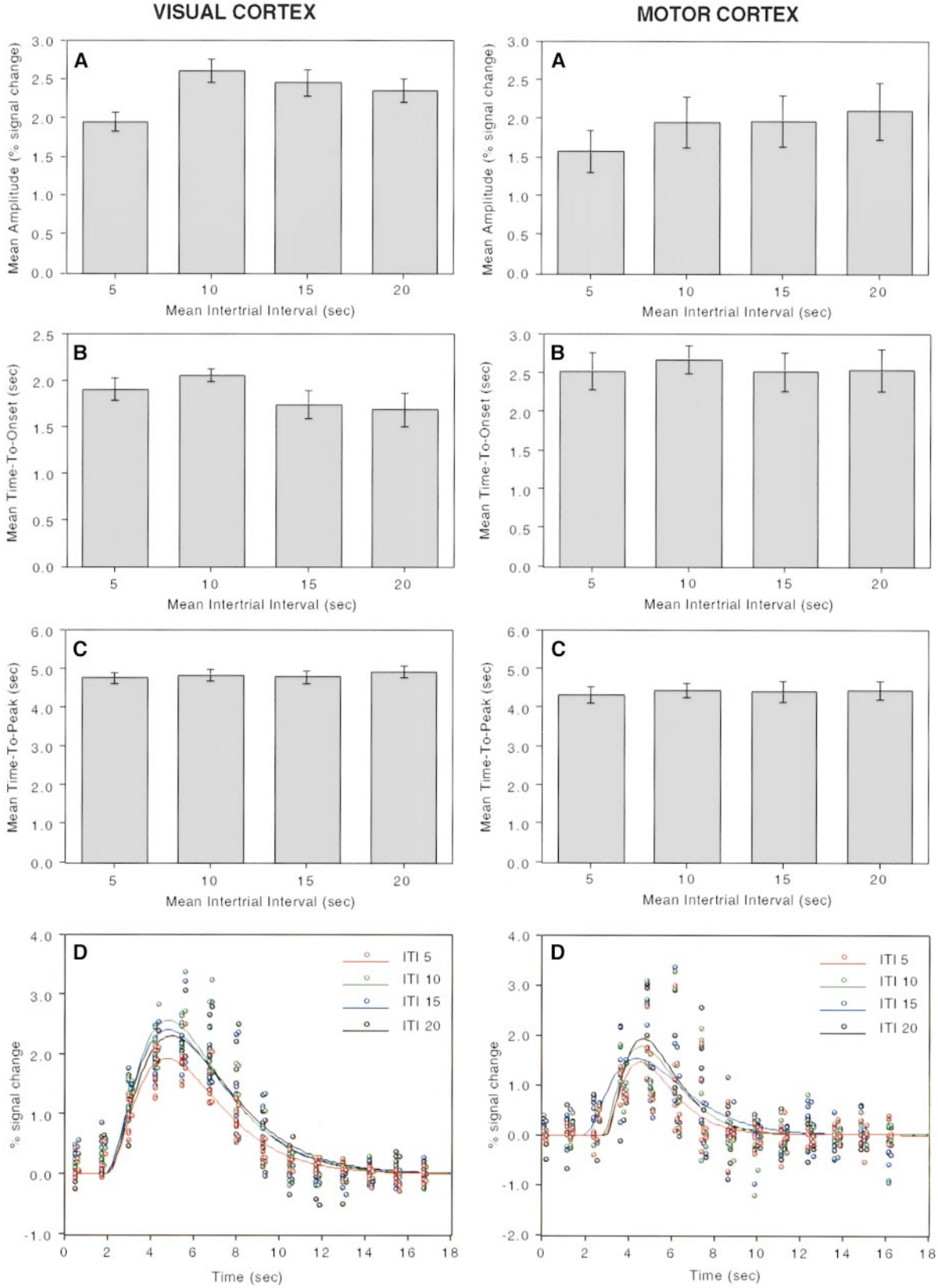


FIG. 5. Mean parameter estimates for the hemodynamic response are shown for both visual and motor cortex, across stimulus presentation rates. For each graph, a different parameter is plotted on the *y* axis (A, amplitude in percentage signal change; B, time to onset in seconds; and C, time to peak in seconds). The four presentation rates, in terms of their intertrial interval, are graphed separately on the *x* axis. Error bars represent standard errors of the mean. The bottom, D, graphs all of the data from all subjects together and shows the best fit to each stimulus presentation rate using a solid line. This graph may differ slightly from the mean estimates in A, B, and C, as those are obtained for each subject and then averaged.

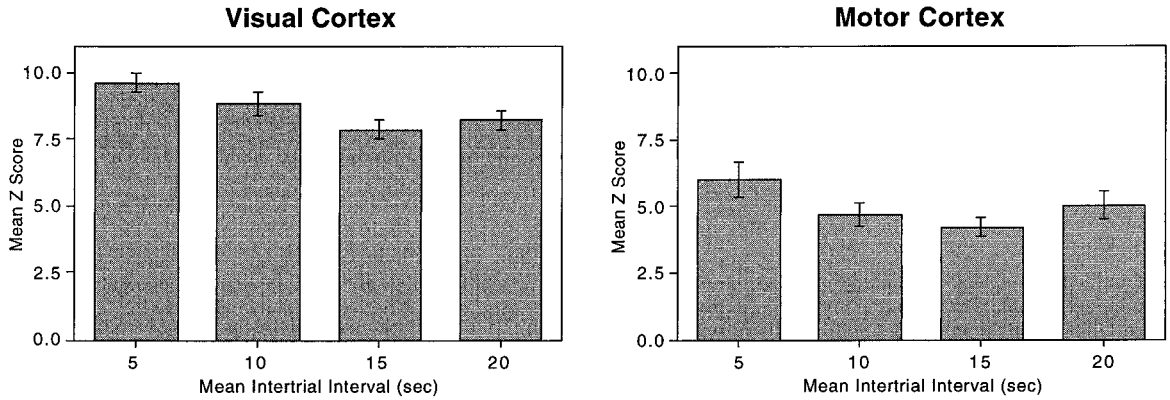


FIG. 6. Power as estimated by mean *Z* score is plotted across the four presentation rates for visual (left) and motor (right) cortex. Error bars indicate standard errors of the mean.

plitude in motor cortex regions [ANOVA $F(7,3) = 2.42$, $P = 0.09$]. Similarly to the visual cortex regions, the fastest trial presentation rate qualitatively showed a decreased amplitude with respect to the slowest trial presentation rate (25% below the estimate for the widely spaced trials; Fig. 5A).

No consistent effects of rate for the estimates of response timing (time to onset, time to peak) were observed. An effect on *time-to-onset* estimate was observed in the visual cortex data [ANOVA $F(7,3) = 3.48$, $P < 0.05$] (Fig. 5B). Post hoc comparisons showed that the mean time to onset of 2.05 s at the second fastest presentation rate (mean ITI 10 s) was significantly longer than the mean time to onset of 1.74 s at the rate with a mean ITI of 15 s [two-tailed *t*-test; $t(7) = 2.61$, $P < 0.05$] and than the mean time to onset of 1.68 s at the slowest rate (mean ITI 20 s) [two-tailed *t*-test; $t(7) = 2.54$, $P < 0.05$]. No such significant effect on time to onset was observed in motor cortex [ANOVA $F(7,3) = 0.25$, $P > 0.85$]. No effect on *time to peak* was observed for either region. The estimated time to peak was similar across all rates; there was no effect of rate in visual cortex [ANOVA $F(7,3) = 1.39$, $P > 0.25$] nor in motor cortex [ANOVA $F(7,3) = 0.39$, $P > 0.75$] and no post hoc comparison reached significance (Fig. 5C).

As can be observed in Fig. 5A, the influence of trial presentation rate on amplitude was quantitatively modest despite its significance. Comparing the most extreme cases in visual cortex (the second fastest rate and the fastest rate), the amplitude decreased by 25% (2.61 to 1.95%); comparing the fastest rate to the slowest rate showed an amplitude reduction of 17% (2.35 to 1.95%). In terms of statistical significance associated with detecting a response (used here as an estimate of power), the increased number of events during the fastest rate well outweighed the modest reduction in amplitude. In every subject, the *Z* score obtained for runs including data collected at the fastest rate was higher than for any other rate (Fig. 6). Thus, for a fixed run length, among the trial presentation rates tested here, there was a clear power advantage for the fastest rate. If the experimental goal is response detection, these results would suggest faster is better (as theoretically suggested by Burock *et al.*, 1998, and Buckner and Braver, 1999). However, at the fastest rates there may be a modest loss of signal amplitude (Fig. 5A).

Sampling Procedure Modestly Affects Response Estimation for Visual Cortex

Analyses were conducted on the data from experiment 2 to characterize hemodynamic response properties as a function of sampling procedure. Again, in order to check the validity of the methods for characterizing the hemodynamic response, the model estimate based on the γ function was applied to each subject's visual cortex gold standard response. As can be seen visually, the model fit predicted the empirically derived data well (Fig. 7), yielding a mean amplitude estimate of 2.52%, a mean time to onset estimate of 2.06 s, and a mean time-to-peak estimate of 4.94 s.

The effect of sampling procedure was examined by comparing runs in which there was Sparse sampling (2.68 s) to runs in which there was Interleaved sampling (effective sampling rate of 1.34 s). Two possible estimates for each of the Sparse and the Interleaved sampling procedures were available for each subject, owing to the crossing of four separate runs (see Fig. 3). These estimates are referred to as Sparse–Immediate, Sparse–Delay, Interleaved–One, and Interleaved–Two. "Immediate" and "Delay" refer to when, after the start of image acquisition, the stimulus occurred; "One" and "Two" are arbitrary terms that refer to the two separate but nominally identical estimates. Critically, all estimates were based on the same amount of data, allowing effects of sampling procedure to be determined while holding constant the amount of data contributing to each estimate.

Sampling procedure modestly, but significantly, affected the estimate of response amplitude [ANOVA

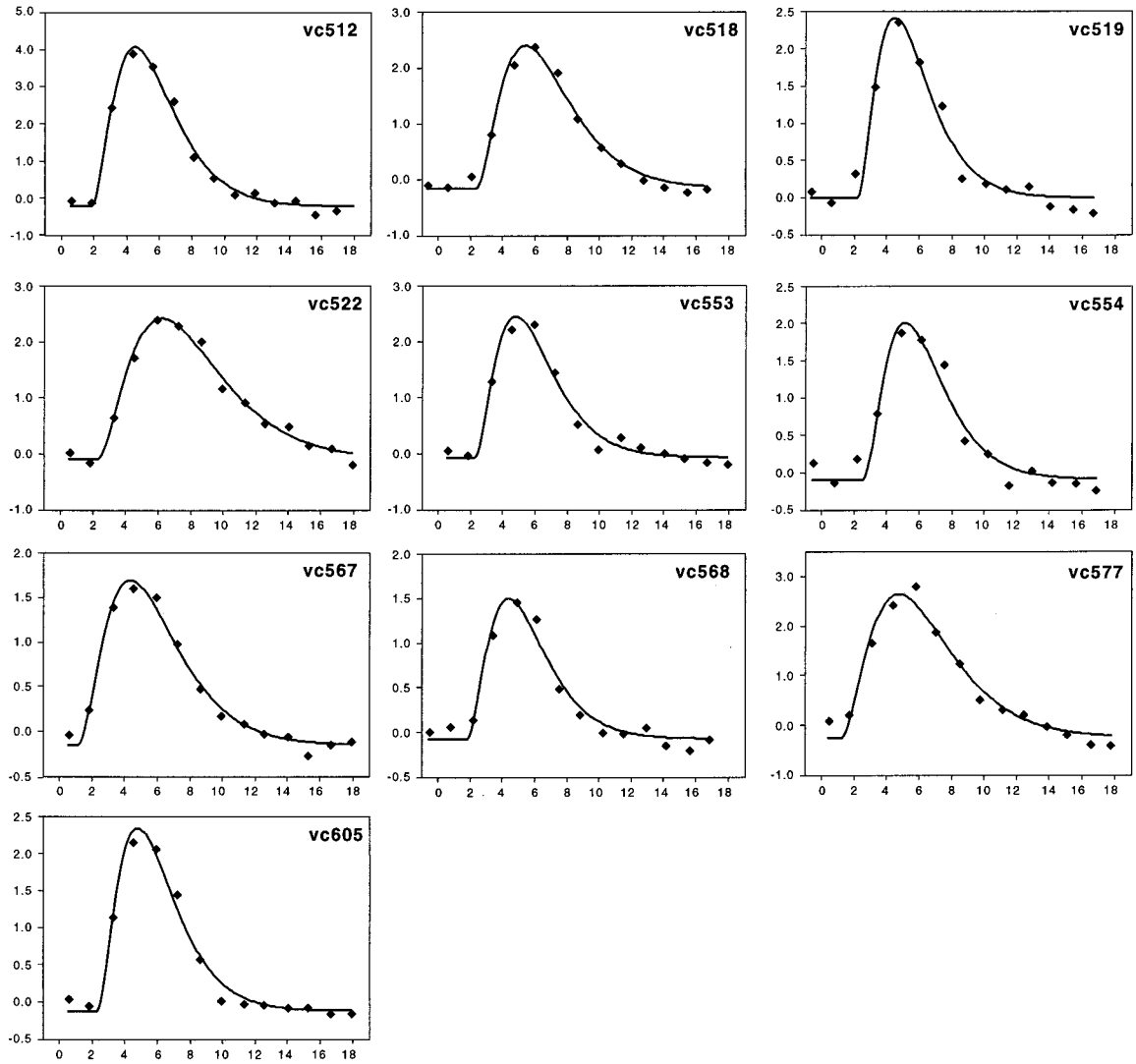
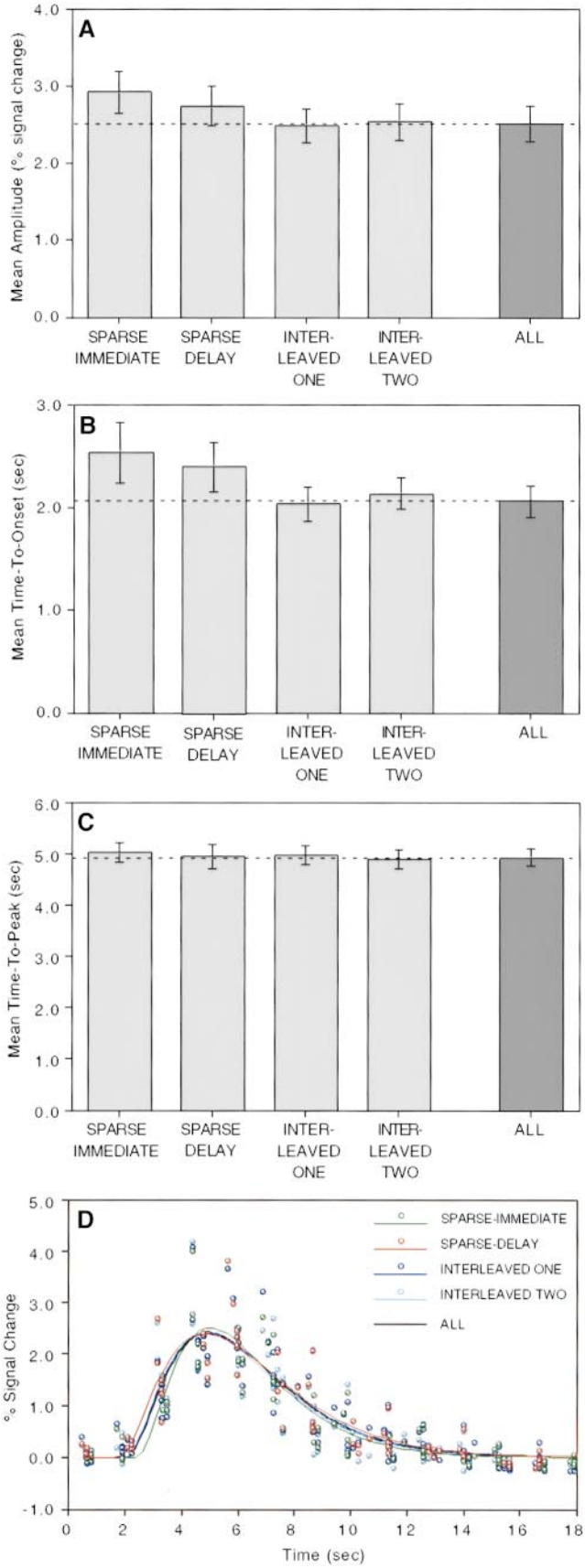


FIG. 7. Visual cortex hemodynamic response estimates are shown for each subject in experiment 2. The *x* axis displays time (in seconds) and the *y* axis displays amplitude (in percentage signal change). Hemodynamic response estimates from the general linear model are displayed as solid diamonds. The lines represent the best fit model based on a γ function.

$F(9,3) = 4.79$, $P < 0.01$] (Fig. 8). *The effect, however, was counterintuitive: Sparse sampling produced overestimates of the response amplitude regardless of when the stimulus occurred during the image acquisition.* In both instances (Sparse–Immediate and Sparse–Delay), the amplitude of the hemodynamic response was overestimated by at least 8% of its value. In contrast, in both instances of interleaved sampling, the amplitude estimate was ±1% of the gold standard estimate value. Post hoc statistical tests supported all of these conclusions. Both Sparse sampling procedures yielded significantly increased estimates compared to either of the Interleaved estimates (all $P < 0.05$, except Sparse–Immediate versus Interleaved–Two was $P = 0.06$). In contrast, Sparse–Delay was not significantly different from Sparse–Immediate and Interleaved–One was not significantly different from Interleaved–Two (both $P > 0.15$).

No significant effect of sampling procedure on response timing was observed [ANOVA $F(9,3) = 2.27$, $P > 0.10$ for time-to-onset estimates and ANOVA $F(9,3) = 0.70$, $P > 0.55$ for time-to-peak estimates) and no post hoc comparison reached significance. Nonetheless, a qualitative analysis suggested a common trend of overestimation of the time-to-onset estimate in the two Sparse conditions, while no such trend was seen in either of the two interleaved conditions (Fig. 8B). Quantitatively, the greatest difference in time-to-onset estimates was between the gold standard estimate and the Sparse–Immediate estimate (0.47 s). Conversely, no such qualitative trend could be discerned for the time-to-peak estimates.



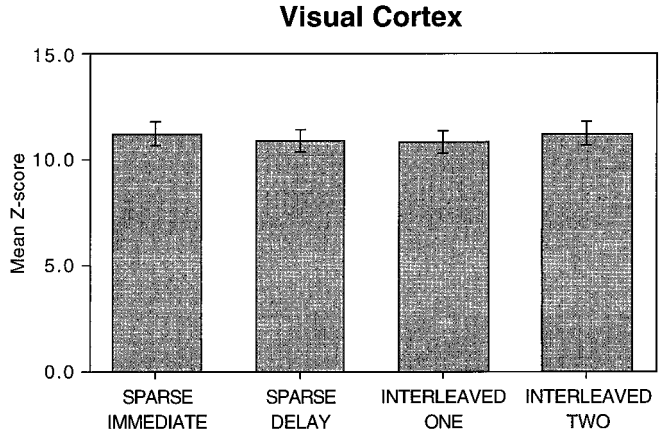


FIG. 9. Power as estimated by mean *Z* score is plotted across the four sampling procedures. Error bars indicate standard errors of the mean.

In terms of statistical significance associated with detecting a response (used here as an estimate of power), the mean *Z* scores were very similar for all sampling procedures, suggesting that, within the parameter constraints of this study, all forms of sampling would be equally likely to *detect* a response (Fig. 9).

Amplitude Estimates Are Unrelated to Timing Estimates for Visual Cortex

A natural question to ask about the hemodynamic response is the relation between the amplitude of the response and the timing of the response. One possibility is that the two are related: a larger hemodynamic response takes longer to build and is slower to decay, while smaller responses might evolve more rapidly. Another possibility is that the two are unrelated and reflect independent quantities that vary across subjects. Both experiments 1 and 2 provide data that could be used to answer this question. For both experiments, the visual cortex gold standard estimates of response

FIG. 8. Mean parameter estimates for the hemodynamic response are shown for visual cortex, across the different sampling procedures. For each graph, a different parameter is plotted on the *y* axis (A, amplitude in percentage signal change; B, time to onset in seconds; and C, time to peak in seconds). The different sampling procedures are graphed separately on the *x* axis. Error bars represent standard errors of the mean. The first two columns in each graph come from the Sparse sampling procedures, the next two from the Interleaved sampling procedures. The rightmost, darkly filled bar represents data from the gold standard estimate using all data. The dotted line in each graph represents the mean as determined by the gold standard estimate. The bottom, D, graphs all of the estimates from all subjects together and shows the best fit to the estimates using a solid line. These solid lines thus represent that shape of the hemodynamic response that would be estimated for each sampling procedure if all of the data, across subjects, were pooled. This graph may differ slightly from the mean estimates in A, B, and C as those are obtained for each subject and then averaged.

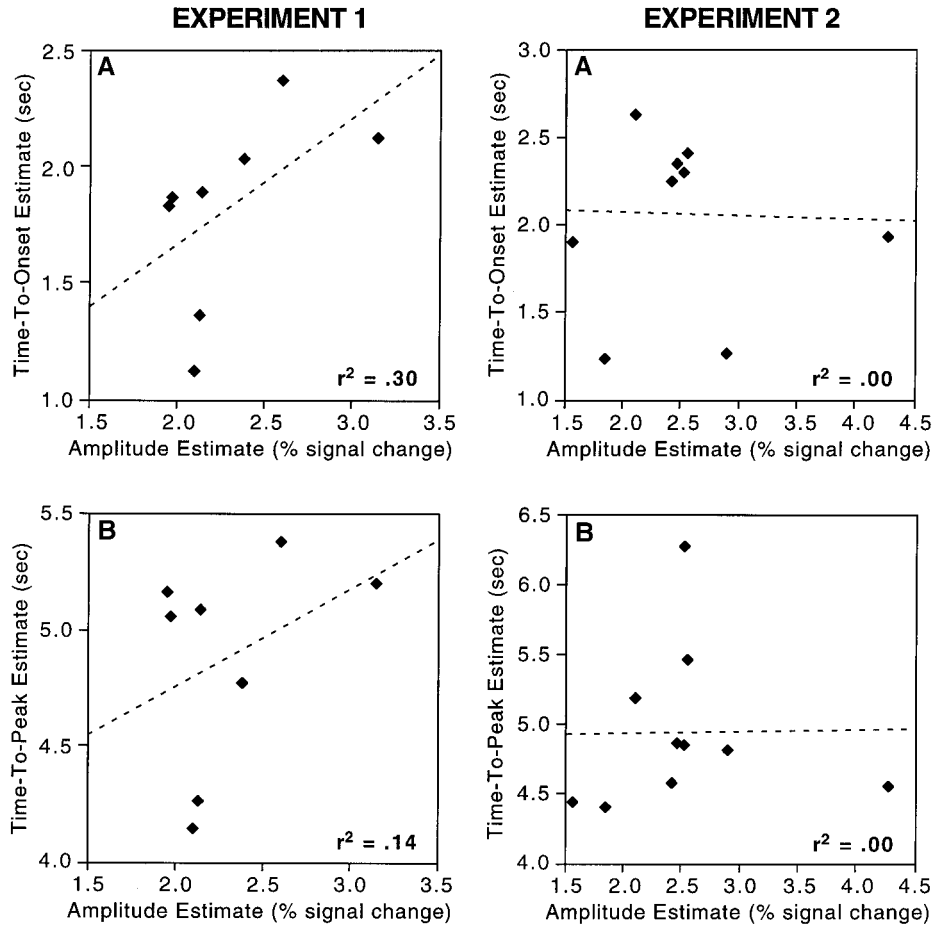


FIG. 10. The relations between separate parameter estimates are plotted for visual cortex for both experiments. Each solid diamond represents data from a single subject. The graphs labeled A plot the relation between estimated amplitude and time to onset and the graphs labeled B plot the relation between estimated amplitude and time to peak. The regression line (dotted line) and correlation (bottom right of each) are also displayed. Note that there is little consistent relation between the amplitude estimates and either estimate of timing.

amplitude, time to onset, and time to peak were examined to determine the degree of correlation across subjects. For experiment 1, the data came from the four runs (two interleaved run pairs) that contained trials spaced widely apart. For experiment 2 the data came from the four runs that all contained rapidly presented trials (again two interleaved run pairs). Results are shown in Fig. 10.

As can be seen visually there was negligible relation between response amplitude and timing (time to onset and time to peak). Larger amplitude responses *did not* significantly predict slower time to onset of the hemodynamic response, in either experiment 1 or experiment 2 ($r^2 = 0.30$ and 0.00, respectively; both $P > 0.15$). The modest, nonsignificant, correlation in experiment 1 appears to be carried largely by a single subject. Similarly, larger amplitude responses *did not* significantly correspond to slower time to peak of the hemodynamic response. For experiments 1 and 2, $r^2 = 0.14$ and 0.00, respectively (both $P > 0.35$). The lack of predictive power was not due to the instability of the estimates themselves as is shown in the next section. Rather, the lack of significant correlation between response amplitude and timing appears more likely to be due to the fact that the two are unrelated when regional hemodynamic responses are estimated. As a further illustration of this point, Fig. 11 shows the hemodynamic response for each individual subject in experiments 1 and 2. In these data sets, the largest amplitude response (indicated as A) occurred in the presence of a response evolving within the average time frame, while the slowest evolving response (indicated as B) occurred within a response of average amplitude.

Amplitude and Timing Estimates Are Extremely Reliable for Visual Cortex

Centrally important to estimates of response amplitude and timing is the reliability of such estimates. For example, it is possible that the lack of predictive power between response amplitude and timing observed in

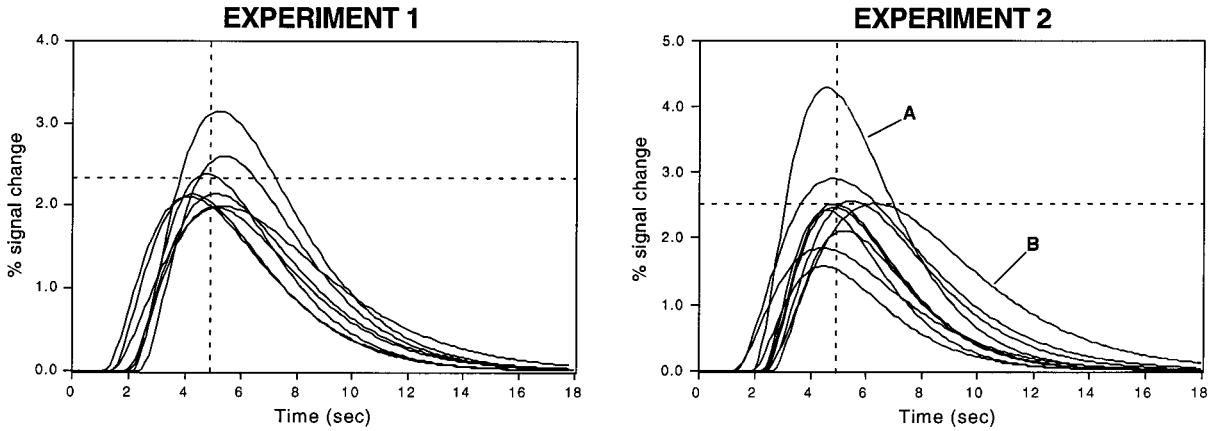


FIG. 11. The across-subject variability of the hemodynamic response is illustrated by plotting each subject's estimate on the same graph. The left plots the 8 subjects from experiment 1 and the right plots the 10 subjects from experiment 2. The dotted lines represent the mean amplitude and time to peak (averaged across subjects) for each experiment. Of particular interest is the visualization of the lack of correlation between amplitude and timing estimates. Larger amplitude responses do not necessarily lead to longer temporal evolution, as shown clearly by two subjects in experiment 2. The subject labeled A has an extremely high amplitude response, yet evolves with a temporal evolution near the mean. In contrast, the subject labeled B has the longest time to peak and second longest time to onset, yet has an amplitude estimate similar to the mean.

the preceding section was due to the instability of the estimates. Data from experiment 2 were analyzed to address this issue. Experiment 2 contained two separate interleaved run pairs (Interleaved–One and Interleaved–Two), which were each collected identically within the same subjects. These two run pairs could thus be used to determine the reliability of the hemodynamic response estimates. Results are shown in Fig. 12.

For all estimates high reproducibility was obtained: for response amplitude $r^2 = 0.98$, for response time to onset $r^2 = 0.60$, and for response time to peak $r^2 = 0.95$ (all $P < 0.001$). These strikingly high correlations (especially for amplitude and time to peak) stand in stark contrast to the lack of significant correlation between amplitude and time-to-onset estimates and between amplitude and time-to-peak estimates. Thus, while the estimates of amplitude and timing (time to onset and time to peak) are highly reliable, they appear to be completely uncorrelated with each other in the context of the present study. Of the three forms of estimate, amplitude and time to peak were the most stable.

Response Properties in Visual Cortex Failed to Predict Properties in Motor Cortex

A further question that can be asked is how well responses estimated in one region can predict response characteristics in another region. That is, does an individual with a high-magnitude response in visual cortex tend to have a high-magnitude response in motor cortex? To answer this question the amplitude, time-to-onset, and time-to-peak estimates from experiment 1 were correlated across regions (using the gold standard estimate from the conditions with a mean ITI of 20 s). No correlations were significant (Fig. 13). There was a modest magnitude of correlation in amplitude ($r^2 = 0.39$; not significant $P = 0.1$) but it was in the opposite direction as would be expected: increased visual cortex amplitudes predicted lower motor cortex amplitudes. The lack of significance and odd direction of correlation cast doubt on the existence of an actual relation between responses in different regions in the present data. The r^2 for time to onset and time to peak were 0.01 and 0.02, respectively. Thus, knowing the amplitude and delay of a hemodynamic response in visual cortex for a subject tells the experimenter little about the characteristics of the response in motor cortex, at least insofar as the analysis is applied to healthy young adults.

Relative Estimates of Response Timing Can Be Used to Infer Relative Offsets in the Timing of Neural Activity

Absolute estimates of hemodynamic response timing may not always be sufficient for making inferences about the timing of neural activity between brain regions. Variance in the timing of the response across regions can be considerable, even when nearly adjacent voxels are considered. However, the extreme stability of the timing of the hemodynamic response within a region still leaves open the possibility of making inferences about *relative* changes in the timing of neural activity within a region. That is, for a given region, the hemodynamic response (whatever its temporal profile) may shift in time to reflect changes in the timing of an underlying neuronal response. Experiment 2 provides data to test this possibility.

Experiment 2 counterbalanced two separate motor response conditions which involved pressing a key in

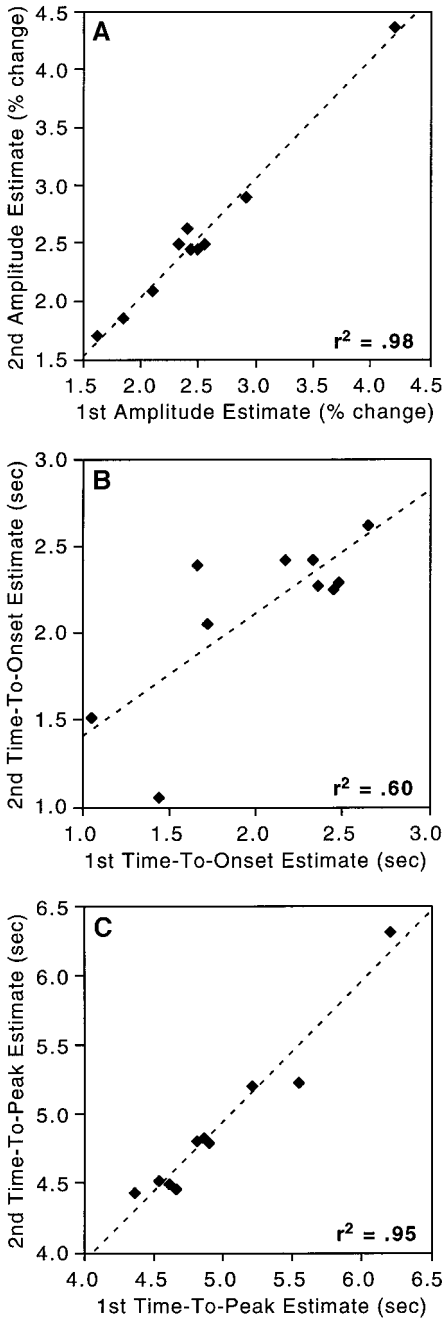


FIG. 12. The reliability of the parameter estimates is plotted for the second experiment, in which two independent Interleaved data sets were acquired for each subject. Each filled diamond represented data from a single subject. Each graph represents the reliability of a separate parameter estimate, plotting the estimate for the first data set in a given subject against the value for the second data set. (A) Amplitude in percentage signal change, (B) time to onset in seconds, and (C) time to peak in seconds. Ideally, the correlation would be 1.00 and all data points would fall along the diagonal; movement along the diagonal reflects between-subject variance. For amplitude (A) and time to peak (C) estimates, this ideal is nearly achieved. The time to onset (B) is less stable, but still highly correlated from one measurement to the next.

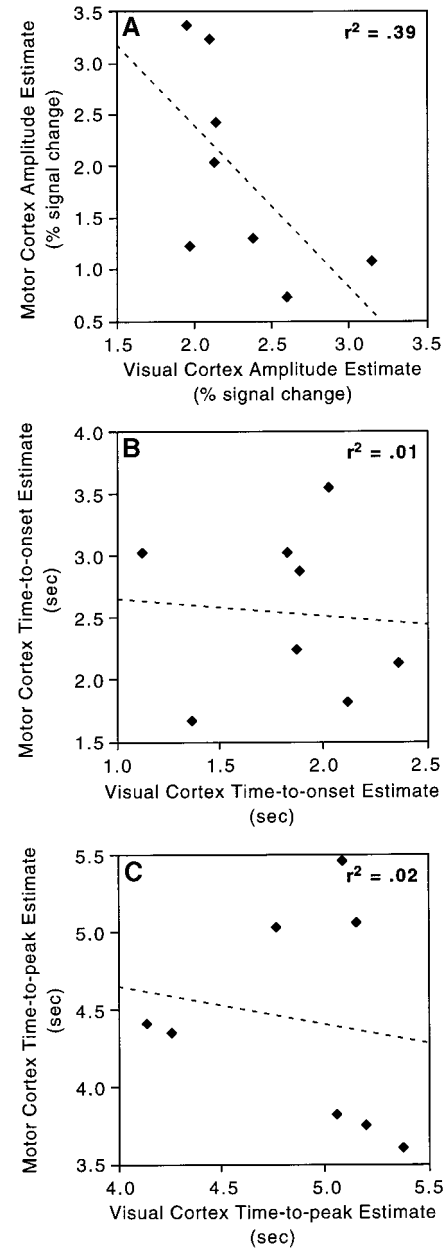


FIG. 13. The relation of the parameter estimates across brain regions (visual and motor) is plotted for the second experiment (A, amplitude in percentage signal change; B, time to onset in seconds; and C, time to peak in seconds).

response to the Onset or Offset of the visual stimulus causing the motor response for a given hand to be temporally offset across runs. Two separate conditions were used, in which the subjects were instructed to press with their right hand at stimulus Onset and with their left hand at stimulus Offset or vice versa. In relation to the question of timing differences between Onset and Offset responses, it should be noted that, behaviorally, the Offset responses were significantly faster for both the right-hand-first and the left-hand-

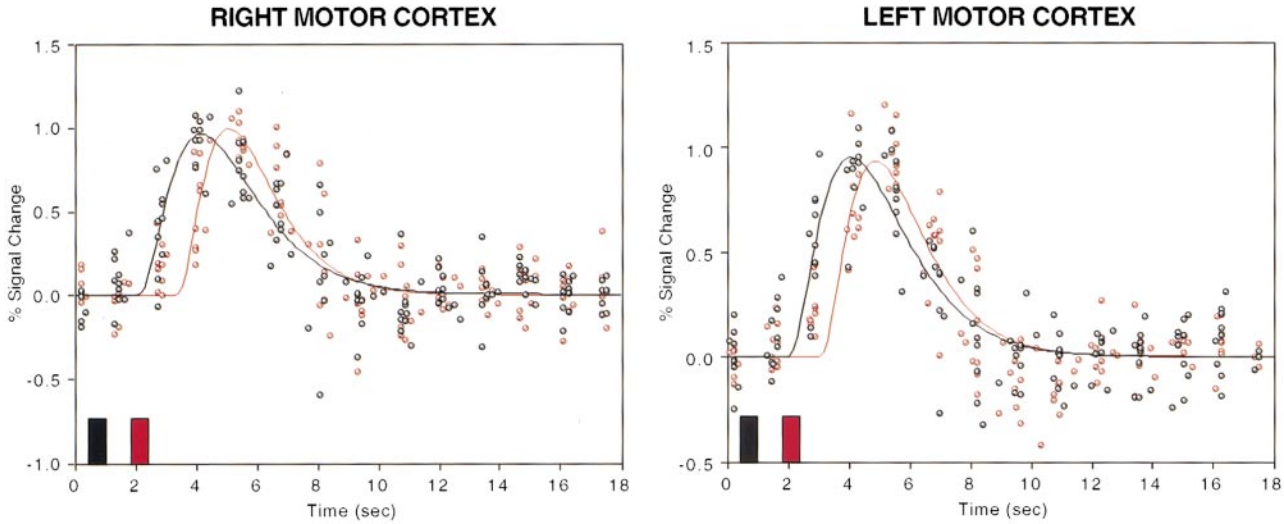


FIG. 14. Timing offsets in motor cortex for experiment 2 are displayed separately for right and left motor cortex. The *x* axis represents time (in seconds) and the *y* axis represents signal change (in percentage). In each graph, the earlier curve represents the pooled estimate for those conditions in which the contralateral motor response was made first (at the Onset of the visual stimulus), while the latter curve represents the analogous estimate for when the contralateral motor response came second (at the Offset of the visual stimulus). The relative positions of the responses are shown schematically by solid bars at the bottom. A significant offset in the hemodynamic response is clearly evident in both right and left motor cortex, consistent with the ordering of the motor responses.

first conditions, likely indicating preparation or anticipation of the Offset responses [$t(9) = 3.30$ and 4.12, respectively; both $P < 0.01$]. For the right-hand-first (left motor cortex) condition, the "Onset" response occurred 310 ms after stimulus Onset and the "Offset" response 285 ms after stimulus Offset; for the left-hand-first (right motor cortex) condition, these response times were 344 and 292 ms, respectively.

To answer the question of whether hemodynamic response estimates can reflect changes in underlying neuronal activity, timing (time-to-onset and time-to-peak) estimates were made for both the left and the right motor cortex regions comparing directly those estimates based on Onset responses to those estimates based on Offset responses. Estimates of timing within each motor region showed a significant change in timing between Onset and Offset conditions. For the right motor cortex, the estimated time to onset shifted from 2.23 to 3.10 s [$t(9) = 4.09$, $P < 0.005$], while the estimated time to peak shifted from 4.25 to 4.98 s [$t(9) = 9.33$, $P < 0.0001$]. Similarly, for the left motor cortex, the estimated time to onset shifted from 2.08 to 3.15 s [$t(9) = 6.15$, $P < 0.0005$] while the estimated time to peak shifted from 4.08 to 4.79 s [$t(9) = 5.24$, $P < 0.0005$]. Thus, the estimated hemodynamic response within motor cortex shifted significantly by 0.75 s to 1 s when the response occurred to Offset as opposed to Onset of the visual stimulus. This value is quite plausible given the paradigm constraint that the Offset responses could be prepared in advance of their execution (and the finding that Offset responses were significantly speeded). While providing only a rough estimate, these results when submitted to a power analysis imply that a relative offset in hemodynamic response timing of as little as 100 ms could be detected in conditions similar to the present study 50% of the time at $\alpha = 0.05$. Figure 14 shows the averaged hemodynamic response across subjects with the temporal shift between the Onset and the Offset response clearly visible.

A further extension of this kind of analysis that may provide information about *relative* timing offsets between regions is possible (Friston *et al.*, 1998). For example, motor cortex may change the timing of its activity more than visual cortex (each relative to their own timing baseline). Relative timing offsets may allow inferences about which regions are involved in a cognitive or behavioral operation and in what order.

To illustrate this possibility, estimates of the time to onset and time to peak from the present data set were subjected to a region (visual cortex, left motor cortex, right motor cortex) $\times$ condition (right hand first, left hand first) ANOVA. The logic was as follows. Regions whose hemodynamic response timing is affected by the condition manipulation likely reflect those regions participating in the process that differed across conditions (in this instance motor programming and execution) *and/or* are downstream from regions affected by the process. Regions that are unaffected by the condition manipulation would be those earlier in the processing hierarchy and/or unrelated to the process being manipulated. In the present example, the prediction would be a region $\times$ condition interaction, with post hoc analyses showing motor cortex to be affected by the condition

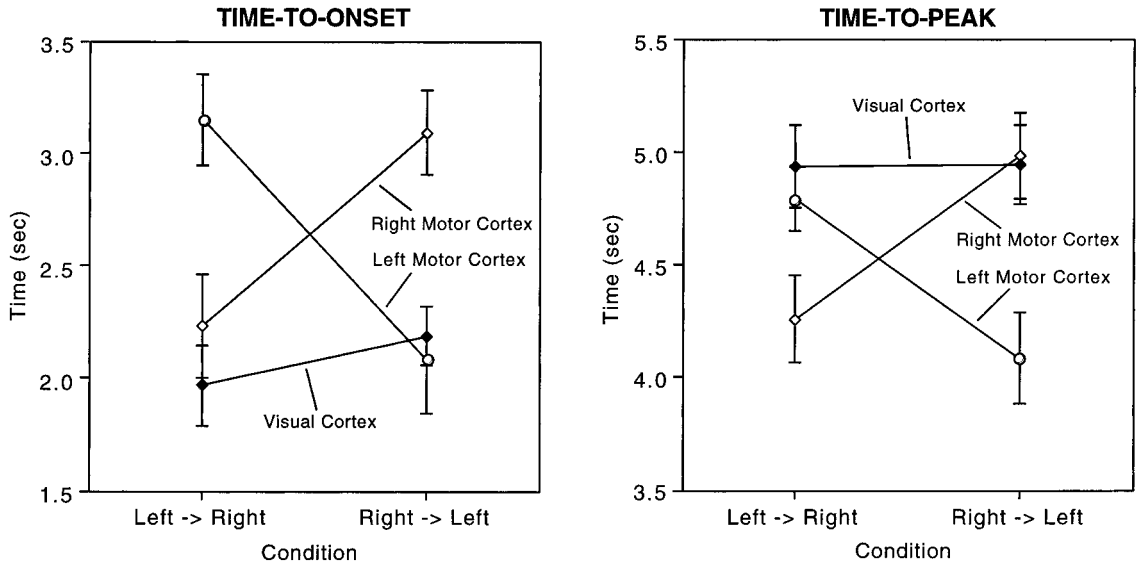


FIG. 15. Parameter estimates of time to onset (left) and time to peak (right) are plotted for each region, for each of the separate motor response conditions. The two conditions (plotted on the *x* axis) indicate whether a left-hand response was made first, followed by a right-hand response (left → right) or vice versa (right → left). A clear and significant interaction is observed for both forms of parameter estimate. Visual cortex does not change timing in relation to motor response condition, while motor cortex does. Motor cortex shows a crossover interaction in that right motor cortex responds fastest when a left key press is made first and left motor cortex responds fastest when a right key press is made first. Error bars indicate standard errors of the mean.

manipulation (whether a right or a left key press was made first) and visual cortex unaffected. The main effect of region would be uninteresting and involve the multiple possible influences for baseline differences in hemodynamic delay. Critically, the interaction of region × condition would be significant only if the condition manipulation influenced response timing above and beyond the baseline differences in regional timing.

Figure 15 illustrates the interaction graph. A clear, significant interaction of region × condition existed for both time-to-onset and time-to-peak estimates [$F(2,27) = 35.32$, $P < 0.0001$ for time to onset and $F(2,27) = 58.50$, $P < 0.0001$ for time to peak]. The pattern lends itself to a straightforward interpretation. Visual cortex is unaffected by whether the first key press is made with the right or left hand. In contrast motor cortex is directly affected by which hand makes the first key press. Left motor cortex is fastest when the right hand presses first; right motor cortex is fastest when the left hand presses first. The inference would be that motor cortex is involved in the process and its involvement comes after that of visual cortex, which is completely unaffected by the condition manipulation.

A further point is worth making with regard to the *absolute* timing of the response across regions. While the time-to-onset estimates behaved in a manner generally consistent with the expected time course of brain activity (visual cortex preceding motor cortex), it did not do so consistently. A clear violation of the expected pattern was observed: visual cortex appeared to onset after left motor cortex in one condition. This seems unlikely to reflect a true assessment of neuronal activity. Thus, the *absolute* estimate of the time to onset should be interpreted cautiously. *Absolute* estimates of the time to peak are even more difficult to interpret. While the motor response is presumably brief in time, the visual response used here evolves over a longer period of time so that the peak response can occur later in visual cortex than in motor cortex.

DISCUSSION

The hemodynamic response was extracted from rapidly presented trials across two separate ER-fMRI studies using linear estimation. A number of clear observations emerged that can be summarized as follows:

(1) The hemodynamic response can be estimated during rapid-presentation ER-fMRI paradigms using linear estimation methods without making assumptions about the shape of the response. Moreover, these estimates are possible in the context of whole-brain imaging and MR sampling rates as sparse as one acquisition every 2.68 s.

(2) For experiment 1, the visual cortex response in the context of rapidly presented trial events began at 1.90 s (time to onset), peaked at 4.75 s (time to peak), and reached an amplitude of 1.95%. For experiment 2, these estimates were 2.06 s (time to onset), 4.94 s (time to peak), and 2.52% (amplitude). These values were

similar, but not identical to, estimates based on widely spaced trials.

(3) Trial presentation rate had a modest, but significant, effect on the estimated response amplitude for visual and motor cortex. In particular, rapidly presented trials (~5 s apart on average) showed a 17% (visual cortex) and 25% (motor cortex) reduction in estimated amplitude compared to trials spaced in time. It is unclear whether the reduction is due to hemodynamic response saturation or to differences in underlying neuronal activity across rates.

(4) Despite the modest amplitude reduction at fast trial-presentation rates, the power for detecting a response was significantly greater at fast rates owing to the increased number of trials possible. In other words, the increased number of trials outweighs the reduction in response amplitude, insofar as power in detecting a response is the goal.

(5) Sampling procedure had a modest, but significant, effect on the estimated response amplitude for visual cortex. Sparse sampling, where measurements were made every 2.68 s, showed an 8% overestimate compared to denser sampling (effective sampling rate 1.34 s). In terms of statistical power, both sparse and dense sampling procedures were equivalent.

(6) The estimated amplitude and timing (time to onset and time to peak) of the hemodynamic response were stable across separate data sets collected in the same subject, for the same region (r^2 = 0.98, 0.60, and 0.95 for amplitude, time to onset, and time to peak, respectively). Thus, the amplitude and time-to-peak estimates were nearly perfectly correlated from one data set to the next, positioning them as extremely reliable measures of the hemodynamic response, at least insofar as good signal-to-noise properties exist in the data.

(7) The estimated timing (time to onset and time to peak) of a region's hemodynamic response could *not* be used to predict its amplitude. The two components of the response appeared unrelated on the spatial scale of brain regions.

(8) Estimates of response properties in visual cortex did not predict response properties in motor cortex, suggesting that, across subjects, the regional variation in response is substantially greater than any global factors influencing response properties that vary across young, normal subjects. That is, if there is tendency for one subject to have globally larger and/or slower hemodynamic responses than another subject, such a tendency could not be detected in the present data.

(9) Estimates of *relative* change in timing of the hemodynamic response within a region could be detected for a temporal difference of under a second. When motor response was delayed, a clear temporal shift of the hemodynamic response in motor cortex was observed. Preliminary power analysis suggests the limit for detecting a temporal offset within a region, using our procedures involving whole-brain data acquisition and a TR of 2.68 s, may be as little as 100 ms.

(10) Relative timing changes between regions could be used to make inferences about which regions contributed to motor response programming. Specifically, in the present study, an interaction between visual and motor cortex was found in relation to motor response delay, with a significant effect of timing identified only for motor cortex. This analysis empirically suggests that motor cortex predicts the delay in response; visual cortex does not. Moreover the presence of a delay in motor cortex and not in visual cortex suggests that the progression of processing goes from visual to motor cortex hierarchically in this paradigm. While such a finding is easily predicted based on known roles for visual and motor cortex, the empirical finding was driven by the timing estimates across regions and their relation to behavioral data, not preexisting knowledge. Thus, analysis of relative timing across regions may be a powerful method for determining the processing contributions of brain regions where their role in a cognitive or behavioral process is less well understood.

Implications

The present observations have a number of practical and conceptual implications. These relate to the four areas outlined in the Introduction: hemodynamic response summation, variance of the hemodynamic response, hemodynamic response sampling, and hemodynamic response estimation. In addition, the results have important implications for a possible new use of functional MRI involving the ordering of processes between brain regions based on relative timing effects. Each of these areas is discussed separately.

Hemodynamic Response Summation

Consistent with the near-linear summation properties previously observed in fMRI studies using BOLD contrast (e.g., Boynton *et al.*, 1996; Dale *et al.*, 1997), the shape and properties of the hemodynamic response remained roughly the same across presentation rates (see Fig. 5D). In the fastest presentation conditions in this study (minimum ITI 2.5. s; mean ITI 5.0 s), in which there was maximum overlap across trials, the estimates of the hemodynamic response were *similar* to those for trials spaced widely apart. Such a finding reinforces the empirical observation that fast presentation rates can be used in cognitive neuroimaging studies (Buckner *et al.*, 1998a; Clark *et al.*, 1998; Wagner *et al.*, 1998).

There was evidence for saturation of the response in that the amplitude of the response at the fastest rate was between 17% (visual cortex) and 25% (motor cortex) reduced relative to the slowest rate (see Fig. 5A). This reduction in amplitude was present after the he-

modynamic response was estimated within the general linear model to remove overlap across trials. The present data do not distinguish between the possibility that the observed reduction in response amplitude represents a change in the underlying neural response (e.g., a form of habituation or interaction across adjacent events) or whether the neuronal response is constant and the hemodynamic response itself saturated.

Almost all previous studies of hemodynamic response summation have noted some form of nonlinearity when temporally extended or overlapping events were considered. Nonlinear summation has sometimes been quite pronounced at extremely fast trial presentation rates (e.g., Friston *et al.*, 1997). The finding of modest amplitude reduction in the present study is consistent with the observation that some amount of saturation can occur at rates in the range of one trial every few seconds. However, the consequence of amplitude reduction was marginal in the present data set, and the data suggest that response summation is sufficiently linear to use rapid presentation paradigms. Robust responses were detected at all rates and, due to the fact that considerably more trials are available at faster rates, the power to detect a response was greatest at the fastest rate. The timing of the hemodynamic response remained largely stable across presentation rates, in terms of both time to onset and time to peak.

Variance of the Hemodynamic Response

Three kinds of variance related to the hemodynamic response were explored: variance across data sets for the same region within a subject, variance across subjects for a given region, and variance across regions. The first kind of variance (pertaining to the same region within a subject) presented the most optimistic finding: for a given region, the hemodynamic response was nearly identical from one data set to the next. Figure 12 illustrates this point most directly. Estimates of amplitude and time to peak were nearly perfectly correlated between separate data sets ($r^2 = 0.98$, $r^2 = 0.95$). The statistically significant correlation for time to onset was lower, but nonetheless quite high ($r^2 = 0.60$). Another place where this extreme stability could be seen was when separate estimates were compared for data averaged over subjects, in which every subject contributes to each estimate. Figure 8D illustrates such a comparison. When the Interleaved sampling procedure was used, the two separate estimates produced hemodynamic responses that overlapped nearly completely. The extreme stability of the hemodynamic response within a region, for a given subject, was exploited in estimating temporal offsets across regions, as will be discussed below.

The second kind of variance—between subjects for a given region—showed more variation but still revealed considerable central tendencies across subjects. Figure 11 shows the hemodynamic response for a region in visual cortex for each subject. As can be seen visually, the amplitude of the visual response tended to be around 2% and varied with a range of 1.95 to 3.15% in experiment 1 (widely spaced trials) and between 1.57 and 4.28% in experiment 2; the standard deviations were 0.40 and 0.73%, respectively. Time to onset and time to peak also showed strong central tendencies. This range of variation suggests that it is reasonable to average across subjects with the assumption that a group of 10 or more subjects would closely approximate a modal response for a given region (such as is done with many event-related data analysis procedures). The error induced would be relatively small, on average. However, there is sufficient variance that for precise estimates either large samples of subjects would be required to make comparisons across groups or within-subject designs should be adopted.

Variance across regions, even within the same subjects, was found to be sufficient to present a formidable challenge to interpretation of absolute timing parameters across regions. Considering regions in visual and motor cortex, there was almost no relation between amplitude or timing estimates between regions (Fig. 13). That is, knowing the amplitude and delay of a subject's response in visual cortex did little to inform one about those parameters of the response in motor cortex. Moreover, the absolute estimates themselves appeared to have only a rough relation to the likely ordering of activity within the regions. For example, in one condition, motor cortex appears to onset (in terms of estimated hemodynamic response parameters) earlier than visual cortex (Fig. 15, left, right → left condition). This inability to make predictions across regions, or to reveal consistently interpretable relations between absolute measurements across regions, may be due to the underlying differences in vasculature (Lee *et al.*, 1995; Robson *et al.*, 1998) or to as yet undetermined factors.

The practical upshot of these findings is that the present data do not support the possibility of using *absolute* measurements of the hemodynamic response in one region to predict or interpret measurements in another region (in healthy young adults).

Hemodynamic Response Sampling

Experiment 2 allowed a direct comparison of two sampling procedures. In the first procedure, hemodynamic response estimates were obtained for data acquired with systematically varied delays between the image acquisition and the trial presentation (Josephs *et al.*, 1997). Varying the delay allows one to increase the effective sampling rate by systematically acquiring the data at different points during the hemodynamic response. In our implementation, the stimulus was either presented at the beginning of the whole-brain

image acquisition or delayed by 1.25 s. We call this "interleaved" because the resulting hemodynamic response estimates can be interleaved to reconstruct a continuous estimate (Fig. 3). In the second sampling procedure the stimulus always appeared at the same time relative to the image acquisition.

The theoretical reason for employing an interleaved procedure is the desire to estimate the true shape of the response with as high temporal sampling resolution as is practically possible (Josephs *et al.*, 1997; Price *et al.*, 1999). The present study allowed a direct examination of the utility of such a procedure by holding constant the amount of data contributing to a given hemodynamic response estimate and manipulating whether or not the sampling used an interleaved procedure. What advantage, if any, does a procedure with interleaved sampling have over fixed sampling?

For the most commonly used forms of analysis in which response detection is the goal, the benefit of interleaved sampling appears minimal. All procedures yielded estimates of the hemodynamic response that were roughly similar (Fig. 8). However, this finding should not be generalized to instances of sparser sampling (e.g., 4–5 s or more), which have been shown to have marked effects even in blocked-task paradigms (Price *et al.*, 1999). Nonetheless, in the present study, a sampling rate of 2.68 s was found to consistently estimate the response to within several tenths of a percentage of its amplitude, and within about one-half second for all timing estimates.

However, the interleaved sampling procedure was consistently better at estimating the exact value for response amplitude and timing (specifically the time to onset). Two separate measures indicated this improvement. The first relates to how close the estimates for each sampling procedure were to the gold-standard estimates obtained from the pooled data with optimal temporal sampling. The interleaved estimates were both within one-tenth of a second for time to onset and time to peak and within one-tenth of one percent for amplitude (e.g., see Figs. 8A and 8B). In contrast, the sparse sampling estimates, which did not involve interleaved sampling, were nearly one-half of a percent and one-half of a second different from the gold standard estimates. While it is impossible in the present context to know whether the gold standard estimate truly represents the most valid estimate of the underlying response, it is our best guess and, holding the amount of data contributing to an estimate equal, interleaved sampling provided estimates closer to this gold standard.

The second measure that indicated an improvement for the interleaved sampling procedure was its reliability across separate data sets. As noted, two independent interleaved data sets were acquired in each subject, allowing two independent estimates of the response for an averaged group of subjects. The mean visual cortex estimates for amplitude, time to onset, and time to peak for the first data set were 2.49%, 2.03 s, and 4.98 s, respectively. These estimates for the second independent data set were 2.54%, 2.13 s, and 4.90 s, respectively. The largest difference was for the estimated time to onset, which was less than one-tenth of one second. Furthermore, the estimated shapes of the hemodynamic responses nearly overlapped across the two separate data sets (Fig. 8D). Thus, a benefit of the interleaved sampling procedure is conveyed to the degree that an application requires the extra level of precision in the hemodynamic response estimate. One possible application will be discussed below under Ordering of Processes between Brain Regions Based on Relative Timing Offsets. Other applications also exist (e.g., characterization of temporally fine aspects of the hemodynamic response such as the "pre-dip," Hu *et al.*, 1999, or the "post-undershoot," Buxton *et al.*, 1999). If precise amplitude and timing estimations are not required, a sampling rate of about $2\frac{1}{2}$ s would appear sufficient for most rapid presentation ER-fMRI applications that attempt to *detect* the robust positive deflection of the BOLD hemodynamic response.

Hemodynamic Response Estimation

A central component of the procedures employed in the present studies was automatically estimating the amplitude and timing of the hemodynamic responses. The basic model was a three-parameter γ function with an added delay (time to onset) parameter (Dale and Buckner, 1997; extended from Boynton *et al.*, 1996). The raw estimates of the hemodynamic response were fit to this function using a least-squares procedure. Estimates of amplitude, time to onset, and time to peak were then derived (see Fig. 2). Because of the importance of these estimates to the present paper, and the broad need for developing stable methods for quantifying event-related fMRI data, it is worth discussing the successes and failures associated with this procedure.

Overall, the data were well fit by a simple γ function. Figures 4 and 7, for example, show the raw data superimposed on the best fit model for each of the subjects. Most of the variance is accounted for and the fit appears face valid, as the model's peak approximates the data peak, and the model's timing estimate approximates the temporal changes in the data. However, two deviations from the model can also be observed, although these would not be expected to affect the amplitude or time-to-peak estimates.

First, the model failed to account for a poststimulus undershoot that was present in many data sets (e.g., the first three subjects, vc734, vc750, and vc751, in Fig. 4). This undershoot has been observed in event-related fMRI data previously (Boynton *et al.*, 1996; Dale and Buckner, 1997; Buckner *et al.*, 1998a) and may reflect a temporally lagged component of the hemodynamic

response relating to blood volume change (Mandeville *et al.*, 1996; Buxton *et al.*, 1998). Regardless of the origin, the model based on a single γ function did not account for the poststimulus undershoot. Indeed, given the basic form of a γ function, there is no way that it can model a second inflection in the data, such as is observed for the extended components of the response undershoot (Fransson *et al.*, 1998a,b). Second, on the rising portion of the response, the raw data showed an increase in signal intensity that was more spread out than provided for by the model. This could often be seen at about 2 s after the stimulus onset (e.g., vc750 and vc751 in Fig. 4 or vc518 and vc519 in Fig. 7). This latter component relating to the rise of the curve may have influenced the time-to-onset estimate, which was the least stable of the quantified variables, as discussed above in the context of variability.

One caveat about the fitting procedures employed is that, although the response estimates were initially computed in an assumption-free manner, regions for analysis were selected based on statistical maps generated by cross-correlation to a γ function. In this regard, voxels showing responses of significantly different shape—that deviate from a single peaked, relatively transient form—would be missed by the present analyses. Thus, the description and appropriateness of the fitting procedure employed may not generalize to all circumstances and responses. In the present study, there was a bias toward identifying a certain class of hemodynamic response shapes which, to our knowledge, is appropriate to visual and motor cortex and likely appropriate for many cortical regions.

Ordering of Processes between Brain Regions Based on Relative Timing Offsets

Perhaps the most significant implication of the aforementioned observations is the possibility of ordering the temporal cascade of processing across brain regions, a possibility raised previously by Menon and colleagues (1998). The present study was able to detect a significant interaction across the timing of regions (visual and motor cortex) in relation to whether a response was made first with one hand, then with the other. Specifically, as would be expected, the analyses showed an interaction between visual and motor cortex. Visual cortex showed no effect of response ordering while motor cortex was significantly influenced by the order of response, showing an increased delay when the contralateral response took longer. This result is not surprising but has potentially broad implications. Event-related fMRI is able to detect temporal offsets within regions and to further contrast *relative* temporal offsets across regions.

The emphasis on "relative" is important here because the absolute timing estimates seemed to vary in a sometimes unpredictable manner that may reflect differences in underlying vasculature. Thus, while the present results show how powerful evaluation of relative timing changes across regions may be, the results also cast doubt on always using the *absolute* measures of timing as reliable indices (see Friston *et al.*, 1998, for a similar point). Caution is suggested since some absolute timing estimates revealed delay ordering that seemed implausible in the present paradigm (e.g., motor cortex activating earlier than visual cortex). Furthermore, using *relative* timing estimates makes several implicit assumptions about the regions active in a task, namely, that the regions are hierarchically related to one another and that they directly participate in task completion. Examination of relative timing is likely to be a powerful tool but only for those circumstances in which reasonable assumptions can be made about the underlying functional anatomy.

The present study employed whole-brain functional imaging in the context of routinely used imaging parameters (a low-field 1.5-T scanner and 16-slice whole-brain acquisition across a TR of 2.50 or 2.68 s). The results obtained with these imaging parameters, which are similar to parameters used by many laboratories employing fMRI, suggest that examining temporal relations across brain regions should be possible by modifying the behavioral paradigms and image analysis procedures. Specifically, using some form of procedure to effectively increase the sampling rate (our interleaved procedure or that of Josephs *et al.*, 1997) coupled with a model fitting approach to estimate response times, appears sufficient to estimate relative delays of less than a second. There is a wide range of cognitive neuroscience questions that would benefit from this form of analysis. In addition, such procedures may serve as an anchor point for making between-modality comparisons between fMRI and MEG/EEG or fMRI and optical imaging methods.

ACKNOWLEDGMENTS

We thank Amy Sanders for help with data collection, Bill Kelley for help with implementation of the behavioral component of the study, and Erbil Akbudak, Abraham Snyder, and Thomas Conturo for support and development of the MRI procedures. Todd Braver provided valuable comments on an early draft of the manuscript. R.L.B. thanks Bruce Rosen and Robert Savoy for discussion that made this work possible. This work was supported by grants from the McDonnell Center for Higher Brain Function (to R.L.B.), NIH Grants MH57506 (to R.L.B.) and NS32979 (to S.E.P.), and a contract from the government (to Henry Roediger).

REFERENCES

Aguirre, G. K., Zarahn, E., and D'Esposito, M. D. 1998. The variability of human BOLD hemodynamic responses. *NeuroImage* **8:** 360–369.

Bandettini, P. A. 1993. MRI studies of brain activation: Dynamic characteristics. In *Functional MRI of the Brain*, pp. 144–151. Soc. of Magn. Reson. in Med., Berkeley, CA.

Bandettini, P. A. 1999. The temporal resolution of MRI. In *Functional MRI* (C. T. W. Moonen and P. Bandettini, Eds.), pp. 205–220. Springer-Verlag, Mauer, Germany.

Blamire, A. M., Ogawa, S., Ugurbil, K., Rothman, D., McCarthy, G., Ellerman, J. M., Hyder, F., Rattner, Z., and Shulman, R. G. 1992. Dynamic mapping of the human visual cortex by high-speed magnetic resonance imaging. *Proc. Natl. Acad. Sci. USA* **89:** 11069–11073.

Boynton, G. M., Engel, S. A., Glover, G. H., and Heeger, D. J. 1996. Linear systems analysis of functional magnetic resonance imaging in human V1. *J. Neurosci.* **16:** 4207–4221.

Buckner, R. L., Bandettini, P. A., O'Craven, K. M., Savoy, R. L., Petersen, S. E., Raichle, M. E., and Rosen, B. R. 1996. Detection of cortical activation during averaged single trials of a cognitive task using functional magnetic resonance imaging. *Proc. Natl. Acad. Sci. USA* **93:** 14878–14883.

Buckner, R. L., and Braver, T. S. 1999. Event related functional MRI. In *Functional MRI* (C. T. W. Moonen and P. Bandettini, Eds.), pp. 441–450. Springer-Verlag, Mauer, Germany.

Buckner, R. L., Goodman, J., Burock, M., Rotte, M., Koutstaal, M., Schacter, D. L., Rosen, B., and Dale, A. M. 1998a. Functional–anatomic correlates of object priming in humans revealed by rapid presentation event-related fMRI. *Neuron* **20:** 285–296.

Buckner, R. L., and Koutstaal, W. 1998. Functional neuroimaging studies of encoding, priming, and explicit memory retrieval. *Proc. Natl. Acad. Sci. USA* **95:** 891–898.

Buckner, R. L., Koutstaal, W., Schacter, D. L., Dale, A. M., Rotte, M. R., and Rosen, B. R. 1998b. Functional–anatomic study of episodic retrieval. II. Selective averaging of event-related fMRI trials to test the retrieval success hypothesis. *NeuroImage* **7:** 163–175.

Burock, M. A., Buckner, R. L., Woldorff, M. G., Rosen, B. R., and Dale, A. M. 1998. Randomized event-related experimental designs allow for extremely rapid presentation rates using functional MRI. *NeuroReport* **9:** 3735–3739.

Buxton, R. B., Wong, E. C., and Frank, L. R. 1998. Dynamics of blood flow and oxygenation changes during brain activation: The Balloon Model. *Magn. Reson. Med.* **39:** 855–864.

Buxton, R. B., Wong, E. C., and Frank, L. R. 1999. The post-stimulus undershoot of the functional MRI signal. In *Functional MRI* (C. T. W. Moonen and P. Bandettini, Eds.), pp. 253–262. Springer-Verlag, Mauer, Germany.

Clark, V. P., Maisog, J. M., and Haxby, J. V. 1998. fMRI study of face perception and memory using random stimulus sequences. *J. Neurophysiol.* **79:** 3257–3265.

Cohen, J. D., MacWhinney, B., Flatt, M., and Provost, J. 1993. PsyScope: A new graphic interactive environment for designing psychology experiments. *Behav. Res. Methods Instr. Comput.* **25:** 257–271.

Cohen, J. D., Perlstein, W. M., Braver, T. S., Nystrom, L. E., Noll, D. C., Jonides, J., and Smith, E. E. 1997. Temporal dynamics of brain activation during a working memory task. *Nature* **386:** 604–607.

Conturo, T. E., McKinstry, R. C., Akbudak, E., Snyder, A. Z., Yang, T. Z., and Raichle, M. E. 1996. Sensitivity optimization and experimental design in functional magnetic resonance imaging. *Soc. Neurosci. Abstr.* **22:** 7.

Dale, A. M., and Buckner, R. L. 1997. Selective averaging of rapidly presented individual trials using fMRI. *Hum. Brain Mapp.* **5:** 329–340.

D'Esposito, M., Zarahn, E., Aguirre, G. K., and Rypma, B. 1999. The effect of normal aging on the coupling of neural activity to the BOLD hemodynamic response. *NeuroImage* **10:** 6–14.

Dymond, R., Norris, D. G., Pollmann, S., and Zysset, S. 1999. Application of double voxel functional spectroscopy to event-related cognitive experiments. *Magn. Reson. Med.* **41:** 217–223.

Fransson, P., Krüger, G., Merboldt, K. D., and Frahm, J. 1998a. Temporal characteristics of oxygenation-sensitive MRI responses to visual activation in humans. *Magn. Reson. Med.* **39:** 912–919.

Fransson, P., Krüger, G., Merboldt, K. D., and Frahm, J. 1998b. Physiologic aspects of event related paradigms in magnetic resonance functional neuroimaging. *NeuroReport* **9:** 2001–2005.

Fransson, P., Krüger, G., Merboldt, K. D., and Frahm, J. 1999. Temporal and spatial MRI responses to subsecond visual activation. *Magn. Reson. Med.* **17:** 1–7.

Friston, K. J., Fletcher, P., Josephs, O., Holmes, A., Rugg, M. D., and Turner, R. 1998. Event-related fMRI: Characterizing differential responses. *NeuroImage* **7:** 30–40.

Friston, K. J., Josephs, O., Rees, G., and Turner, R. 1997. Nonlinear event-related responses in fMRI. *Magn. Reson. Med.* **39:** 41–52.

Glover, G. H. 1999. Deconvolution of impulse response in event-related BOLD fMRI. *NeuroImage* **9:** 416–429.

Hu, X., Yacoub, E., Le, T. H., Cohen, E. R., and Ugurbil, K. 1999. Event related functional MRI. In *Functional MRI* (C. T. W. Moonen and P. Bandettini, Eds.), pp. 243–252. Springer-Verlag, Mauer, Germany.

Josephs, O., Turner, R., and Friston, K. 1997. Event-related fMRI. *Hum. Brain Mapp.* **5:** 243–248.

Kim, S. G., Richter, W., and Ugurbil, K. 1997. Limitations of temporal resolution in functional MRI. *Magn. Reson. Med.* **37:** 631–636.

Konishi, S., Yoneyama, R., Itagaki, H., Uchida, I., Nakajima, K., Kato, H., Okajima, K., Koizumi, H., and Miyashita, Y. 1996. Transient brain activity used in magnetic resonance imaging to detect functional areas. *NeuroReport* **8:** 19–23.

Lee, A. T., Glover, G. H., and Meyer, C. H. 1995. Discrimination of large venous vessels in time-course spiral blood-oxygen-level-dependent magnetic-resonance functional neuroimaging. *Magn. Reson. Med.* **33:** 745–754.

Mandeville, J. B., Marota, J., Keltner, J. R., Kosofsky, B., Burke, J., Hyman, S., LaPointe, L., Reese, T., Kwong, K., Rosen, B. R., Weissleder, R., and Weisskoff, R. 1996. CBV functional imaging in rat brain using iron oxide agent at steady state concentration. In *Proceedings of the Society of Magnetic Resonance Fourth Scientific Meeting and Exhibition*, Vol. 3, p. 292.

McCarthy, G., Luby, M., Gore, J., and Goldman-Rakic, P. 1997. Infrequent events transiently activate human prefrontal and parietal cortex as measured by functional MRI. *J. Neurophysiol.* **77:** 1630–1634.

Menon, R. S., Luknowsky, D. C., and Gati, G. S. 1998. Mental chronometry using latency-resolved functional MRI. *Proc. Natl. Acad. Sci. USA* **95:** 10902–10907.

Pollmann, S., Wiggins, C. J., Norris, D. G., von Cramon, D. Y., and Schubert, T. 1998. Use of short intertrial intervals in single-trial experiments: A 3T fMRI study. *NeuroImage* **8:** 327–339.

Press, W. H., Teukolski, S. A., Vetterling, W. T., and Flannery, B. P. 1992. *Numerical Recipes in C: The Art of Scientific Computing.* Cambridge Univ. Press, New York.

Price, C. J., Veltman, D. J., Ashburner, J., Josephs, O., and Friston, K. J. 1999. The critical relationship between the timing of the stimulus presentation and data acquisition in blocked designs with fMRI. *NeuroImage* **10:** 36–44.

Robson, M. D., Dorosz, J. L., and Gore, J. C. 1998. Measurements of the temporal fMRI response of the human auditory cortex to trains of tones. *NeuroImage* **7:** 185–198.

Rosen, B. R., Buckner, R. L., and Dale, A. M. 1998. Event related fMRI: Past, present, and future. *Proc. Natl. Acad. Sci. USA* **95:** 773–780.

Ross, M. H., Yurgelun-Todd, D. A., Renshaw, P. F., Maas, L. C., Mendelson, J. H., Mello, N. K., Cohen, M. D., and Levin, J. M. 1997. Age-related reduction in functional MRI response to photic stimulation. *Neurology* **48:** 173–176.

Savoy, R. L., Bandettini, P. A., O'Craven, K. M., Kwong, K. K., Davis, T. L., Baker, J. R., Weiskoff, R. M., and Rosen, B. R. 1995. Pushing the temporal resolution of fMRI: Studies of very brief visual stimuli, onset variability and asynchrony, and stimulus-correlated changes in noise. In *Proceedings of the Society of Magnetic Resonance Third Scientific Meeting and Exhibition*, Vol. 2, p. 450.

Schacter, D. L., Buckner, R. L., Koutstaal, W., Dale, A. M., and Rosen, B. R. 1997. Late onset of anterior prefrontal activity during true and false recognition: An event-related fMRI study. *NeuroImage* **6:** 259–269.

Snyder, A. Z. 1996. Difference image versus ratio image error function forms in PET–PET realignment. In *Quantification of Brain Function Using PET* (D. Bailey and T. Jones, Eds.). Academic Press, San Diego.

Taoka, T., Iwasaki, S., Uchida, H., Fukusumi, A., Nakagawa, H., Kichikawa, K., Takayama, K., Yoshioka, T., Takewa, M., and Ohishi, H. 1998. Age correlation of the time lag in signal change on EPI–fMRI. *J. Comput. Assisted Tomogr.* **22:** 514–517.

Vazquez, A. L., and Noll, D. C. 1998. Nonlinear aspects of the BOLD response in functional MRI. *NeuroImage* **7:** 108–118.

Wagner, A. D., Schacter, D. L., Rotte, M., Koutstaal, W., Maril, A., Dale, A. M., Rosen, B. R., and Buckner, R. L. 1998. Building memories: Remembering and forgetting of verbal experiences as predicted by brain activity. *Science* **281:** 1188–1191.

Zarahn, E., Aguirre, G., and D'Esposito, M. 1997. A trial-based experimental design for fMRI. *NeuroImage* **6:** 122–138.