**Title**:

Using retinotopic mapping and simultaneous EEG-fMRI to examine the anatomical source of healthy human intra/inter-subject EEG signal variability

**Author**:

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**Aim**:

To use retinotopic mapping and simultaneous EEG-FMRI to compare how the EEG signal strength variability in response to selective visual field stimulation relates to macroscopic cortical/head anatomy as measured through structural MRI.

**Background**:

Electroencephalography (EEG) is a functional measure of human brain activity. EEG is thought to measure the electrical potential originating from dipoles created by synchronous synaptic input to pyramidal cell neurons orientated perpendicular to the cortical surface in the brain (Nunez and Srinivasan, 2006). However, as the EEG signal is recorded from the surface of the scalp, several anatomical factors play a role in determining EEG signal strength, including but not limited to factors such as skull thickness, cortical folding patterns, and distance from scalp to brain.

EEG measures the broad band signal (0-100+Hz) emanating from the brain, and this signal is commonly divided into multiple non-overlapping frequency bands. Two of the most commonly studied bands are the alpha/beta (8-25Hz) and the gamma (30-80Hz) frequency bands.

Due to advances in EEG hardware and signal processing techniques, it has become more popular recently to examine the gamma range EEG activity. For example, psychological disorders such as schizophrenia and autism have been linked to corresponding changes in the gamma rhythm (Sun et al., 2012). The statistical power of these studies is hampered, however, by the fact that there is enormous variability within a healthy population in response to a simple stimulus (Figure 1A, 1B). In fact, some healthy participants show no gamma response whatsoever to a full field grating stimulus (Figure 1B, subject #3) while others show changes of up to 6db in the gamma range (Figure 1B, subject #1). While this example focuses mainly on gamma, the alpha/beta frequency range shows a similar degree of inter-subject variability.

The source of this alpha/bet and gamma variability across a healthy population is poorly understood. A variety of anatomical and neurotransmitter-based correlates to the healthy human inter-subject EEG signal having been proposed, including but not limited to the following: 1) GABA receptor density predicts gamma peak power and frequency (Kujala et al., 2015), 2) GABA neurotransmitter concentrations predicts gamma peak frequency (Muthukumaraswamy et al., 2009), 3) cortical surface area predicts gamma peak frequency (Schwarzkopf et al., 2012), and 4) cortical thickness predicts event-related alpha modulation (Provencher et al., 2016). However, these experiments suffer from a number of pitfalls, including a low number of participants (10-20 in most cases), and failure to account for independent variables such as age. For example, in the cortical thickness vs EEG study (Provencher et al., 2016) a highly significant correlation between EEG event-related alpha modulation and cortical thickness was reported, but it is clear from the plots that the correlation between thickness and EEG power modulation was driven entirely by age.

In this study, I propose to use retinotopic mapping with simultaneous EEG-FMRI to perform a more systematic investigation into the structural basis of healthy human EEG variability. The human visual system is well delineated retinotopically, and retinotopic mapping is routinely performed using FMRI experiments, though this has not been attempted with EEG. However, recent MEG literature (Nasiotis et al., 2017) and our own preliminary results (Figure 1C) have indicated that EEG-type measurements are sensitive enough to detect differences in retinotopic location of a visual stimulus. By stimulating many different retinotopic areas separately, it becomes possible to control the precise area of functional activation, which in turn allows to compare the same brain area across many different subjects, or many brain areas across the same subject. In this manner, the structural dependence of EEG scalp measurements can be more accurately assessed.

**Summary of problem**: Anatomical features not related to brain function may obscure functional differences in brain activity across a population or within an individual. To better understand the anatomical source of EEG scalp measurement variability, I propose to use simultaneous EEG-FMRI experiments to map the retinotopic organization of the human visual cortex in a small group of subjects, to compare EEG signal strength with structure across a variety of cortical regions and head geometries.

**Experimental approach**:

The experimental approach of this study focuses on taking advantage of the retinotopic organization of the human visual cortex to acquire multiple measures within a single subject, in order to circumvent the problem of acquiring many different participants. If 30 discrete retinotopic areas are stimulated in each subject, and 10 subjects are acquired, a total of 300 (30x10) brain areas and corresponding EEG responses can be isolated, giving much greater statistical power to the experiment.

To make this possible, retinotopic mapping experiments will be performed using simultaneous EEG-FMRI. This will allow to precisely delineate the anatomical region associated to each retinotopic stimulus location, while simultaneously measuring the EEG response. The EEG responses to each stimulus configuration can then be compared with the corresponding anatomical patch isolated by the FMRI. While in principle simultaneous EEG-FMRI is not necessary (EEG and FMRI retinotopic mapping can be performed separately), in practice ensuring that both modalities are acquired in identical stimulus conditions adds value to this type of study, by ensuring that the EEG and FMRI responses originate from the exact same cortical tissue.

FMRI acquisition: As mentioned earlier, the EEG signal is thought to arise from dipolar-like neuronal arrangements in the cerebral cortex. Therefore, to properly compare anatomical variability with EEG, individual cortical patches must be isolated using FMRI. This includes cortical regions which are highly folded, and tightly compressed, which using a traditional blood oxygen level-dependent (BOLD) FMRI sequence (3.5mm resolution) becomes impossible due to partial volume effects and the inherent thickness of human cortex (~3mm thick in visual cortex). Furthermore, the biophysical nature of gradient-echo (GE) BOLD means the signal is highly skewed towards large draining veins which are situated between cortical folds, further hampering efforts to distinguish between adjoining folds using GE BOLD. Therefore, special image acquisition and processing steps must be employed to ensure sufficient data quality to perform this analysis. One such study already exists (Scheeringa et al., 2016) where laminar FMRI was performed in a simultaneous EEG-FMRI setup at 3T. In this acquisition, a high resolution (0.75mm isotropic, TR=3.8 seconds) 3d EPI acquisition over a limited field of view was employed after performing localizer scans to isolate visual cortex in each participant. The authors were then able to map functional activation to individual cortical layers, solving the adjoining cortex PVE/draining vein problem.

Retinotopic mapping: To ensure maximal signal to noise ratio, the retinotopic stimulus will need to be piloted extensively. However, due to the different temporal and spatial resolution of EEG and FMRI, different stimuli may work better for either modality. The classical rotating checkerboard which works well for FMRI may not be the optimal stimulus for inducing a strong frequency specific EEG response. Preliminary results indicate that a drifting grating masked by a rotating checkerboard work well for both EEG (Figure 1C) and FMRI experiments so although more effective stimuli may exist for mapping EEG outside the scanner, this stimulus can be used to acquire retinotopic maps in both signals simultaneously.

Structural measures: T1 and dual echo UTE images (Hu et al., 2014) will also be acquired. The T1 will allow to map the cortical gray/white matter interface, and serve as anatomical reference for the high resolution 3d EPI FMRI sequence, to localize changes specific to the gray matter. The T1 will also allow to map the orientation of each patch of cortex, by taking the vector normal to the gray/white matter interface provided by the Freesurfer (Fischl, 2012) segmentation program. The UTE will provide additional anatomical information including the skull thickness and electrode locations (Butler et al., 2017).

Acquiring gamma band responses inside an MRI scanner is not a well established technique, but it has been attained both in our own data (Figure 1D) using average artifact subtraction (AAS) to remove gradient artifacts, and in other centers (Scheeringa et al., 2011) using interleaved acquisitions. Therefore we do not consider simultaneous EEG-FMRI a major obstacle in the acquisition of high quality EEG gamma power responses to the retinotopic stimulus. The ballistocardiogram artifact is a major contaminant of the alpha/beta frequencies, but a combined AAS and independent component analysis (ICA) denoising approach is capable of removing artifacts in that range as well.

The simultaneous EEG-FMRI experiment will have a duration of approximately 1 hour, in order to strike a balance between subject comfort and number of trials. The anatomical images will be acquired first (9 minutes), followed by the functional localizer (4 minutes) and the remainder of the experiment (45 minutes) will be spent acquiring high resolution 3d-EPI FMRI in response to retinotopic mapping stimuli.

EEG cap will be a 64 channel BrainVision easyCap, with MR-compatible amplifiers. Applying the cap and preparing the subject for the experiment takes 45 minutes, so the total experimental duration will be under 2 hours per subject. Subjects will be compensated for their time with course credit or financially.

**Discussion**:

The goal of this project is to use functional MRI to precisely map and isolate areas of cortical activation in response to retinotopic stimulation, and then examine how structure can predict variability in EEG signal strength responses to the same stimulus.

This work has a number of advantages over previous attempts to link EEG to structure. Firstly, the experimental approach will allow for intra-subject comparisons of EEG signal strength to structure. Using retinotopic mapping, 30 different areas will be isolated within each subject, and compared to EEG signal responses to that stimulus configuration. This will allow to examine not only the source of inter-subject structural variability by comparing EEG responses in a single area across participants, but also intra-subject variability by comparing EEG responses in a single participant across multiple brain areas. This is important because the anatomical sources of inter and intra subject variability may not be the same. For example, within a single subject, skull thickness may be relatively homogenous over the occipital lobe, so skull thickness may not play a role in intra-subject EEG variability. However, across participants skull thickness may play a more important role, due to varying skull thickness from subject to subject. Another important benefit of performing structure-EEG correlations in a single subject is that behavioral (subject attention) and technical (electrode-scalp impedance) confounds, which may play a role in inter-subject variability, are minimized.

Secondly, the study will provide insight into the structural dependence and spatial specificity of the different EEG frequency bands, which has received almost no attention in the literature. The two bands of major interest for this study are the alpha/beta (8-25Hz) and the gamma (30-80Hz) bands. While both of these bands show strong modulation in response to a visual stimulus, their retinotopic specificity and dependence on structure may be quite different, which is to say, anatomical parameters which have large effects on the strength of the gamma rhythm may not be as important in explaining alpha/beta rhythm variability. The EEG alpha rhythm has long eluded investigators, and differences between alpha and gamma responses to selective visual stimulation may provide clues towards its origin.