BOLD responses in human auditory cortex are more closely related to transient MEG responses than sustained ones

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\textbf{Running head}: BOLD versus MEG time series in auditory cortex
Abstract

BOLD-fMRI and MEG signals are both coupled to postsynaptic potentials (PSPs) but their relationship is incompletely understood. Here, the wide range of BOLD-fMRI and MEG responses produced by auditory cortex was exploited to better understand the BOLD-fMRI/MEG relationship. Measurements of BOLD and MEG responses were made in the same subjects using the same stimuli for both modalities. The stimuli, 24-s sequences of click trains, had duty cycles of 2.5, 25, 72 and 100%. For the 2.5% sequence, the BOLD response was elevated throughout the sequence while for 100%, it peaked after sequence onset and offset and showed a diminished elevation in between. On the finer time-scale of MEG, responses at 2.5% consisted of a complex of transients, including N1m, to each click train of the sequence, whereas for 100%, the only transients occurred at sequence onset and offset between which there was a sustained elevation in MEG signal (a sustained field). A model that separately estimated the contributions of transient and sustained MEG signals to the BOLD response best fit BOLD measurements when the transient contribution was weighted 8 - 10 times more than the sustained one. The findings suggest that BOLD responses in the auditory cortex are tightly coupled to the neural activity underlying transient, not sustained, MEG signals.
Introduction

Post-synaptic potentials (PSP) are thought to drive much of the blood oxygen level dependent (BOLD) response which can be mapped with millimeter spatial resolution by functional magnetic resonance imaging (fMRI) (Logothetis et al., 2001). They are also the source of the high temporal-resolution signals detected with MEG (Creutzfeld et al., 1966; Hämäläinen et al., 1993). The common activity (PSPs) underlying both fMRI BOLD and MEG signals and the differential sensitivity of these techniques for the spatial and temporal details of this activity, respectively, imply that a composite and highly informative view of post-synaptic brain activity may be obtained by "fusing" fMRI and MEG data so as to benefit from both. While PSPs likely underlie both BOLD and MEG responses, there are a number of factors that preclude a simple relationship between the two modalities. For example, significant uncertainty stems from cancellation of excitatory and inhibitory PSP (EPSP & IPSP) in MEG, as well as from cancellation between neighboring sites, depending on details of the individual anatomy (Ahlfors & Simpson, 2004). Currently, there is no explicit model to relate BOLD fMRI and MEG responses at the macroscopic level, leaving considerable uncertainty for approaches aiming at their integration.

The present study examined the relationship between BOLD-fMRI and MEG by comparing BOLD and MEG signal time-courses in the auditory cortex (AC). In response to a prolonged sequence of sound stimuli, the BOLD response in AC can range from being elevated throughout the sequence to highly phasic, with prominent peaks after sequence onset and offset (Harms & Melcher, 2002). These response patterns depend on temporal parameters of the sequence, such as the interval between stimuli
comprising the sequence (inter-stimulus interval, ISI), the duration of the individual stimuli, or stimulus repetition rate (Harms and Melcher, 2002; Harms et al., 2005; Giraud et al., 2000). These factors also influence MEG signals from AC (Hari et al., 1982; Imada et al., 1997), which, like BOLD responses, comprise both sustained elevations and transients (Hari et al., 1980; Pantev et al., 1996), but on a much finer temporal scale. The rich variety of BOLD and MEG responses to sounds make the auditory cortex an excellent test-bed to study the relationship between the two. While a few studies have compared MEG and fMRI measures from AC (Woldorff et al., 1999; Mathiak et al., 2002; Jääskeläinen et al., 2004; Lin et al., 2004; Ahveninen et al., 2007; Gutschalk et al., 2007), none has exploited the uniquely wide variety of waveforms produced by this brain area.

In this study, BOLD-fMRI and MEG were measured in the same subjects using the same stimuli so the two types of data could be directly compared. Stimuli were sequences of click trains, which are known to produce robust responses in both MEG and fMRI (e.g. Gutschalk et al., 2004; Harms et al., 2005). In order to produce a wide variety of BOLD time courses and MEG source waveforms, the click trains were presented in sequences with four different duty cycles controlled by adjusting ISI and train duration. The results indicate that transient MEG activity, rather than sustained, is predominantly reflected in the BOLD signal time courses.
Methods

Subjects

We report data from 6 subjects (mean age 27.7, range 20-35 years, 3 female, 3 male).

Subjects provided written informed consent before their participation. The study was approved by the review boards of Massachusetts Eye and Ear Infirmary, Massachusetts General Hospital, and Massachusetts Institute of Technology.

Stimulus presentation

The stimuli were presented in a block paradigm during both fMRI and MEG; 24 s of binaural sound stimulation were alternated with silent periods of equal duration.

Stimulus level at the ear was 75 dB SPL. During fMRI, stimuli were presented via piezoelectric headphones (GEC Marconi, UK). The scanner's coolant pump was switched off during fMRI to eliminate the background acoustic noise it produces (Ravicz and Melcher, 2001). For MEG, the stimuli were delivered via foam earpieces connected to 3-m-long plastic tubes. The sound was created by a speaker system outside of the magnetically shielded room (UNIDES ADU1b, Helsinki, Finland). During both MEG and fMRI, subjects watched a self selected silent movie without subtitles and were asked not to attend to the auditory stimuli.

Eight click trains were presented during each 24-s stimulation period resulting in a stimulus onset asynchrony (SOA) of 3 s. Train duration, held constant during any given 24-s period, was either 75 ms, 750 ms, or 2150 ms. The clicks of each train were presented at a rate of 500 Hz. An additional stimulus condition comprised a continuous, 24-s train of 500-Hz clicks. Thus, there were four stimulus conditions with duty cycles of
2.5%, 25%, 72% or 100%. The stimulus conditions, separated from one another by 24-s periods of no stimulation, were presented in pseudo-randomized order.

The stimuli for this experiment were generated at a 24-kHz sampling rate. The duration of each click was one sample. Each generated 24-s sequence was low-pass filtered at 10 kHz (high-frequency roll-off: 18 dB/oct.).

**Data Acquisition**

MRI data were acquired with a 3T head-and-neck scanner (Magnetom Allegra, Siemens, Erlangen, Germany) equipped with a standard, bird-cage head coil. First, two whole-head anatomical MPRAGE sequences were acquired (sagittal in-plane resolution 256x256; field of view (FOV) = 256x256 mm; slice thickness 1.3 mm). Based on these images, the volume for functional imaging was chosen as 11 near-coronal slices (perpendicular to the Sylvian fissure) covering auditory cortex from the posterior end of planum temporale to the anterior aspect of the superior temporal gyrus, including Heschl's gyrus in both hemispheres. For co-registration, T2-weighted anatomical images were obtained for the same volume with a high in-plane resolution [387x387; FOV = 200x200 mm]. Functional imaging was performed using an echo-planar imaging sequence [gradient echo; echo time (TE)=30 ms; flip angle=90°; in plane resolution = 64x64; FOV = 200x200 mm; slice thickness=4mm; gap=1.32mm]. The repetition time (TR) between functional acquisitions was 8 s, and the acquisitions were compressed into ~1 sec long clusters to prevent effects of imager acoustic noise on brain activation (Edminster et al., 1999; Hall et al., 1999). To allow for the reconstruction of the time course of activation, the timing of image acquisition relative to the sound stimulus was staggered by two seconds across stimulation periods (Belin et al., 1999).
MEG data were acquired with a Neuromag Vectorview system (Elekta Neuromag Oy, Helsinki, Finland), which comprises 204 planar gradiometers and 102 magnetometers at 102 positions, equally spaced around the head. The system is housed in a six-layer magnetically shielded room with active shielding (Cohen et al., 2002). The data were sampled continuously at 600 Hz with a 160-Hz low-pass filter, and without high-pass filtering (direct coupled, DC). The data were averaged across all presentations of a given condition, excluding epochs with amplitudes exceeding 5000 fT/cm (considered artifact). Prior to MEG, four head position indicator (HPI) coils were fixed to the subject’s head and their location was digitized relative to landmarks on the head surface. The positions of the HPI coils were detected by the Neuromag system at the beginning of the MEG session allowing registration of the location and orientation of the head with respect to the MEG sensor array. Eye movements were recorded from four electrodes fixed to the outer canthi and above and below the left eye.

**Data Analysis**

Activation maps for the fMRI data were based on a multivariate, linear regression analysis using basis functions designed to capture the range of BOLD response time courses produced by auditory cortex (Harms and Melcher, 2003). All epochs of stimulation were contrasted with epochs of no stimulation. The resulting activation maps were coregistered with the whole head T1-weighted volumes, which were processed with Freesurfer (Athinoula A. Martinos Center for Biomedical Imaging, Charlestown, MA) to create an inflated projection of the cortical surface (Fischl et al., 1999). The analysis was restricted to the AC; voxels within this regions of interest (ROI) that were significantly activated (p<0.001; not corrected for multiple comparisons) were used to
compute activation time courses after excluding any voxels with a signal change exceeding ±8% (These were considered artefactual). Grand-average time courses were subsequently generated by averaging the time courses of each single subject.

In the source analysis of the MEG data, we employed a single-compartment boundary-element forward model of the head with the inner skull surface reconstructed from the MPRAGE data (Hämäläinen and Sarvas, 1989). Two dipoles were fit iteratively and simultaneously using the XFit software (Elekta Neuromag Oy, Helsinki), starting with the two dipoles positioned symmetrically about the midplane in left and right superior temporal lobe, respectively. No constraints for symmetry were used in the iterative fitting procedure. The final dipole solutions were then used to define a spatial filter to derive source waveforms over the whole 24-s epoch (Scherg, 1990). The spatial filter was defined by dipoles fit to the N1m in a grand average across the individual trains of all stimulus conditions and across presentations. The dipole positions and orientations were fit for a 15-ms time windows centered on the peak of each N1m waveform. The dipoles were fit to the N1m, because it provided better signal to noise ratio than other alternatives and accordingly the most stable estimates dipole location. Dipole locations for the N1m and sustained fields were not systematically different from each other. Importantly, there were only minor variations of relative source magnitudes for N1m and sustained fields, depending on whether N1m or sustained-field dipoles were used. This insensitivity to precise dipole fit is in agreement with previous MEG studies using click train stimuli, where the N1m and the sustained field generally showed similar source configurations, such that decompositions based on the N1m and the sustained field result in similar source waveforms (Gutschalk et al., 2004). Once a spatial filter was determined for a given subject and experiment, it was used for all stimulus conditions of
a data set. Grand-average waveforms were generated by averaging the source
waveforms obtained separately in each single subject. For side-to-side presentation with
the fMRI activation, dipole positions were displayed on the cortical surface using the
MNE software (Athoula A. Martinos Center for Biomedical Imaging, Charlestown, MA).

Low-frequency, external artefacts in the magnetometers were corrected with a signal-
subspace projection (SSP), including 3 orthogonal topographies corresponding
approximately to the three components of homogeneous magnetic fields as detected by
the magnetometers. These topographies were also included in the inverse matrix of the
two-dipole model. Additionally, drifts in the baseline epoch succeeding each stimulus of
a sequence were modeled by SSP, including one topography for gradiometers and
another, similarly defined topography for magnetometers. This correction of slow
artefacts assumes that the activity always returns to baseline within ~500 ms. The
validity of this assumption was supported by the post-stimulus return to baseline of
signals averaged across the individual stimuli of a sequence, as well as by similar
previous data from other investigators (Picton et al., 1978; Lammertmann and
Lütkenhöner, 2001; Pantev et al., 2004). Finally, the topographies removed were not
dipolar and not well explained by the AC source model. For the continuous, 24-s
stimulus (100%), an additional SSP was only applied at the end of the whole block, in
the interval 2–5 s after stimulus offset.

Models of the BOLD time courses

For a quantitative comparison of BOLD and MEG measurements, the rectified MEG
source waveforms were convolved with a standard hemodynamic response function
(HRF). We compared two HRFs: HRF 1 was adapted to match earlier BOLD fMRI
studies of human AC activation (Robson et al., 1998; Glover et al., 1999; Harms and Melcher, 2002). This HRF had a peak latency of four seconds that was then followed by a negative undershoot with trough latency of twelve seconds. The amplitude of the negative undershoot was 20% of the positive peak. HRF 2 was a gamma function, as in Dale and Buckner (1997) with delta=2.25s and tau=1.25s, leading to a peak latency of four seconds. This HRF comprised only positive values.

To evaluate the differential contribution of transient and sustained components, the MEG response to individual stimuli, i.e., individual click trains, was divided into epochs that included: (A) the onset response, (B) the sustained response, (C) the offset response, and (D) the period after the offset response to a click train and before the onset response to the next. The epochs were overlapping and multiplied with 50-ms raised cosine windows at their intersections. The off epoch, during which the MEG noise fluctuates around the baseline level, was set to zero and not included in the fit, because we considered its contribution implausible. The offset-response epoch was additionally high-pass filtered at 3 Hz (24dB, zero-phase shift Butterworth filter) to help separate the offset response from the sustained field. The contribution of the components was reconstructed with a least-squares fit, minimizing the difference between the simulated BOLD response and the measured one. For plausibility, the fit was constrained to positive weights.
Results

**BOLD activation maps and MEG source locations in auditory cortex**

Maps of significant BOLD activity, shown for each subject in Figure 1, revealed widespread activation in the AC, including Heschl’s gyrus, planum temporale, the circular sulcus, and the superior temporal gyrus. MEG dipole locations are superimposed on BOLD activation maps for each hemisphere and subject in Figure 1. The dipole locations generally fall in AC, but are variably located in either Heschl’s gyrus or planum temporale. This variability may be related to the fact that the widespread activation in these cases includes gray matter with a range of orientations, different orientations across subjects, resulting in changes in the "center of mass" of activity reflected by the net dipole fit.

**Dependence of BOLD time courses on duty cycle and stimulus type**

The time course of the BOLD response for each stimulus condition is shown in Figure 2. Since the time courses showed only small systematic differences across anatomically defined regions of interest within the AC, Figure 2 shows only time courses for the whole AC. Note that time courses for the various stimulus conditions were all calculated from the same voxels that is, those showing activation in Figure 1.

The data in Figure 2 illustrate progressive changes in the BOLD time course with increasing stimulus duty cycle. The time course for the condition with the shortest click trains (resulting in a sequence with the lowest duty cycle - 2.5%) is elevated throughout the 24-s duration of the sequence with only a small peak shortly after sequence onset. Conversely, the continuous 24-s click train (duty cycle = 100%) produces a highly phasic
time course with prominent peaks just after the onset and offset of the sound, with a comparatively weak (<1% signal change) intervening sustained elevation in BOLD signal. The 25%- and 72%-duty-cycle conditions show time courses between the two extremes defined by the 2.5 and 100% duty cycle time courses. The sustained elevation in signal is slightly stronger for the 72% duty cycle, but the difference is subtle. The shape of the time courses for a given duty cycle was generally very reproducible across subjects, as reflected by the small standard errors in Figure 2.

**Dependence of MEG source waveforms on duty cycle and stimulus type**

MEG source waveforms for the same stimuli and subjects used for fMRI are shown in Figure 3. Each trace shows the entire response to a 24-s long stimulus averaged over subjects and hemispheres. The MEG source waveforms, like the BOLD time courses, show clear changes with increasing stimulus duty cycle. For instance, the 2.5% duty cycle condition shows a transient response to each of the eight click trains comprising the 24-s stimulus with the signal returning to baseline from one response to the next. In contrast, the 100% duty cycle condition (clicks throughout the entire 24 s of the stimulus) produced a long sustained field with the only transients being at the onset and after the offset of the clicks. In the case of the 25% and 72% duty cycle, a transient onset response, sustained field, and offset response are observed for each of the click trains of the sequence.

A detailed analysis of the MEG responses to the individual click trains of sequences is provided in Figure 4. The most prominent peak of the transient response to click train onset was the N1m (labeled in Fig. 4b; black circles in Fig. 4a). It was followed, except in the 2.5% duty cycle condition, by a sustained field of the same polarity (labeled SF in
Figure 4b; white circles in Fig. 4a). The amplitudes of these components were not constant, but decreased across the successive click trains of a sequence (Fig. 4a). The first N1m elicited during a sequence had approximately the same amplitude for all duty cycles. For the subsequent trains of the sequence, the N1m decreased in amplitude, most prominently for the 72%-duty-cycle condition.

A decline in amplitude over the course of a sequence was also observed for the sustained field, with the greatest decline occurring for the continuous clicks (100% duty cycle) condition; the sustained-field source amplitude decreased to half its initial value by the end of the sequence. Some decrease of sustained-field amplitude is also observed for the 72% duty cycle, while nearly no decrease is seen for the 25% duty cycle.

Between the N1m and sustained field, a prominent P2m (latency around 200 ms) is observed from the second click train on. This P2m became prominently more positive from the first to the second click train and then continued to become slightly more positive across subsequent trains of the sequence. The increased positivity of P2m could reflect diminished cancellation of P2m by the N2m wave, which could only be defined in the response to the first click train of each sequence (Figure 4b).

In contrast to the transient onset response to each click train, the offset response varied little from one train to the next in a sequence, as shown in Figure 4c and d. The offset response consisted of peaks N1m, P2m and N2m, which were most prominent for the 100% and 72% duty cycles. These peaks were fairly equal in prominence across successive trains. With the presence of N2m, the overall morphology of the off response more closely resembled the onset response to the first click train than the onset.
response of subsequent trains of a sequence (compare respective panels in Figure 4b and 4d). The N1m of the off response to the continuous, 24-s click train (100% duty cycle; Figure 4, c and d, lower right) approaches the size of the onset N1m for this condition (Figure 4, a and b, lower right). The peak-to-peak amplitude of the off response (N1m to P2m) for each of the 72% duty cycle click trains is nearly as strong as for the 100% duty cycle. It appears, however, that the relative amplitude of N1m and P2m differs, such that the N1m is larger for the 100% duty cycle, whereas the P2m is larger for the 72% duty cycle. Because the transient off response overlaps with the decrease of the sustained field, the exact amplitude relationship of these waves varies depending on the baseline definition used to measure their amplitude.

Comparison of BOLD and MEG responses

Several trends are clear from a comparison of the BOLD time courses in Figure 2 and the MEG source waveforms of Figures 3 and 4:

(1) A clear BOLD response was produced by the 2.5% duty cycle sequence while the MEG response to this stimulus consisted almost exclusively of transients.

(2) The sustained elevation of the BOLD time courses and the sustained field in the MEG waveforms do not increase and decrease in accordance with one another. The lack of covariation between the two can be seen from the 2.5% and 100% duty cycle conditions: While the BOLD response in the 2.5% duty cycle condition is mainly a sustained elevation (Figure 2, top left), there is little or no sustained field in the MEG waveform for this stimulus (Figure 3, top). At the same time, the BOLD response for the 100% duty cycle condition (Figure 2, bottom right) shows slightly less sustained
elevation (on which the on and off peaks ride) than the 2.5% condition, while the sustained field in MEG is greatest for this condition (Figure 3, bottom).

(3) While a distinct BOLD off peak was only observed for the 100% duty cycle condition, prominent MEG offset responses with similar peak-to-peak magnitude were seen following each individual click train in the 72% duty cycle sequence as well as at the end of the 100% duty cycle sequence. Thus, the relationship between the MEG and BOLD offset phenomena is not obvious. There are some subtle differences in the response morphology, between the 72% and 100% duty cycle conditions, which might be of some importance, however. For example, the $N_{1m}$ increases from 72% to 100% duty cycles (most prominent when measured from the sustained field level), but at the same time the $P_{2m}$ is more prominent for the 72% condition.

(4) The transient MEG response to the onset of the first click train of a sequence differs from that of subsequent trains raising the possibility that the neural activity responsible for this difference may play a role in the strong BOLD onset transient.

**A model relating MEG activity to BOLD time courses**

Several alternative models were used to further investigate the relationship between the BOLD time courses and MEG source waveforms. The first (Model 1) convolved a hemodynamic response function (HRF 1; Figure 5f) with a rectified version of the MEG source waveform for each stimulus condition (Figure 5e). The result was scaled to provide the best linear fit between measured and simulated BOLD time courses. The simulated BOLD time courses are superimposed on the measured ones in Figure 5a. The same scale factor was applied to all four conditions. The fit is not satisfactory,
particularly for the lower duty cycle conditions. The poor fit arises from the relatively large area under the sustained field compared to transient components of the MEG source waveform, which makes the sustained field dominate the fit: Preventing an overestimate of the BOLD time course in the 100% and 72% duty cycle conditions leads to an underestimate in the 2.5% and 25% conditions. Note that the fit was not significantly improved by using the alternative HRF 2 (Figure 5c), which was based on a gamma function (Figure 5g). Implementing the model in individual subjects did not improve the fit either, as can be seen in Table 1 where the results of two implementations are given: (1) one based on BOLD and MEG source waveforms averaged across hemispheres and subjects (table 1, left) and (2) another based on individual data for each subject averaged between hemispheres (table 1, right). For the latter, the values given in the table, including residual variance (RV), were determined separately for each subject and then averaged across subjects.

A far better fit to the data is achieved when the onset and offset transients of the MEG data on the one hand, and the MEG sustained field on the other, are separated (orange and blue, respectively in Figure 5e) and allowed separate weightings in the least square fit (Model 2). The results from this weighted model using HRF 1 are shown in Figure 5b. Most obviously, the weighted model improves the explanation of the 2.5% and the 100% duty cycle conditions. In the case of the 2.5% condition, the amplitude of the measured BOLD time course is better matched. For the 100% duty cycle condition, the latency of the BOLD onset, previously overestimated, is now correct. The reduction in residual variance from the unweighted to the weighted model was 20.5% for the empirical HRF 1 and 15.3% for the gamma function, HRF 2, cf. Table 1. The relative weighting assigned to transient MEG components was eight to ten times greater than the weighting
assigned to sustained MEG activity. The dominance of transient MEG activity over
sustained activity in accounting for the BOLD time courses can be seen from the
separate traces for the transient (dashed orange trace) and sustained (dashed blue)
contributions in Figure 5b and d. Note that this same result – a dominance of transient
MEG signals in explaining of the BOLD response using model 2 - was also observed for
eight additional listeners, who participated in pilot experiments reported in
supplementary materials (available online).

While the sustained part of the BOLD response was similarly well estimated using either
of the two HRFs, the empirical HRF 1 performed better in modeling the transient BOLD
onset response, which was not well modeled when the gamma-function, HRF 2 without
undershoot was used (compare Figure 5b and 5d). On the other hand, the empirical
HRF 1 produces some undershoot after sequence offset, while the undershoot in the
measured BOLD was quite variable across listeners, and therefore the overall residual
variance is not much different between the empirical and the gamma-function HRF.

In the model just described, contributions of the onset and offset MEG transients were
assumed to contribute with the same weighting, the result being a notable failure to fit
the prominent offset response in the BOLD time course for the 100% duty cycle
condition. This motivated a variation (Model 3) in which the onset and offset transients
of MEG activity were assigned separate weighting factors, resulting in three free
parameters, two for MEG transients and one for the sustained field. This model
produced only a minimal improvement over the previous weighted model (model 2),
yielding less than 1% reduction in residual variance (Table 1). This lack of improvement
is primarily because of the prominent offset responses following each click train in the
72% duty cycle condition; the weighting of these off responses had to be low enough to avoid over-estimation of the BOLD time course prior to sequence offset, which resulted in too low a weighting to account for the BOLD off peak. Thus, the weighting of onset and offset transients remained relatively balanced in Model 3 despite being free to differ.

We also considered the possibility of cancellation between N\textsubscript{2m} and P\textsubscript{2m} at sequence onset (Model 4). In particular, it was assumed that P\textsubscript{2m} would have been as prominent in the onset response to the first click train as in the response to subsequent trains except that a positive wave partially canceled it, resulting in an attenuated P\textsubscript{2m} and in N\textsubscript{2m}. Thus, the onset response to the first click train was assumed to have two contributions, each of which was independently rectified and weighted in the model. For this separation of onset responses, the N\textsubscript{2m} was estimated as the half-wave rectified difference between the response to the first and the last four click trains in the interval after the N\textsubscript{1m} and before the sustained field onset (150 – 300 ms). An average of the N\textsubscript{2m} estimated for duty cycles 2.5 – 75 % was used for the 100% condition, for which N\textsubscript{2m} could not be similarly estimated. The estimated N\textsubscript{2m} was then subtracted from the onset transients in Model 3 thus revealing the putative P\textsubscript{2m} evoked by the first click train.

In sum, one component of the onset transients included the waves P\textsubscript{1m}, N\textsubscript{1m}, and P\textsubscript{2m} evoked by all subsequent click trains, while the second component included only the N\textsubscript{2m}, putatively evoked by the first click train only. This separation of onset transients (Model 4) resulted in a better fit to the transient BOLD responses (Figure 6), but reduced the residual variance by only 2 – 7% compared to Model 3 (table 1). However, the BOLD off response was still poorly fit and the weighting of the MEG sustained field was far less than that of transient MEG components.
Because previous studies comparing evoked responses in the somatosensory cortex with BOLD (Nangini et al., 2008; Nangini et al., 2009) and optical imaging (Franceschini et al., 2008) reported that squaring the evoked-response data considerably improved modeling of hemodynamic measures, we tested whether using the squared instead of the rectified MEG data yielded similar improvements in fit here. The results are summarized in the bottom half of Table 1: overall there were no strong or consistent differences between squared and rectified. And finally, with the MEG signal squared instead of rectified, the sustained MEG response was still assigned a substantially lower weighting than the transient responses (Table 1), and the BOLD off peak could not be generated from the MEG signal.

Discussion

Our results indicate that the neural activity giving rise to transient MEG responses to sound is likely a major, and perhaps primary, contributor to BOLD responses of auditory cortex; the sustained field measured with MEG appears to contribute much less to the BOLD signal produced by auditory cortex. This conclusion is based on the following observations: (i) A stimulus producing transient MEG responses with little or no sustained field (the 2.5% duty cycle sequence) also produced a robust, sustained elevation in BOLD signal. (ii) The BOLD responses to a range of stimuli that produced transient and sustained MEG activity in varying proportions were simulated faithfully when the contribution of transient activity to the BOLD signal was weighted 8-10 times more than sustained activity. This latter result was confirmed by the experiments described in the supplemental materials.
Our conclusion regarding the relationship between transient vs. sustained MEG signals and BOLD response is based on two assumptions: (i) The neural activity underlying transient MEG responses, and not some other activity correlated with it, is the generator of the BOLD signal (but see Brugge et al., 2009 for an example of gamma-band activity observed in intracranial recordings that is temporally correlated with long-latency transients); (ii) The neural activity contributing to the BOLD responses also contributes measurably to MEG signals. This assumption is supported by data suggesting that both BOLD responses and long-latency MEG signals like those recorded here are driven by PSPs (Creutzfeld et al., 1966; Hämäläinen et al., 1993; Logothetis et al., 2001). In addition, there is evidence that the two responses may be driven by activity in similar cortical layers. In particular, studies in monkey AC have suggested that long-latency responses such as N₁ recorded from the scalp via EEG (the counterpart of N₁m in MEG) are mainly related to activity in supragranular layers of cortical gray matter (Javitt et al., 1994; Steinschneider et al., 1998). In addition, studies of somatosensory cortex relating hemodynamic measures to local field potentials (LFP), a measure of extra-cellular synaptic potentials, suggest that supragranular LFP activity is more closely coupled to the hemodynamic response than infragranular LFP activity (Franceschini et al., 2008). Thus both the MEG and the BOLD responses of the present study may be due to PSPs in supragranular cortical layers.

While we compared MEG and BOLD responses to specific stimuli, there are reasons to believe that our conclusions have broader pertinence. Most importantly, the form of MEG and BOLD responses seen here for sequences of click trains are typical for many types of stimuli. For instance, a transient N₁m-P₂m complex is a typical MEG response to noise or tone bursts of short duration, as well as brief click trains, as is the emergence
of a sustained field with increasing noise or tone duration (Picton et al., 1978; Gutschalk et al., 2002; Hari et al., 1980; Pantev et al., 1996). In BOLD, sequences of repeated noise or tone bursts, for instance, produce the same kind of responses seen here for sequences of click trains (Harms and Melcher, 2002; Seifritz et al., 2002; Harms et al., 2005). It is possible but unlikely that this rich variety of responses arises from completely different neural mechanisms. Therefore, the insights from the present study are likely to be generally applicable to similar responses produced by other stimuli.

**Neural activity underlying the MEG and BOLD responses**

The pyramidal, cortical neurons thought to generate the PSPs from which MEG signals arise (Creuzfeld et al., 1966; Hämäläinen et al., 1993) are also the targets of single neuron recordings from auditory cortex. One such study is especially pertinent to interpreting the data presented here. The study, by Lu et al. (2001), dissociated two populations of sustained firing neurons in extra-cellular single cell recordings in awake monkeys, one that fires in a manner synchronized to the sound stimulus and strongly decreases its activity for stimulus repetition rates above ~20 Hz and another that increases its rate above ~20 Hz, but is not synchronized to the stimulus. In MEG, the two populations could both be reflected in the sustained field in principle, but at the high click rate of 500 Hz used in this study, no significant sustained response from synchronized neurons would be expected, leaving only the non-stimulus synchronized neurons of AC to respond throughout most of each click train. In contrast, many neurons that are not spiking during the sustained portion of the train would be expected to produce action potentials at train onset (Wang et al., 2005). It would therefore appear that the transient and sustained MEG responses of the present study could have been
driven by different populations of neurons: those producing onset responses (and possibly stimulus-synchronized activity in general) on the one hand, and those responding in a sustained, non-time locked fashion on the other hand. Given our finding that transient MEG responses contribute far more effectively to the BOLD response than the sustained field does, it follows that the population of AC neurons that responds in a sustained, non-stimulus locked manner may have contributed little to the BOLD responses of the present study while stimulus-synchronized neurons and other neurons responsive to stimulus onsets may account for the bulk of the BOLD response. This suggestion must be tempered, however, by the fact that MEG and fMRI are not directly coupled to spiking activity but rather to the PSPs driving the spiking activity.

Importantly, spiking arises from an interplay between excitatory PSPs (EPSPs) and inhibitory PSPs (IPSPs) that may manifest differently in MEG and BOLD signals. How EPSPs and IPSPs shape the different characteristics of AC neurons is still being unraveled, but some findings are potentially important for the different observations made here in MEG and fMRI: In many auditory-cortex neurons, EPSPs and IPSPs are closely co-tuned in space and time (Wehr and Zador, 2003; Tan et al., 2004). EPSPs precede IPSPs by less than a millisecond, and spikes are generated within this temporal gap only (Wehr and Zador, 2003). Closely-timed EPSPs and IPSPs such as these will produce canceling contributions to MEG signals, but likely additive contributions to the BOLD response since both EPSPs and IPSPs have a hemodynamic demand (but see below). Thus, if the activity of co-tuned neurons was more prevalent during the transient MEG onset response than during the sustained field, it would help explain why the transients contribute more strongly to the BOLD signal. Other neuronal populations in the AC have been reported that show broader frequency tuning of IPSPs than EPSPs to
produce lateral inhibition (Wu et al., 2008). De la Rocha et al (2008) suggested that these two neuronal types (co-tuned and lateral inhibition) are intermingled and that the lateral-inhibition type produces broadly tuned onset neural firing and subsequent, more frequency-specific sustained firing. The latter features are characteristic of the specific, sustained firing units reported by Wang et al (2005) of which the non-stimulus locked, sustained-firing neuron type observed with fast click trains (Lu et al., 2001) is supposedly a subtype. A consequence for MEG could be that the more broadly tuned IPSPs would not be completely canceled by EPSPs such that the non-stimulus locked neurons would produce a more sustained MEG response than the co-tuned type. In summary, different synaptic mechanisms producing different amounts of cancellation between EPSPs and IPSPs might underlie the strong weighting of MEG transients over sustained fields that was needed to account for the BOLD responses of the present study.

The preceding discussion makes a common assumption: that EPSPs and IPSPs contribute similarly to the BOLD response based on their energy demand (Logothetis et al. 2001; Viswanathan and Freeman, 2007; Niessing et al 2005). But it is worth noting that this view has recently been challenged by findings of vessel constriction at sites of isolated IPSP activity (Devor et al., 2007), suggesting that EPSPs and IPSPs might oppositely and very directly control perfusion. In this case, the balance of EPSPs and IPSPs would need to be considered for the generation of the BOLD response, which would have strong implications for the integration of BOLD and MEG. The link between neurons and local perfusion is thought to be mediated by astrocytes and is best established for the excitatory transmitter glutamate (Schumm et al., 2008). There is some evidence for an involvement of GABAergic interneurons (Cauli, 2004), with
subtypes producing both vasodilation or vasoconstriction. Thus, while a relationship between MEG and fMRI can be expected based on EPSPs, the role of IPSPs is unclear at this point. Several scenarios could therefore be constructed to explain the data of this study: For example, if the BOLD response was primarily coupled to EPSPs, while the sustained field reflected IPSPs (cf previous paragraph), little direct correlation between the MEG sustained field and BOLD response would be expected. Conversely, if EPSPs and IPSPs were antagonistic for BOLD then a closer correlation with MEG than expected might be possible.

**BOLD offset response: no clear correlate in MEG**

When the contribution from transient MEG activity was strongly weighted in our simulations of BOLD responses, the simulated responses provided a good explanation for much of the measured BOLD responses, but not the off-peak occurring after sequence offset. The reason for this discrepancy remains unclear at present, but several explanations might apply. Perhaps the most tempting would be to attribute the off-peak entirely to the hemodynamic mechanisms coupling neural activity to the measured BOLD signal. For example, we cannot rule out the possibility of a non-linear relation between neural activity and BOLD response accounting for the off-peak, i.e., a situation in which the increase in BOLD exceeds what one would expect from the driving increase in neural activity, a scenario supported by empirical data (Devor et al., 2003).

An alternative possibility is that the neural activity driving the BOLD off-peak is only subtly reflected in the MEG signal or is not reflected at all. This could occur if the BOLD off-peak reflected activity in neuronal populations that are not temporally synchronized or spatially aligned in a manner suitable for contributing effectively to the MEG signal.
could also be that PSP contributions to the MEG signal cancel out, as discussed above, and reinforce one another in the BOLD signal. An indication of such a process is the observation that the N₁m-off is larger for the 100% compared to the 72% condition, whereas the P₂m-off is smaller. Considering that a decrease of the P₂m-off is unlikely here, one might suggest that this is rather an indication of enhanced (cortical) surface-negative activity, respective the N₁m-off and N₂m-off. Inclusion of such interpretation of the MEG signal peaks in the model would significantly enhance the explanation of the BOLD offset transient.

**Significance for future fMRI studies**

The physiological basis of the BOLD response is of general interest for the design and interpretation of many fMRI experiments. Experiments where fMRI is combined with MEG or EEG need to consider the differential contribution of separate evoked response components to BOLD, especially when source models for MEG/EEG are constrained by BOLD data (Dale et al., 2000). For instance, the sustained activity might be of more interest than transient activity, but reflect only a minor fraction of the overall BOLD magnitude. Wang et al. (2005) have shown that sustained firing units in AC reflect specificity for higher level sound features. Most of these neurons also generate transient onset responses, but these transient responses are non-specifically observed across many neurons. Our data suggest, that these feature specific, sustained firing neurons may only contribute weakly to the BOLD response in fMRI, and the consequential low sensitivity for these neurons will need to be considered for the design of studies seeking feature specificity in AC with fMRI.
**Conclusion**

We investigated the relationship of MEG and BOLD time series at the auditory cortex using click trains with parametrically varied click trains as stimuli. Our results demonstrate that MEG time series cannot be transformed into BOLD time series by convolution with hemodynamic response functions without additional constraints. One such constraint suggested by our study is to weight transient MEG activity more strongly than sustained (near DC) activity contributions to the BOLD signal. Further studies of the hemodynamic coupling to transient versus sustained activity at the microscopic level are required to more fully understand the neurophysiological underpinnings of our functional imaging data.

**Grants**

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References


**Figure Captions**

**Figure 1.** BOLD activation maps and MEG source location for individual subjects. Each panel shows inflated views of the left and right hemispheres of a given subject. Light and dark gray indicate portions of the cortical surface lying within gyri and on sulci, respectively. Bold activation maps (color) are based on a contrast between all stimulus conditions combined and epochs without stimulation. In the maps, significance of the difference between conditions is coded on a red (p <= 0.001) to yellow (p <= 0.00001) scale. The activation is largely confined to Heschl's gyrus (HG), planum temporale (PT), the circular sulcus and the superior temporal gyrus (STG), the regions for which BOLD time courses were analyzed. The locations of current dipoles fit to the MEG data are indicated with white circles.

**Figure 2.** Time courses of the BOLD responses in auditory cortex to each stimulus sequences with different duty cycles, indicated by the percentages above each panel. The gray shading indicates the 24-s duration of each sequence. The individual click trains comprising each sequence are depicted near the bottom of the gray shading. Time courses (% signal change vs. time) were determined for the significantly activated voxels of each hemisphere then averaged across hemispheres and subjects to yield the thick trace in each panel. Thin traces indicate the mean plus and minus one standard error of the mean. Importantly, the same voxels were used to calculate the time courses for all of the stimuli of the experiment for a given subject.
Figure 3. MEG source waveforms as a function of duty cycle as indicated by the percentage to the left of each waveform. The source waveforms are an average across hemispheres and subjects. An indication of standard error has been omitted for clarity. A schematic stimulus is shown below each trace. The gray shading marks the time interval 0 – 24 s for a convenient comparison with the BOLD data in figure 2.

Figure 4. Details of the MEG onset (a-b) and offset (c-d) responses for each successive click train of a sequence. Panels a and c show the mean amplitudes (and standard errors) across subjects measured at the peak. Sustained field amplitudes were measured as the maximum in the interval from 400 ms after the onset until the end of the click train. For the continuous click train (100% duty cycle), the time interval was 400 – 3000 ms with respect to train onsets in the other conditions. Panel a includes the onset components N1m (black circles), P2m (gray circles), and the sustained fields (white circles). Panel c includes the components N1m-off (black squares) and P2m-off (gray squares). Panels b and d show the response waveforms evoked by the eight successive click trains of a sequence aligned to train onset (b) or offset (d). The response evoked by the first click-train of the sequence is plotted in black, the response evoked by the second by a bold gray line, and those evoked by the 3rd to 8th by a thinner gray line. The most prominent change of the onset response occurs from the first to the second train: while an N2m is observed between the N1m and the sustained field for the first stimulus, all subsequent stimuli evoke a P2m instead.
Figure 5. Comparison of BOLD responses derived from MEG signals (red and blue) and the BOLD responses actually measured (black). The unweighted model (model 1, a, c) convolved the rectified MEG data (e) with standard, empirical hemodynamic response functions (HRF1, f; HRF2, g). This model underestimates conditions with short sustained fields in MEG, or overestimates conditions with long ones. When the transient (orange in e) and the sustained fields (blue in e) are separately convolved with the HRFs (model 2, panels b, d), and their relative contribution is determined by a least-squares fit, the BOLD data are explained best when the sustained field contribution is weighted less than the transient contribution by a factor of 8-10 (cf. table 1). Sustained and transient contributions to the modeled BOLD response are plotted as blue and orange dashed lines, respectively, in panels b and d.

Figure 6. Measured BOLD responses (thick black trace) superimposed on BOLD responses derived by convolving MEG signals with HRF1 (a, red) or HRF2 (b, blue). The MEG signals were divided into four separately weighted components, three transient and one sustained (model 4) each of which was then convolved with the HRFs. The resulting contribution of each MEG component to the derived BOLD response is shown by the dashed traces.
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<th>Table 1. Weights and residual variance (RV) of MEG model for the BOLD response (ROI = whole auditory cortex)</th>
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**MEG full-wave rectified:**

**MEG squared:**

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