**Does EEG-FMRI measure neurovascular coupling?**

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**Abstract**:

The alpha/beta (10-25Hz) and gamma (40-90Hz) frequency bands are the most well-known and extensively investigated frequency bands in human and non-human primate neurophysiology, due to their prominence in a wide range of cognitive and perceptual experiments. In recent years, these bands have been linked to the blood-oxygen level dependent functional magnetic resonance imaging (BOLD FMRI) signal both invasively and non-invasively, and it is now widely believed that neural activity represented by frequency specific power fluctuations underlie hemodynamic responses. The purpose of this work is to act as a “stress test” on the notion of a frequency specific relationship between neurophysiological measurements and BOLD. To this end, we manipulate frequency specific power in alpha/beta and gamma while measuring EEG and BOLD simultaneously. We manipulate gamma with a grating using different levels of spatial randomization (SR), to systematically suppress the narrow-band gamma response while holding BOLD constant. We also manipulate alpha/beta with an eyes-closed/eyes-open paradigm, to observe the state dependence of EEG-BOLD coupling. By controlling frequency specific power in this way, we show that the variance in the BOLD signal explained by gamma or alpha/beta power is dependent on the power in different bands, and power itself varies widely as a function of brain state or stimulus type. These results suggest that frequency specific EEG-BOLD correlations may reflect more the demands of the stimulus or brain state rather than inherent hemodynamic coupling mechanisms specific to a certain frequency band.

**Introduction**:

It has been suggested that different frequency bands in the neurophysiological signal may represent different aspects of neural activity (Logothetis, other LFP/MUA). Generally, the lower range of the frequency spectrum of a microelectrode recording (<300Hz), also referred to as the local field potential (LFP) is assumed to reflect synaptic activity at the dendrites, or input to a neuronal circuit (ref), while the higher range (>600Hz) containing high frequency spikes is assumed to reflect neuronal spiking or output from a circuit (ref, Kevin chapter), also referred to as multi-unit activity (MUA). Due to the different biophysical mechanisms underlying their generation, these different aspects of neural activity carry different metabolic burdens. For instance, while it is known that the vast majority of the brain’s energy goes towards maintaining the membrane potential of neuronal cells (ref), this membrane potential is not degraded solely by the depolarizing action of all or nothing axonal action potentials, but rather, any depolarization of the membrane potential however slight must be restored by the work of Na/K+ pumps (ref). In fact, it is hypothesized that action potentials or MUA account for only a minority of energy burden in the primate brain (ref), with the majority of brain energy dedicated to restoring the small depolarizing actions of dendritic synaptic potentials (Ref), which are represented predominantly in the lower frequency range (LFP). The fact that LFP and MUA represent different aspects of neural activity, each with different energy requirements, has led to the hypothesis that certain frequency bands may be more or less correlated to metabolic markers such as blood oxygen level-dependent functional magnetic resonance imaging (BOLD FMRI) (Ref).

Much work has been done on the frequency specificity of neurophysiological correlates of BOLD FMRI (logothethis, goense, lachaux, mukamel, niessing, shmuel, Scheeringa, Harvey, maier). While the original work focused on the importance of a single frequency band in the mid-gamma range (40-90Hz) (Logothetis), the same study and other similar studies observed correlations of similar magnitude between LFP and BOLD across a much wider range of frequencies (Shmuel, Goense) up to and including MUA. The 40-90Hz (gamma) band was selected for special attention presumably due to the slightly higher than average correlation coefficient observed in that range (than in MUA), and subsequent studies (refs) seemed to have confirmed this although exceptions exist (Lima).

However, it has also been shown that the frequency spectrum of neurophysiological activity is stimulus/state dependent (refs, gamma burst), and regionally specific (refs). For example, in human EEG during periods of relaxed wakefulness with eyes closed, a prominent peak in the alpha range (8-13Hz) emerges (Berger). During certain sleep stages however the alpha rhythm in the same scalp electrode is replaced by lower frequency activity in the delta (0-2Hz) range (Ref), and significant attenuation of the alpha peak has also been reported merely by the opening of the eyes (refs). More spatially specific invasive neurophysiological investigations have also found vast differences in the frequency spectrum across brain areas, for example, the hippocampus is known for its characteristic theta (2-8Hz) rhythm (ref), while the motor cortex is the province of beta (15-30Hz) oscillations (ref). The visual cortex, where the majority of invasive NVC studies take place, is known primarily for the gamma oscillations (40-90Hz) (ref) as well as for the aforementioned alpha rhythm which can be observed from the surface of the scalp using EEG.

Due to the wide range of frequency specific activity observed across brain regions, and over time in the same region, it seems unreasonable to expect any one frequency band (such as gamma) to be the exclusive generator of hemodynamic responses. Furthermore, while the distinction between LFP and MUA is clear-cut in terms of the underlying neural activity, gamma power can manifest in a variety of ways, for example, in electrocorticography and LFP recordings (wandell/Winawer, jia), both narrow and broad-band gamma responses were noted, and while both types of response result in increased gamma power, the underlying physiological processes or neural circuits involved in generating both narrow band/broadband are probably not the same (winawer review).

Narrow band gamma oscillations appear to be primarily a stimulus driven phenomenon, indeed, recent studies both invasive and non-invasive (Zhou, Jia, Winawer, Muthu) have demonstrated the stimulus dependence of narrow band gamma oscillations. This same work on the stimulus specificity of gamma in the macaque has demonstrated that gamma power can be strongly biased by the synchrony of underlying neural activity (Jia, Zhou) which is stimulus dependent. In particular, under certain stimulus conditions (increased spatial randomization of a grating) it was shown that stimulus driven narrow-band gamma power or the “gamma bump” can be reduced by up to 60% while leaving spike rates in the region unchanged (Jia). In the same study, it was also shown that when the amplitude of the “gamma bump” is normalized by total gamma power, the bump is reduced by 90%, suggesting that decreases in the gamma bump may be offset by increases in other surrounding frequencies or “broadband activity”. Due to the fact that spatial randomization has actually been shown to *increase* the BOLD signal in primate V1 in some cases (ref), this type of stimulus manipulation offers a unique opportunity to study the relationship between gamma power and BOLD.

According to some studies (niessing, Logothetis, Scheeringa) which argue for a tight relationship between frequency specific gamma and metabolic responses, abolition of the gamma bump using SR should result in strong suppression of the BOLD response, while other studies (muthu, wandell, lima?) suggest supressing gamma could result in limited or no changes in BOLD. One of the primary goals of the study therefore was to investigate how trial to trial EEG-BOLD coupling in the gamma range is affected when gamma power is suppressed using SR.

The other major band of interest, but more specific to non-invasive neurovascular coupling studies in the human, is the alpha/beta band (10-25Hz). Spontaneous fluctuations in the alpha/beta bands have been correlated with BOLD on numerous occasions (many refs), but most of these studies were carried out in the eyes-closed resting state condition, in order to ensure a robust alpha peak. These studies mostly came to the same conclusion: after convolving alpha power with a hemodynamic response function (HRF), alpha and BOLD were anti-correlated in occipital and parietal cortices (refs). Little work has been done on alpha-BOLD coupling in brain states other than resting state eyes-closed, and it is unknown if this frequency specific coupling persists across a wider range of brain state.

The second primary goal of this study was therefore to test the state dependence of alpha/beta-BOLD coupling. To this end, we employed a continuous visual stimulation paradigm, which is known to significantly attenuate the alpha/beta peak (Berger) alongside the standard resting state eyes closed paradigm, and examined the effects of alpha peak attenuation on neurovascular coupling in the alpha/beta bands.

**Methods:**

**Subjects:** nine subjects (3 female) with no history of neurological or psychological symptoms and normal or corrected-to-normal vision took part in the experiment. The experiment was carried out according to the ethical guidelines of the institute. One subject was unable to complete the movie portion of the experiment, and one subject was unable to complete the event-related portion of the experiment, so respective data segments from these two subjects were excluded from the analysis.

**Experiment and stimuli:** data were acquired over four separate runs, two event-related runs each 8.5 minutes, a movie run of 8.5 minutes and a resting state run of 5 minutes. The data acquisition was structured in this way to allow short breaks for the subject and allow communication between experimenter and subject between runs. Stimuli were viewed through a mirror mounted on the head coil inside the bore, reflecting the screen of an MRI-compatible monitor placed outside the bore. **Event-related paradigm:** Three types of grating manipulation made up the visual stimuli used in the event-related runs. The first was an “unperturbed” grating which consisted of a full field two-dimensional vertical sin wave function spanning 3 cycles per visual degree, and drifting from left to right at 6 cycles per second. The second stimulus type was a sin wave grating with all the same parameters as the first, except 10% of the grating’s pixels were swapped with other randomly selected pixels in the grating, in order to spatially randomize the unperturbed grating. This was done purposefully to reduce neural synchronization in the gamma range as has been shown in multiple invasive recordings (Zhou, Jia). The third grating type was a completely randomized grating, in which 100% of the pixels had been swapped, which amounts to a white noise image. This was done to suppress completely the gamma response, while holding alpha/beta constant, as has been established in previous studies and pilot recordings. These stimuli were presented for 32 trials each over the course of two event-related runs, each trial consisting of a 5 second period during which the drifting grating was present, followed by a 3-5 second (randomly jittered) offset period where only background luminance was present. A red crosshair was present for the entirety of the event-related runs, which the subjects were instructed to maintain fixation on and refrain from blinking during periods in which the grating was present. **Movie paradigm:** the movie stimulus consisted of a continuous 8.5 minute black and white segment of a popular action movie, with a red central crosshair overlayed. Subjects were instructed to maintain fixation on the crosshair for the duration of the 8.5 minute recording. **Rest paradigm:** The screen was turned off, and subjects were instructed to lie still with their eyes closed in the complete darkness of the scanning environment.

**MRI/FMRI acquisition:** single shot Cartesian full brain BOLD FMRI images were acquired in the anterior-posterior direction at a voxel size of 3.75mm isotropic over 33 transverse slices in a 240x240x123.75mm field of view, with a flip angle of 50 degrees and TE of 30ms. Multi-band factor 3 and SENSE factor 2 were employed, resulting in a volume repetition time (TR) of 693ms. T1-weighted anatomical images were acquired at 1mm isotropic over a 240x240x150 field of view, using a TR of 7.9ms, a TE of 3.5ms, and a flip angle of 8 degrees.

**EEG acquisition:** Scalp signals were acquired on a 64 electrode BrainProducts cap sampling at 5000Hz, with the reference at Fz according to the 10-20 coordinate system. The cap was positioned by the experimenter such that electrode Oz was immediately superior to the inion in each subject, but no special measurements were taken to ensure cap placement. Prior to starting the recording, the impedances at each of the scalp electrodes was reduced to below 20kOhm, and the impedance at the ECG channel, which was positioned medial and inferior to the left scapula, was reduced below 5kOhm.

Prior to placing each subject in the scanner, an 8-minute recording was done outside the scanner. For this experiment, subjects were seated upright in a standard office chair, viewing a CRT display at approximately arm’s length as they participated in a passive fixation and hand movement task. A similar grating stimulus to the unperturbed grating was shown to the subjects at this time, in order to compare the quality of gamma band responses inside and outside the scanner. After completing the outside the scanner experiment, subjects were placed in the scanner with the EEG amplifiers positioned behind the subject’s head, in the bore. The cables connecting the cap to the amplifiers were fixed in place by positioning sandbags underneath and around the cables, in order to minimize vibration artifacts in the EEG data due to gradient switching of the scanner. The helium pump was disabled during functional acquisitions. Prior to the experiment, the subjects were instructed briefly on the importance of proper fixation, and the importance of keeping still during the experiment.

**EEG pre-processing:** EEG processing was done in matlab using in-house scripts. EEG data acquired inside the scanner was corrupted with the typical gradient, ballistocardiogram, and motion artifacts common to all simultaneous EEG-FMRI experiments. These artifacts were removed in three separate steps. **1) Gradient artifact removal:** As this to our knowledge is the first simultaneous EEG-FMRI study using continuous multiband BOLD with the aim of examining gamma power, special care was taken to properly remove the gradient artifacts. The gradient artifacts were removed using average artifact subtraction (AAS) (Allen) on a slice-by-slice basis. An 80 artifact average sliding window approach was employed, such that each artifact occurrence was corrected by subtracting the Gaussian weighted mean of the 80 surrounding artifacts, with artifacts closer in time to the current artifact receiving higher weighting due to the Gaussian shape of the averaging window. The window size (80) and standard deviation (40) of the Gaussian window were carefully selected to optimize the subtraction for the slice time used in the experiment. The sliding window approach removes low frequency power due to the close spacing of the average artifact selection, so to mitigate this effect the low frequency power (<5Hz) was filtered out prior to the AAS procedure, and then added back after. Figure 1A shows the result of the gradient artifact subtraction procedure in a single subject. **2) Ballistocardiogram attenuation:** The term attenuation is used here instead of removal, as it is extremely difficult to completely remove the BCG artifact due to its variable physiological nature. The ballistocardiogram was attenuated using AAS, but instead of a sliding window approach, a more selective averaging approach was used due to the cyclic temporal variability of the heart-beat which is timed to the respiratory cycle (ref). First, each separate heartbeat occurrence was detected by running independent component analysis (ICA) on gradient-subtracted data and then running peak detection on the ICA component most resembling the BCG artifact. Peak detection was performed on this ICA component, instead of the electrocardiogram (ECG) channel due to the fact that the ECG was sometimes corrupted by motion artifacts making peak detection more difficult in some segments, and while head channels were also corrupted by motion artifacts, ICA was better able to separate these from the underlying BCG signal which resulted in a cleaner BCG peak detection. After automatically detecting each peak occurrence in this fashion, the BCG component was epoched in the 1.5s around each peak, and the sum-squared-difference (SSD) was computed between each BCG epoch and all other BCG epochs, and stored in a matrix. This gave a measure of the “closeness” of each BCG artifact to all other BCG artifact epochs. Then, each channel of raw data was epoched according to the peak indices detected on the BCG ICA component, and AAS was performed on the raw channel data by selecting indices of the 30 “closest” BCG epochs from the SSD matrix, and subtracting the corresponding raw data mean from the raw channel epoch. This was repeated over all BCG artifact occurrences. The BCG-attenuated data was then decomposed using ICA (more details in pre-ICA filtering), to isolate the alpha/beta and gamma range brain activity and remove the residual BCG artifact. **3) Motion artifact removal:** the motion artifact is less well known and rarely explicitly accounted for in simultaneous EEG-FMRI studies, but has nevertheless been shown to bias EEG-FMRI correlations in a significant way and result in misleading interpretations (Mullinger) and so must also be removed. While the post BCG-attenuation ICA decomposition was able to isolate a significant portion of the motion artifacts, due to the non-repeating and non-stationary nature of the motion artifact, some brain-related components were significantly corrupted by the motion artifact in some subjects, especially in the higher frequencies. The motion removal step is described further in the **spontaneous fluctuation correlation** section of methods.

**Pre-ICA filtering:** in order to help the ICA algorithm obtain weights more optimal for extracting gamma band responses from non-invasive scalp signals, it has been suggested (Scheeringa) to use pre-ICA filtering in specific frequency ranges, and after obtaining weights on pre-filtered data apply those weights to the unfiltered data to obtain broadband signals using optimal weights for extracting gamma band responses. We followed a modified version of this approach by pre-filtering BCG-attenuated data in the 10-15Hz and 40-70Hz frequency range before running ICA. As a further step, the 40-70Hz power was multiplied by a scalar factor of 3 in order to compensate for the fact that low frequency power is naturally much amplitude than high frequency power. By filtering and pre-multiplying in these specific ranges prior to running ICA, we were able to obtain strong stimulus specific induced gamma oscillations both inside and outside the scanner in all eight subjects for which the event-related data was analyzed (Figure 1D).

**BOLD-FMRI pre-processing:** minimal pre-processing was performed on BOLD images; the images were first motion corrected for inter-TR rigid body head movements, then corrected for echo planar imaging-related distortions using a reverse phase-encoding image, and finally band-pass filtered between 0.02 and the Nyquist frequency (0.7215Hz) to remove low frequency drifts due to gradient heating or other artifacts. No smoothing was performed due to the relatively large voxel size (3.75mm isotropic). A region of interest was selected based on the event-related BOLD time course in each voxel correlation with an HRF-convolved stimulus design time series. Voxels with correlations >0.3 were selected for further analysis, and the signal in those voxels was averaged to create a single time course for comparison with the EEG signal power.

**Spontaneous fluctuation correlations:** to compare the spontaneous fluctuations in the BOLD-FMRI signal with EEG power fluctuations during movie and rest, EEG was first separated into independent components as described above in **pre-ICA filtering**, and components exhibiting sustained narrow band responses to the grating in the alpha/beta and gamma frequency ranges were selected for further analysis (these same components were assumed to contain activity relating to spontaneous fluctuations in the resting state/movie conditions). This subset of components was then filtered in 100 equally spaced frequency bands, from 1-100Hz, in 6Hz wide windows around each band. Each band was then rectified and down-sampled to the same temporal resolution as the FMRI (0.7215Hz). **Motion removal from the spontaneous fluctuation data:** at this point, the next step is to typically compute cross-correlations between EEG power and FMRI time courses, to produce cross correlation functions for the different conditions (movie and rest). However, as demonstrated by (Mullinger) this type of continuous correlation analysis can be susceptible to motion artifacts, especially in the higher frequencies, so we took the following steps to mitigate these types of artifact. First, due to the fact that the motion artifacts were spread out over many ICA components, the spectrogram of all 64 components was averaged to create a single “motion spectrogram” accentuating the large broadband transients resulting from rigid-body head motion inside the scanner. Motion artifacts were then detected frequency-by-frequency by masking any points on the spectrogram with power greater than the mean of the power in that particular band plus the standard deviation of the power in that band across time. This resulted in a binary spectrogram mask, with time-frequency points corresponding to motion set to 1, and all other points set to zero. Finally, the binary motion spectrogram was used to mask the spectrogram from the narrow-band components used in computing the cross correlations with BOLD, and the points corrupted by motion were replaced using cubic mesh interpolation on the surrounding points (Figure 1B). **Cross-correlation:** after interpolation of motion artifacts, the cross correlation between EEG power and BOLD could be performed without any significant bias due to transient broadband motion artifacts. At each frequency band, a cross correlation (Spearman’s rho) between EEG power in that band was computed in increments of 0.693 seconds, from -12 to 12 seconds, for each EEG component, resulting in a 100, 30 (frequency, time) cross correlation matrix for each component. These matrices were then averaged across components to produce a single cross-correlation matrix for each subject. This procedure was applied for both movie and rest acquisitions.

**Event-related single trial correlations:** single trial coupling between EEG power and BOLD was computed separately for each of the three stimulus types. Single trial EEG power was obtained in a manner similar to the spontaneous correlation procedure; components were selected based on their narrow band response to the stimulus (the same components were used for the spontaneous and event-related correlations), filtered in 100 evenly spaced bands from 1-100Hz, rectified, and down-sampled to FMRI temporal resolution. Power in each separate band was then convolved with the canonical hemodynamic response function (HRF), and the data was epoched according to stimulus type. For each stimulus type, HRF-convolved EEG power was normalized by subtracting the baseline preceding that trial, and then correlated across trials (Pearson’s rho) with BOLD which had been baseline normalized in the same way. This procedure was repeated across all frequency bands, yielding correlation coefficients from 1-100Hz for each of the three stimulus types.

**Event-related spectral perturbation:** to visualize the stimulus-induced effects of the event-related acquisitions more clearly, event-related spectral perturbation (ERSP) was computed using a multi-taper approach (ref). Stimulus data segments were pre-multiplied with five orthogonal tapers prior to computing the Fast Fourier Transform (FFT) and resulting FFTs were averaged across the 5 orthogonal tapers to increase the SNR of the power estimations. These ERSPs were computed for visualization purposes only and not used in the coupling analysis.

**Results:**

Simultaneous EEG-FMRI experiments were carried out to test the state and stimulus dependent relationship between EEG and BOLD. Two continuous coupling experiments were conducted (8.5 minutes movie eyes open and 5 minutes rest eyes closed) and 17 minutes of an event-related single trial coupling experiment was conducted using three different visual stimulus types (0%SR, 10%SR, and 100%SR). Shorter experiments were carried out outside the scanner to ensure data quality.

**Inside vs outside gamma range data quality:** The gradient artifact was removed successfully from all channels (Figure 1A) and the BCG artifact was significantly attenuated in all channels such that the subsequent ICA decomposition was able to recover component weight maps and power spectrums similar to those obtained outside the scanner (Figure X). Critically, single subject ERSP in response to the grating was preserved inside the scanner (Figure 1C), and strong single trial narrow-band gamma modulation was observable (Figure X), again demonstrating the success of the gradient denoising procedure in removing high-frequency artifacts. Although significant differences were observed between frequency specific power inside and outside the scanner in response to the grating (Figure 1E) the overall shape of the power spectrum remained nearly identical and the differences were likely due to the ~4Hz shift in peak gamma frequency which can be attributed to slightly different viewing conditions from CRT monitor outside the scanner to the coil mounted mirror inside the bore (the gamma peak frequency has been shown to be highly stimulus dependent (Maunsell)). The spectrogram of a single subject’s neural activity during one 8.5 minute scan is also shown before and after the motion removal process (Figure 1B) to demonstrate the quality of the continuous recording inside the scanner.

**Event-related single trial coupling:** 300 TRs of simultaneously acquired EEG power and BOLD time-series are displayed (Figure 2A) in a single subject during the event-related paradigm. Visually induced gamma and alpha/beta responses are plotted to demonstrate the coupling between frequency specific power and BOLD, as can be seen strong BOLD responses are noted even in complete absence of gamma power increases for the 100%SR stimulus. Grand average ERSP for the three stimulus types were computed to show differences across all frequencies and especially the complete abolition of power in the narrow-band gamma range due to spatial randomization of the grating (Figure 2B, Figure 2C). In contrast, the BOLD response was similar across all three stimulus types (Figure 2D), demonstrating a striking difference in stimulus selectivity between narrow band gamma and BOLD.

While the difference in stimulus selectivity of narrow band gamma and BOLD is striking, it only confirms previous results and does not preclude the possibility of single trial coupling between gamma and BOLD; small differences across trials in response to 100%rnd may still correlate with differences across trials in the BOLD response. Therefore, single trial correlations between EEG and BOLD were computed separately across the three stimulus conditions. As hypothesized, differences due to stimulus type in single trial coupling were noted in the bands most affected by the stimulus manipulation (Figure 2E), which in this case was the narrow band gamma. We observed a decrease in single trial coupling between 0%rnd and 100%rnd from 40-90Hz, which was most significant in the narrower 40-60Hz range, or the peak of the gamma band response to the unperturbed grating. To quantify this effect, we examined the EEG power dependent relationship between event-related single trial coupling and BOLD, first by correlating the average power (Figure 2F, top left) across all stimuli with average coupling (Figure 2F, bottom left) across all stimuli. This correlation showed that frequency specific modulation explained almost 90% of the frequency-specific coupling (r^2=0.87, p=0) (Figure 2F, right). Then, to see if differences in coupling across stimulus types could also be explained by differences in power modulation across stimulus types, we correlated power differences from 0%SR to 100%SR (Figure 2G, top left) with coupling differences from 0%SR to 100%SR (Figure 2G, bottom left), which is to say, the changes in EEG power introduced by 100%SR were correlated with the same changes in coupling (across frequencies). While this relationship was again highly significant (p=9e-07), the majority of the variance in coupling differences across stimulus type could not be explained by power differences due to stimulus type (r^2=0.22) (Figure 2G, right).

To examine the spatial pattern of the alpha/beta and gamma correlations with BOLD, we convolved the respective band-limited power time-series with an HRF, and correlated this with the BOLD signal in each voxel. This analysis revealed that while both low and high frequency correlations were restricted to the occipital lobe (Figure 2H), high frequency gamma correlations appeared to be much more spatially specific, while the alpha/beta correlations were spatially extensive, covering the entirety of the occipital pole. To our knowledge, this is the first report of non-invasive gamma-BOLD coupling during a continuous acquisition scheme (EEG recorded simultaneously with gradient acquisition).

**Spontaneous coupling:** EEG and BOLD were recorded simultaneously during the resting state eyes closed (EC) and eyes-open passive viewing (EO) states. As expected, the alpha (8-13Hz) peak during EC was much more pronounced than for EO, and the beta (15-25Hz) peak present in EC was completely suppressed by EO (Figure 3A). Cross-correlation matrices were computed by comparing frequency specific EEG power with BOLD time course in a region of interest (ROI). **Eyes-closed resting:** For EC, the low frequencies were dominated by a strong anti-correlation at a time lag of 5-10s (Figure 3B) peaking around 9 seconds (Figure 3D). This anti-correlation was not restricted to alpha, but extended from 10-35Hz although it was strongest in the peak alpha/beta frequencies (Figure 3F). The anti-correlation was mirrored by a slightly weaker positive correlation around 0s time lag, with a similar spectral profile. No significant correlation was present in the higher frequencies (40-100Hz) during resting state EC (Figure 3E) at either positive or zero time lags. **Eyes-open passive viewing:** the strongest correlations were again anti-correlations in the low frequency range (Figure 3C), but for EO the peak-latency of the anti-correlations was almost 3 seconds earlier (6.2s as opposed to 9s for EC) (Figure 3D), and more restricted to the alpha range (Figure 3F). In the gamma (40-90Hz) frequencies of EO coupling, a significant positive correlation was observed between EEG and BOLD at time=5.5s (Figure 3E), slightly earlier than the low frequency response. To quantify the power dependence of spontaneous coupling, average coupling (across both EO and EC) (Figure 3G, top left) was correlated with average log power (Figure 3G, bottom left) across all frequencies. This analysis revealed that power accounted for a roughly half of the coupling during spontaneous conditions (r^2=0.47, p=2e-15) (Figure 3G, right). To examine differences in spontaneous coupling due to differences in power from EC to EO, we correlated the power differences from EC to EO (Figure 3H, bottom left) with the coupling differences from EC to EO (Figure 3H, top left), finding again a significant correlation, but with differences in power explaining a smaller amount (1/5) of the variance in coupling differences (r^2=0.19, p=5e-6) (Figure 3H, right). The correlation here was negative (rho=-0.43) as opposed to positive for the event-related power differences (rho=0.46), this is due to the fact that power differences from EC to EO were mostly in the low frequency range, while differences from 0% to 100% SR were strongest in the high frequency (gamma) range.

**Spontaneous coupling voxel-wise analysis:** The spatial pattern of EC vs EO coupling was completely different. In the lower frequency range, correlations were much stronger and more widespread during EC (Figure 3I) than during EO. In particular, the medial anterior part of the occipital lobe (lingual gyrus) was strongly coupled to EEG alpha/beta power during EC, while for EO the correlations were more restricted to the lateral occipital cortex and occipital pole (Figure 3I). EEG-BOLD correlations were also noted in the temporal lobe during EC but not for EO (Figure 3I). In the gamma range, spontaneous EEG-BOLD correlations were largely absent during EC, but for EO gamma range correlations were present in the same regions as for the alpha/beta range correlations (Figure 3I).

**Average coupling across all brain states and tasks:** coupling was averaged across all brain states and stimulus types on a frequency by frequency basis, to create a grand average spectral coupling profile (figure). This spectral profile reflects the single trial stimulus-driven coupling and the spontaneous coupling from both EC and EO. The same procedure was repeated for the voxel-based analysis, by averaging correlation coefficients in each voxel across all stimulus types and brain states (figure).

**Discussion:**

Here, the frequency specific EEG-BOLD relationship has been investigated under a variety of brain states and stimulus conditions. We find this relationship to be highly variable, and largely dependent on power, especially in the gamma band which is known to be stimulus selective and synchrony dependent (jia, zhou).

Firstly, the work presents a methodological advance, showing that using state of the art signal processing and denoising techniques, high-quality gamma band responses can be obtained during simultaneous EEG-FMRI experiments with negligible interference from the gradient artifact. Therefore, unlike in previous studies utilizing interleaved acquisition (Scheeringa) which both reduces the amount of data collected and increases the complexity of the experiment design/acquisition scheme, we advocate for continuous recording even in the case of experiments examining high-frequency neural activity inside the scanner. In fact, the main source of interference during simultaneous EEG-FMRI experiments continues to be the BCG artifact, although recent techniques are emerging which may allow for its complete subtraction (harmonic regression). We should also note that the experimental acquisition times here were quite short (5 minutes resting state, 8.5 minutes movie, and 32 trials/stimulus type event-related) as opposed to some other simultaneous EEG-FMRI experiments which recorded simultaneous EEG-FMRI for up to 30 minutes in a single brain state (de Munck, others). The fact that we observed similar correlations to these other studies with just 1/6th of the acquisition time is further evidence for the data quality presented here.

The primary and perhaps most controversial finding of this study is the lack of gamma-BOLD correlation in the resting state, and overall power dependence of frequency specific gamma-BOLD coupling. While multiple invasive recordings have demonstrated a relationship between gamma band activity and the resting state BOLD signal (refs), we find no such relationship in our resting state EEG data. In fact, it seems that some type of stimulus is needed to drive EEG-BOLD correlations in the gamma band. This may be due to the effect of the skull, which acts as a low-pass filter on neurophysiological signals (ref) and so only stimuli which produce highly synchronous activity in the gamma band create large enough signals to be observed on the scalp and hence correlated with BOLD. This is further supported by the fact that using different levels of spatial randomization (SR) the gamma-BOLD relationship can be decreased from r=0.25 to r=0.1 (Figure 2E) at the peak frequency, while the alpha/beta correlations remain unchanged.

A similar case can be made for the correlations between spontaneous fluctuations in the two signals. In the eyes-closed (EC) resting state condition, strong correlations are observed over the entire occipital lobe, and some of the temporal lobe. However, as soon as the subject opens their eyes and begins a passive viewing task (EO) the spontaneous correlations disappear in some areas and are reduced drastically in others. This state dependent spontaneous coupling has also been observed in invasive recordings, although not in the same frequency bands (Leopold) where it was shown that coupling varies as a function of the animal’s behavioral state.

This again raises the question: are EEG/LFP-BOLD correlation really a measure of neurovascular coupling, revealing the neuronal drivers of metabolic activity, or do these correlations simply reflect shared variance with some other, underlying source (Winawer)? The fact that EEG and BOLD are so readily dissociable, and the correlations are so low (rarely exceeding r=0.3, which amounts to less than 10% of the hemodynamic variance explained in the best of cases), suggests that frequency specific neural activity as measured with EEG or even LFP may only “underlie” the BOLD signal by coincidence or in special circumstances. The classical interpretation of neurophysiological signals as “causing” or “underlying” the BOLD response may be a conceptual mistake.

A previous study has argued for independent contributions from alpha/beta and gamma to BOLD, based on the fact that trial-by-trial variability in the two bands is not correlated, but both share a significant trial-by-trial relationship with the BOLD signal (Scheeringa). We find, however, using the same measurement techniques and very similar stimuli, that it is possible to completely suppress high frequency power using SR with little to no effect on BOLD, suggesting the BOLD signal is independent of high-frequency power. Further, we also compute trial-by-trial correlations across all frequency bands and BOLD, and show that coupling can be significantly reduced in the gamma band, while remaining unchanged in the alpha/beta bands, with an unchanged BOLD signal. This supports other findings (muthu, Winawer) which argue that narrow band gamma which arises largely due to synchrony (jia, zhou) has little to no influence on hemodynamic responses. However, the fact remains that narrow-band gamma does correlate on a trial by trial basis with BOLD, for some stimulus types. One explanation for this is that the neural synchrony in the gamma range which gives rise to the prominent spectral bump boosts the SNR in that band enough for single-trial correlations with BOLD to be observed, while the broadband signal which matches BOLD across a wider range of stimulus types (Winawer) may simply be too weak to observe trial-by-trial correlations with EEG and BOLD.

One general principle underlying all the results reported here is the power dependence of EEG-BOLD correlations. In particular, we have shown that 90% of the frequency specific event-related coupling can be explained by frequency specific modulation, which is to say, EEG-BOLD coupling exists primarily as a function of whichever bands were modulated by the stimulus. This seems to run against the argument that specific neurophysiological bands are correlated to BOLD due to an underlying physiological mechanism represented by that band, for example, in the original gamma-BOLD coupling articles (Logothetis) it was argued that the LFP was a frequency band representing neuronal input, which is more metabolically expensive, which was why gamma and BOLD were more strongly correlated than MUA and BOLD. In light of these findings, however, it seems that gamma and BOLD are correlated only when a certain stimulus type induces a strong gamma-band response. The original coupling results, which were based on stimulus driven activity, (Goense, Logothetis) may be explained by a similar type of argument.

However, one might object that other more recent invasive experiments (Logothetis, Leopold) have also reported gamma-BOLD coupling, in the resting state where no stimulus was present to increase gamma SNR. Closer examination of this data however reveals that in one case (Leopold) the strongest correlations were actually in the 0-15Hz range, although this was not discussed, and in the other case (Logothetis) the data also shows that alpha/beta bands contribute an equal amount of information, on a frequency by frequency basis, but by the much wider definition of the gamma band as 40-90Hz the conclusion was reached that gamma was more informative about the BOLD signal.

In any case, the fact that gamma and BOLD can be dissociated by scrambling the pixels of a grating, or that alpha/beta and BOLD can be dissociated simply by opening one’s eyes suggests that “neurovascular coupling” as measured through EEG-BOLD correlations is probably not causal in nature, which is to say, gamma oscillations or alpha/beta desynchronization are not directly related to neurovascular coupling mechanisms causing alterations in blood flow. Rather, these neurophysiological signals (alpha/beta and gamma oscillations) are generated by state or stimulus specific activity in underlying neural circuitry, which is responsible for increases in blood flow and may or may not give rise to alpha/beta or gamma oscillations. A similar type of argument was put forth in a review on the relationship between cognition and gamma oscillations where it was stated (with reference to the multitude of studies correlating gamma oscillations and cognition) “every one of those correlations is expected because cortical gamma signals an activated state of cortical tissue: cognitive activity of the most diverse kinds involve cortical activation” (Merker). In other words, gamma power signals activation, but activation is not always signaled by gamma power, which is why BOLD and gamma are dissociable. Can these conclusions be extended to similar measures performed invasively? We believe that in particular with regard to gamma, the results are likely to be highly similar in invasive preparations due to the observations of (Jia, Zhou). We should add that claims of one or another frequency band “representing” different aspects of neural activity are still, at this point, largely conjecture, and have yet to be demonstrated experimentally.

**Conclusion:** This study should serve as a cautionary tale to those seeking to understand neurovascular coupling by correlating frequency specific neurophysiological power with BOLD. We show here that frequency-specific EEG-BOLD coupling is largely power dependent, and power itself is heavily dependent on stimulus or brain state. Inferences about NVC mechanisms based on frequency specific coupling patterns may therefore not always be well founded.