The kinetics of the BOLD response depend on inter-stimulus time

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Numerous parameters such as subject age, region of activation, and stimulus timing are known to affect the BOLD signal following neural activation. Here, we investigated how differences in the rest time between successive long visual stimuli alter the kinetics of the BOLD signal in the visual cortex. We found that the BOLD rise time varies with the inter-stimulus interval. By taking this into account when performing statistical analyses of BOLD data, we show that a roughly 20% increase in statistical power can be achieved. In addition, the dependence of the BOLD signal rise time on the inter-stimulus interval provides insight into the physiology underlying the post-stimulus undershoot.

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Introduction

Event-related functional MRI has become a popular method for studying patterns of neural activation in a wide variety of mental tasks (Dale and Buckner, 1997; Josephs et al., 1997; Kim et al., 1997; Richter et al., 1997; Rosen et al., 1998). Identification of neural activity is frequently accomplished through methods that assume a standard shape for the extended BOLD response (Friston et al., 1995; Josephs and Henson, 1999; Kruggel and von Cramon, 1999; Hinrichs et al., 2000). However, this simplifying assumption necessarily produces conservative results, and improved statistical power can be achieved through the use of regressors that take into account physiological dependencies of the BOLD response. Here, we investigate the dependence of signal recovery to a steady-state activated equilibrium on the length of the inter-stimulus interval (ISI), defined as the duration of the resting period between successive stimuli.

BOLD signal transients such as the post-stimulus undershoot have been hypothesized to occur in one of two ways: (1) the cerebral metabolic rate of oxygen consumption (CMRO2) and cerebral blood flow (CBF) are closely coupled while the cerebral blood volume (CBV) response lags the CBF and CMRO2, or (2) CBF and CBV are closely coupled while the CMRO2 response lags the CBV and CBF (Buxton, 2002; Lu et al., 2004).

Studies using a rat model show that following the onset of a long stimulus (30 s), an increase in cerebral blood flow (CBF) results in an increase in the venous cerebral blood volume (CBV) that lags the flow increase (Mandeville et al., 1999). After the stimulus is terminated, the return of CBF to a resting rate precedes the return of CBV to baseline levels (Mandeville et al., 1999). According to Mandeville et al. (1999), the time constant for the CBF response to stimulus onset and offset was 2.4 ± 0.8 s and the time constants for the CBV response were 1.9 ± 0.7 s (fast response) and 14 ± 13 s (slow response). The return of CBV to baseline, which can be explained by the mechanics of the vessel walls, lags the return of flow to baseline by approximately 10 s (Mandeville et al., 1999).

When the ISI is shorter than the duration of the post-stimulus undershoot, the subsequent BOLD response to visual (Pollmann et al., 1998) as well as motor stimuli (Bandettini and Cox, 2000) shows decreased magnitude for stimuli lasting a few seconds. Here, we investigate what effect, if any, the post-stimulus undershoot has on the kinetics of BOLD activations to subsequent stimulation.

Materials and methods

Subjects

This experiment was approved by the Princeton University Institutional Review Panel for human subjects. Subjects were recruited from the university community. In a first experiment, we imaged 20 subjects and excluded 7 because of excessive motion (greater than 2 mm head movement). The remaining 13 subjects (mean age 20; range 10–30 years; standard deviation 6 years; 8 male) were scanned a second time to improve signal to...
noise ratio. All resulting 26 data sets are included in the analysis.

In a second, follow-up experiment (see below; also Fig. 4), we scanned 6 subjects (mean age 25; range 22–30; standard deviation 3 years, 3 male) on two separate occasions; we did not have to exclude any data sets in this experiment.

Experimental design

Functional imaging experiments were carried out with a 3-T Siemens Allegra head-only MRI system using a circularly polarized head volume coil. The visual stimuli were displayed on a rear projection screen and viewed by the subjects through a mirror attached to the head coil.

The stimulus paradigm alternated between a monochrome full-field checkerboard, reversing at 8 Hz, and a gray screen as the resting condition. A red cross was displayed in the center of the screen for fixation throughout the experiment. Stimuli were presented using E-Prime software (Psychology Software Tools, Inc., Pittsburgh, PA) on a commercial personal computer.

In the first experiment, the reversing checkerboard stimulus was displayed for periods of 60 s. The resting condition (ISI) was displayed for either 60 s, 20 s, 10 s, 5 s, or 2 s (inspired by Fransson et al., 1998). When the ISI was 60 s, the stimulus paradigm consisted of only 3 periods of oscillating checkerboards and 2 periods of resting condition (in order to limit the time for each run), and for all other ISIs of 4 periods of oscillating checkerboard and 3 periods of resting condition.

In the second experiment, we replicated our results with repeated short-duration stimuli to test if this finding would apply to event-related experimental designs as well. For these experiments, each stimulus block consisted of flashing checkerboards displayed for 1 s at a time separated by 1 s of gray display, for a total of 60 s. The stimulus blocks were separated here by ISIs of 60 s, 20 s, 10 s, 5 s, and 2 s.

Each functional scanning session comprised a total of 10 runs (two for each value of the ISI). In all experiments, we recorded simultaneous timing information on stimulus display through the PC running E-Prime, and on the acquisition of each imaging volume through a digital signal sent by the scanner. This timing information allowed us to perform time-locked averaging for all tasks in post processing of the data.

Imaging parameters

High-resolution (1 mm³) T1-weighted structural images were acquired with an MP-RAGE pulse sequence at the beginning of each scanning session. The calcarine sulcus was identified in these images by inspection. A slab comprising five axial/coronal oblique slices (3 mm thick, 1 mm gap) containing the calcarine sulcus was scanned in the functional imaging experiments. Functional data were acquired with EPI pulse sequences (TR = 297 ms, TE = 30 ms, matrix size 64 × 64 × 5, voxel size 3 × 3 × 3 mm³). We selected a TR of 297 ms because it is the fastest possible temporal resolution achievable on our scanner for five slices and a TE of 30 ms.

Data analysis

Since our focus was on the return of the BOLD signal to the activated state after the resting period and not on the initial rise, we removed the first 50 volumes to account for T1 stabilization. Data were motion corrected using MCFLIRT (Jenkinson et al., 2002) and subsequently analyzed using home-written MATLAB programs (The MathWorks, Inc., Natick, MA). Active voxels were identified through a cross-correlation analysis with a boxcar (on/off) regressor fit to data from experiments with 60 s ISI. This boxcar function consisted of 5 periods of 50 points each and was correlated with 5 periods of 50 volumes from the data. The 50 volumes for correlation with the “on” part of the boxcar function were taken from the end of the steady-state activation period. Similarly, the 50 volumes for correlation with the “off” part of the boxcar function were taken from the end of the resting period.

This correlation procedure mitigates the influence of the hemodynamic response transient periods, and the resultant activation maps are expected to be relatively independent of the exact temporal form of the hemodynamic response. We examined time courses from the following four thresholds: a correlation coefficient of 0.4, 0.5, and 0.6, and also the ten most highly correlated voxels. We carried out a two-way ANOVA on time courses generated from the above-listed thresholds. Group-averaged time courses from these four thresholds were not significantly different from each other (F = 2.002; P = 0.1296) and we finally defined the threshold for significant activation at a correlation coefficient of 0.5, corresponding to t > 8.13 or P < 10⁻¹⁴ (uncorrected for multiple comparisons). This significance level corresponds to P < 10⁻⁶ after Bonferroni correction. For each subject, cross-correlation images of the same size as our functional data were generated using only the two 60 s experiments and the boxcar regressor. To obtain the mask, the cross-correlation images were subjected to a threshold of cc = 0.5, and the voxels that exceeded this threshold were used for all other experiments for that subject. An example cross-correlation image is shown in Fig. 1A.

Voxels that contain draining veins should exhibit delayed BOLD responses and would thus have increased recovery times (de Zwart et al., 2004). To eliminate this artifact, we removed voxels putatively containing draining veins by excluding voxels based on the standard deviation of their time course during the resting period. Voxels with baseline variance exceeding two standard deviations from the mean of all voxels were excluded (Ravi Menon, personal communication). Time courses from active voxels were normalized and time-locked averaged for all experiments within subject for each ISI. A group average was also generated for both experimental conditions (Fig. 1).

Modeling

We first modeled our data with the balloon model (Buxton et al., 1998). In the balloon model, intravascular sources to the BOLD signal (e.g., venous vessels) are modeled as an elastic compartment. The overall BOLD signal is approximated by the following equation (Obata et al., 2004):

\[
\frac{\Delta S}{S} = \frac{V_0}{a_1 (1 - q) - a_2 (1 - v)}
\]

where \( V_0 \) is the resting volume fraction and the parameters \( a_1 \) and \( a_2 \) depend on physiology and imaging parameters (Obata et al., 2004). Changes in the amount of deoxyhemoglobin and blood volume are modeled by the differential equations:

\[
\begin{align*}
\frac{dg}{dt} &= \frac{1}{\tau_0} \left[ f_{in}(t) - \frac{E(t)}{E} - f_{out}(v) \right] q(t) \\
\frac{dv}{dt} &= \frac{1}{\tau_0} \left[ f_{in}(t) - \frac{E(t)}{E} - f_{out}(v) \right] v(t)
\end{align*}
\]
and
d\frac{dv}{dt} = \frac{1}{\tau_0} [f_{in}(t) - f_{out}(v)]

where $q$ is the amount of deoxyhemoglobin, $\tau_0$ is the mean transit time in the capillaries, $f_{in}$ is flow into the voxel, $E$ is the oxygen extraction fraction, $f_{out}$ is flow out of the voxel (we used the nonlinear form of $f_{out}$ used in Mildner et al., 2001), and $v$ is blood volume. All values are normalized to their baseline values (Buxton et al., 1998). Adaptations of balloon model parameters ($a_1, a_2$) for 3 T from Mildner et al. (2001) were used in this study.

The second model used to fit our data differed from the balloon model in how it fit the rise of the BOLD signal. We modeled the BOLD signal time course from the point when the stimulus was presented to the end with a single exponential. This combination of models was able to fully capture our entire data with high significance (goodness of fit is shown in Fig. 2).

A single exponential was used to model the dynamics of the ratio of deoxygenated hemoglobin and oxygenated hemoglobin when inflow increases to a given volume. During the rest period between visual stimulations, we assumed that the ratio of oxygenated hemoglobin (oxHb) to deoxygenated hemoglobin (dHb) is given by $f_{R} ([dHb]/[oxHb])$. Furthermore, we assumed that the venous volume at the time of the second visual stimulus is given by $V$. Because of the vascular property of delayed compliance, CBF and CBV return to baseline levels at different rates (Mandeville et al., 1999). The assumption that the volume is constant over a time interval $\Delta t$, if $\Delta t$ is on the order of tenths of a second (approximately our measurement frequency), is reasonable given that volume changes occur on a much slower time scale than flow and mixing changes.

Let $f_{A}$ be the fraction of dHb to oxHb in the fresh incoming blood supply which fills $V$ with flow rate $F$ (note that in general $f_{A}$ will be less than $f_{R}$ due to reduced oxygen extraction with increased flow rate). The fraction of $V$ replaced by $F$ in an increment of time $\Delta t$ is given by

$$p = \frac{F \Delta t}{v}.$$  

If we assume that the incoming blood supply fully mixes with the existing blood in $V$ in the time $\Delta t$, then the ratio ($f$) of dHb to oxHb will change according to:

$$f(t + \Delta t) = (1 - p) f(t) + p f_{A}.$$
or
\[ f(t + \Delta t) - f(t) = p(f_\alpha - f(t)). \]

By substituting in for \( p \), we arrive at the differential equation
\[ \dot{f} = \frac{F}{V} (f_\alpha - f) \]
whose solution is
\[ f(t) = f_\alpha - (f_\alpha - f_0) e^{-t/F}. \]

From this equation, it is evident that the amount of oxHb increases exponentially while the amount of dHb decreases exponentially when inflowing blood mixes with blood in a given volume. It is also important to note that \( p \) varies depending on the size of \( V \). If \( V \) is small (i.e., the vasculature has had sufficient time to relax or the flow increase arrives towards the end of the post-stimulus undershoot) then \( p \) is large because a given amount of inflowing blood will displace a larger percentage of the blood from the small venous compartment.

Fits for both models were calculated using MATLAB’s “fminsearch” function which is based on the simplex optimization method (The MathWorks, Inc.; Lagarias et al., 1998). Parameters used for modeling were the onset and offset time of both the stimulus and the balloon model, the signal magnitude (defined here as the difference between steady-state activation and the deepest part of the post-stimulus undershoot), \( \tau_u \), an undershoot parameter specific to the balloon model at 3 T (Mildner et al., 2001), and the exponential rise time constant. All time courses were modeled within subject and then the group average was calculated for the rise time constants. Goodness of fit was determined by correlation analysis with the time course data and model function; goodness of fit results are shown in Fig. 2.

**Results**

Figs. 1B and C show the time-locked averages for all experiments averaged across all subjects. The rise time of the BOLD response increases with decreasing ISI as is shown by the slower return to the activated state for time courses with shorter ISI.

In order to better capture and test these results, we first fit acquired BOLD data with the balloon model (Buxton et al., 1998). The flow_in function consisted of two 60-s flow responses separated by 10 s. Both flow responses were assumed to have identical form (Uludag et al., 2004). Though we used a nonlinear form of \( f_{out} \) to ensure transient behavior in the BOLD response (Buxton et al., 1998; Mildner et al., 2001), the balloon model was unable to account for the altered rise times seen in our data as shown in Fig. 3. The balloon model predicts the decreased BOLD signal magnitude but not the increased rise time with shorter ISIs (Fig. 3B).

We modeled the rise of the BOLD signal after onset of a subsequent stimulus as a single exponential to account for how blood flow into a volume affects the ratio of oxHb to dHb through time (see Materials and methods for derivation). This was done for each ISI within subject. Rise time constants increase with decreasing ISI as illustrated in Fig. 4. The time constant for ISIs of 2 s and 5 s is significantly longer than the time constant for the longer ISIs (\( P > 0.001 \) for each paired \( t \) test; time constant not significantly different for 2 s and 5 s ISI, \( P = 0.21 \); time constant not significantly different for ISI of 20 s, 60 s, \( P = 0.12 \)).

The results developed thus far may apply not only to long-duration stimuli, but, assuming that our hypothesized mechanism is accurate, also to blocks of repeated short stimuli (such as the stimulus presentation in Haxby et al., 2001). This latter stimulus pattern is of interest to event-related experimental designs. Based on this reasoning, we conducted a second experiment in which we replaced the continuous 60-s stimulus with a 60-s stimulus block consisting of the alternation of a 1-s stimulus and 1-s rest period. Stimuli blocks were separated by ISIs of 60 s, 20 s, 10 s, 5 s, and 2 s. Fig. 4 shows that the rise time of the BOLD responses to stimuli increases with decreasing ISI regardless of whether the stimulus is continuous or consists of repeated short stimuli.

To determine the effect on data analysis of the increased rise time with decreased ISI, we generated ROC curves for each ISI.
that compare a regressor created from our modeling analysis results, a gamma function (Cohen, 1997), and the SPM hemodynamic response function (Friston et al., 1995; Wellcome Department of Cognitive Neurology, London, UK). To generate the ROC curves, correlation analyses were performed on all runs for all subjects (shown in Fig. 5). We set the “ground truth” to be the number of voxels that correlated with our original regressor, the boxcar function, at a range of $P$ values ($10^{-12}$, $10^{-8}$, $10^{-4}$, $10^{-3}$, $10^{-2}$) for each run for all subjects. The total number of active voxels at each $P$ value was determined for each regressor and the number of true positives was calculated by determining how many voxels overlapped with active voxels from the regression with the boxcar function. False positives were determined by subtracting the number of true positives from the total number of active voxels.

The ROC curves for an ISI of 60 s, 20 s, and 10 s do not show a large difference between the gamma function regressor, the SPM regressor, and the ISI-dependent regressor. However, the ROC curves for the ISIs most commonly used in event-related (ER) fMRI, 5 s and 2 s, show marked differences between the three regressors: the ISI-dependent regressor outperforms both the gamma function and SPM regressor. Our findings that the rise time dependency does not greatly affect regressor performance until the ISI used is 5 s or less are supported by Pollmann et al. (1998) who report that the frequency of activation was not affected until their inter-trial interval decreased below 6 s.

Discussion

Results from data analysis in ER-fMRI experiments depend upon the regressor used. Programs such as AFNI, SPM, FSL, and BrainVoyager all assume a canonical shape for the hemodynamic response function (HRF) used in regressor creation. Numerous studies report that the BOLD response is not constant, but varies regionally (Rostrup et al., 2000; Henson et al., 2002; Huettel and McCarthy, 2001; Obata et al., 2004), with subject age (Richter and Richter, 2003; Riecker et al., 2003), and with experimental design (Pollmann et al., 1998; Bandettini and Cox, 2000). Use of a

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![Balloon model for two 60-s stimuli with 10-s ISI](image1)

![Rise time depends on inter-stimulus interval](image2)

Fig. 3. Balloon model for two 60-s stimuli with 10-s ISI. (A) Second stimulus (red time course) is presented during post-stimulus undershoot of preceding stimulus (blue time course). (B) Realignment of the BOLD response to the second stimulus (red time course) with the BOLD response from the first stimulus (blue time course) illustrates that the balloon model in its current form cannot explain changes in BOLD response rise time.

Fig. 4. Rise time depends on inter-stimulus interval. Y axis: exponential rise time constant. X axis: inter-stimulus interval (s). Continuous 60 s stimulation (diamonds): Rise time constants for each inter-stimulus interval were averaged across all subjects ($n = 13$, 26 data sets). Error bars are SEM. Stimulus blocks of 1 s stimuli and 1 s rest (triangles): Rise time constants for each inter-stimulus interval were averaged across subjects ($n = 6$, 12 data sets). Error bars are SEM. Note that according to our explanation for the origin of the rise time dependency on the ISI, it follows that for a smaller volume the rise time constants are shorter (see Materials and methods).
standard HRF kernel provides a conservative measurement of information retrievable from fMRI data. Our regressor comparison analysis shows that incorporation of the physiology underlying the BOLD response can improve statistical power significantly.

The improved statistical power achieved by taking into account ISI is most obviously relevant for experiments using tasks with long stimulus durations as used in this study. However, our results can be extrapolated to certain event-related experimental designs such as experiments that utilize a series of short stimuli in rapid succession with insufficient rest in between stimuli (i.e., 1 s stimulus, 1 s resting period) alternated with a longer resting period (Haxby et al., 2001). We show in Fig. 4 that the BOLD responses from this event-related design approach the BOLD responses from a stimulus of long duration with short resting periods. In these instances, modifying the temporal dynamics of the regressor used could improve detection of activated voxels at more stringent thresholds.

To account for our observed effect of ISI on BOLD rise time, we hypothesize that the increase in rise time with decreasing ISI can be explained considering how the flow of blood into a volume affects the BOLD signal. We explain our data in terms of CBV lagging CBF and CMRO2 because this explanation relies on fewer assumptions than CMRO2 lagging CBF and CBV. In order to explain our results in terms of CMRO2 lagging CBF and CBV, we would have to make complex assumptions about neuronal firing when ionic gradients are potentially in dis-equilibrium during the shorter ISI experiments (Lu et al., 2004). Instead, we derive a simple explanation for our results considering volume dynamics. In Materials and methods, we derive a differential equation that shows that a flow increase results in an exponential increase in the amount of oxHb and an exponential decrease in the amount of dHb. This behavior provides a physiological explanation for the dependency of rise time on ISI. Inflow into a volume of blood results in instantaneous mixing of the inflowing oxygenated blood with the already present more deoxygenated blood. The inflowing blood also displaces an amount of blood from the venous compartment. As the volume of blood gets larger, the required time for mixing and displacement increases; this is the case when the ISI is short. With a short ISI, the vasculature has insufficient time to return to a baseline state, and the CBV remains elevated. Presentation of a stimulus during the post-stimulus undershoot of a previous BOLD response introduces a flow increase to a large volume of blood and the subsequent BOLD response will have a rise time that is delayed due to mixing effects.

The above scenario can also account for the differences between rise times from a constant 60-s stimulus and a 60-s stimulus block consisting of the alternation of a 1-s stimulus and 1-s rest period. The latter stimulus generates a BOLD response with a post-stimulus undershoot smaller than the post-stimulus undershoot of the former stimulus (i.e., the latter stimulus generates a smaller CBV change than the former stimulus; data not shown). From our explanation for the origin of the rise time dependency on ISI, it also follows that for a smaller volume the rise time constants are shorter.

Based on our hypothesis that the BOLD rise time dependence on ISI is primarily a volume effect, an average time constant of 14.44 s for blood volume changes can be estimated. However, the BOLD signal depends on complex interactions of CBF, CBV, and CMRO2; more detailed measurements of the CBF, CBV, and CMRO2 responses to our stimulus paradigm are needed to determine the precise influence of CBV changes in our results.

In summary, we have argued that the kinetics of the BOLD response to subsequent stimuli presented during the post-stimulus undershoot can be attributed to the exponential decrease in the amount of dHb when oxygenated blood is flowing into a volume. Though our data are well explained by models relying on flow and

![Fig. 5. Comparison of a gamma function regressor, the SPM hemodynamic response function, and the ISI-dependent regressor.](image)
vascular dynamics, we hypothesize that the BOLD post-stimulus undershoot is the result of multiple physiological processes (Lu et al., 2004). Studies interrogating the CBF and CBV response to our stimulus paradigm are necessary and underway.

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References


