Comparison between microscopically and macroscopically hippocampal activity in epilepsy patients

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ABSTRACT
Thirty percent of epilepsy patients do not respond to adequate antiepileptic drugs and for those patients, epilepsy surgery is an option. The invasive measurements of depth electroencephalography are used to localize the seizure onset zone, which is later resected to achieve seizure freedom, but are complicated because of their invasive nature. In this study, dEEG measurements of the hippocampus were compared to anatomically-matched virtual electrodes reconstructed out of MEG signals, to investigate whether restating-state non-invasive measurements reflect resting-state activity that is measured invasively with dEEG. Our findings indicate MEG being a possible clinical replacement for depth EEG in the future.

Keywords
Invasive EEG, MEG, epilepsy, beamforming, virtual electrodes.

INTRODUCTION
Epilepsy is one of the most prevalent neurological conditions worldwide. It is characterized by the unexpected and unpredictable emergence of an abnormal dynamic state of the brain with excessive neuronal firing and synchronization[1]. Temporal Lobe Epilepsy (TLE) is the most common form of partial epilepsy and often associated with mesial temporal sclerosis (MTS). MTS is characterized by a progressive hippocampal cell loss and reorganization of adjacent mesial structures, which might contribute to epileptogenicity[2]. Epilepsy is treated with antiepileptic drugs (AED's) but 30% of the patients do not respond to AED's sufficiently and are diagnosed with refractory epilepsy[3]. For those patients, surgical removal of the brain region responsible for seizure onset might be an option, but hypotheses about the location of this area is needed and sometimes require intracranial or depth electroencephalography (dEEG) recordings[4]. During these recordings, medication is phased out to provoke seizures. Capturing the seizures enables one to localize the SOZ, which is later resected to achieve seizure freedom. This method is a very intensive procedure; therefore ultimately non-invasive methods would be preferred.

Because of the dynamical character of epileptic activity, brain-measuring tools with the highest possible temporal resolution such as magnetoencephalography (MEG) are used in epilepsy investigations and for pre-surgical evaluation[5]. MEG reflects large-scale summated field potentials at the sensors outside the skull that are ultimately caused by excitatory- and inhibitory postsynaptic potentials within the brain. With techniques such as beamforming it is possible to reconstruct the activity detected at the MEG sensors to predefined anatomical locations[6]. A set of atlas based regions of interest that cover the brain will be used and therefore this method can be compared with results obtained using other imaging modalities such as MRI. The hippocampus is often related to and thought to originate TLE, therefore brain measurements in this area are crucial. In this study, hippocampal depth trajectories will be compared with anatomically-matched reconstructed virtual electrodes from MEG, hereby bridging the gap between microscopically and macroscopically recorded hippocampal activity and assessing the questions whether MEG could be an interesting non-invasive alternative for depth EEG during pre-surgical work-up studies in epilepsy patients.

METHODS

Subjects
Nine epileptic patients who underwent epilepsy surgery between 2010 and 2014 at the VU Medical Center were enrolled in this study. The data was gathered from the SEIN VU medical center epilepsy database, containing the records of patients' characteristics and outcomes from diagnostic tests. Patients who underwent surgery with hippocampal trajectories, with pre-operative and post-operative MRI scans and MEG data were included. One-third of the patients had MTS and the number of hippocampal trajectories ranged from one to three. The gender ratio (f:m) was 2:7 and the mean age of the patients was 37,5 years old.

dEEG acquisition
dEEG recordings were made at the VU medical center at the Epilepsy Monitoring Unit (EMU). Locations of the depth trajectories were based on the pre-surgical work-up including MRI and MEG data to localize the SOZ for each patient. Each trajectory consisted of around 8 electrodes. Table 1 shows the amount of hippocampal trajectories for ach patient, which were studied. Two types of dEEG recording conditions were used, one with high antiepileptic medication levels

(dEEG HM) and one with low antiepileptic medication levels (dEEG LM). For each dEEG modality 10 artifact free epochs, which resulted in two times 80 seconds of data. The patients were in resting-state with their eyes open during the used recordings.

MEG acquisition

MEG recordings were made using a 306-channel whole-head neuromagnetometer (Elekta Neuromag Oy Helsinki, Finland) in a magnetically shielded room (VacuumSchmelze GmbH, Hanua, Germany). Twenty artifact-free epochs of MEG data were selected with a length of 4096 samples and a sample frequency of 1250 Hz., which results in 65,536 seconds of data. During the recordings the patients were in a resting-state eyes-closed condition.

Beamforming (virtual electrodes)

The virtual electrodes were anatomically matched to the locations of the depth electrodes on the trajectories during the dEEG recording. The locations of the depth electrodes were marked on a CT-scan, using iPlan RT planning software version 3.0.0 [authored by Brainlab AG]. The Hounsfield scale was used for describing radio density on a CT scan. Depth electrodes, made from metal, which has a high radio density, were visible on a three dimensional view. The visualized trajectories were laid over the MRI. In MRI viewer, point fitting the exact locations for virtual electrodes was done [DICOM Viewer v1.9.16 authored by Medixant; available at **http://www.radiantviewer.com**]. The beamformer approachas described by Hillebrand et al. (2012) was used for spatial scanning.

Frequency analysis

Peak frequencies from different modalities were used as a marker for signal similarities between source-space MEG data, dEEG HM and dEEG LM recordings in the hippocampus. Peak frequencies were calculated with the Fast Fourier Transform (FFT) in brainwave v0.9.151.5 [authored by C.S.Stam; available at **http://home.kpn.nl/stam7883/brainwave.html**].
The signal was filtered in the broadband (0.5 Hz – 48 Hz). The gain was set on 0.25 for MEG and 3.0 for dEEG. The peak frequencies were averaged over all epochs, therefore for each patient, for each hippocampal electrode, for each modality a measurement was compared. The comparison between dEEG HM and MEG was mostly investigated, because during both those measurements patients were on high medication levels. Therefore, this comparison was the best way of testing our hypothesis.

Statistical analyses

The distribution of the data was checked with the Kolmogorov-Smirnov test. To test for group differences, the related-samples Wilcoxon signed rank test was performed. Not only differences between groups, but also similarities between the signals were analyzed. Spearman's rank correlation coefficient was used to measure associations between MEG, dEEG HM and dEEG LM peak frequencies. To test whether the locations of the electrodes on the trajectories influenced the correlations between the modalities, separate Spearman's rank correlation tests were performed on both deeper and superficial located electrodes. Differences between SOZ and other region's are tested with an independent samples Mann-Whitney U test. Statistical testing's were performed in IBM SPSS Statistics version 20 [authored by IBM corp.].

RESULTS

Grouped results

The average peak frequency between groups significantly differed for each modality (dEEG HM: 5.9130 ± SD = 1.095, dEEG LM: 6.1847 ± SD = 1.334, MEG: 7.416 ± SD = 0.987). The means of both the dEEG measurements and MEG significantly differed with $p < 0.001$. The means of dEEG HM and dEEG LM significantly differed with $p = 0.002$. In figure 1 the median peak frequencies and the distribution of each modality are shown.

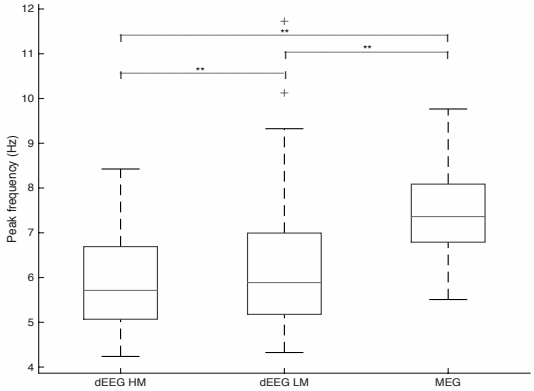

Figure 1. Descriptive statistics of the data groups. (+ = outlier data point, LM = low medication levels, HM= high medication levels, ** = $p < 0.001$)

Overall associations

Between groups associations for hippocampal MEG, dEEG HM and dEEG LM peak frequency measurements were analyzed with the Spearman rank correlation coefficient. Between dEEG HM and dEEG LM a very high and significant correlation was revealed. A modest, but significant correlation between MEG and dEEG HM was found. The association between MEG and dEEG LM was very weak and not significant. The exact correlations between the different modalities are shown in table 1.

Table 1. Overall, superficial and deep correlation coefficients between modalities.

Comparison:	**dEEG HM and dEEG LM**	**dEEG HM and MEG**	**dEEG LM and MEG**
	r	r	r
All	0.506**	0.274**	0.088
Superficial	0.690**	0.352**	0.208
Deep	0.361**	0.205	0.639

(LM = low medication levels, HM= high medication levels, **= $p < 0.001$)

Individual associations

To explore individual differences in the associations between dEEG HM and MEG, within-patient correlations were performed. The associations between dEEG HM and MEG are aggregated for each patient in the graph shown in figure 2. For this comparison four out of nine patients were significantly positively correlated. Exact values for the within patient associations in the comparison between dEEG HM and MEG are shown in Table 2. These correlation coefficients vary among the individual patients, with a range between -0.1 and 0.9. One-second hippocampal data-examples of patient 6 and 8 are shown in figure 2. Signals of patient 6 were positively significant correlated for this comparison. Patient 8 is an example of a patient with a low correlation coefficient between dEEG HM and MEG signals.

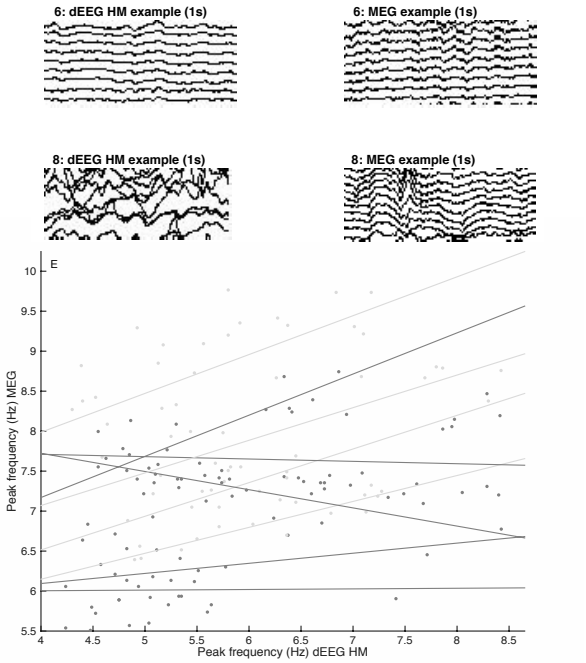

Figure 2. MEG and dEEG signal examples from patient 6 and 8. The graph underneath describes the peak frequency correlations between MEG and dEEG for each patient separately. (HM= high medication levels)

Additional Analyses

Separating electrodes belonging to different trajectories did not affect the correlation coefficients, for the within patient comparison between dEEG HM and MEG. For example, the overall correlation coefficient of patient 6 was 0.628 with $p < 0.001$, whereby the three different trajectories separately showed lower and less significant correlation coefficients (r = -0.238 with $p = 0.570$, r = 0.336 with $p = 0.310$ and r = 0.036 with $p = 0.939$). Taking into account the position of the electrodes is an alternative approach for evaluating the MEG measurements in comparison with the dEEG measurements. The location of the electrodes on the trajectories did not extensively affect the correlation coefficients. Table 2 shows the correlation coefficients for the different comparisons separated for deeper and superficial trajectories.

Peak frequencies in the three different modalities showed a trend to be lower in SOZ trajectories comparing to other regions, despite not reaching statistical significance in all modalities. For MEG no significant difference was found ($p = 0.779$). In dEEG HM a significant difference between SOZ and other region's was found with $p = 0.009$ and in dEEG LM also a significant difference was revealed ($p = 0.006$).

Table 2. Individual correlation coefficients for the comparison between dEEG HM and MEG signals.

Comparison:		**dEEG HM and MEG**
Patient nr.	n	r
1	15	0.527*
2	26	-0.556**
3	5	0.300
4	27	-0.146
5	16	0.415
6	25	0.628**
7	9	0.714*
8	19	0.040
9	15	0.939**

Legend:
Significant positive correlation
Significant/negative correlation

(N = number of hippocampal electrodes, ** = $p < 0.001$, * = $p < 0.05$, HM = high medication levels)

DISCUSSION

A retrospective comparison study between MEG and dEEG signals has been performed in order to investigate the possibilities of non-invasive measurements for replacing dEEG in the pre-surgical work-up of epilepsy patients. MEG signals were reconstructed to the virtual electrodes at the same locations as hippocampal depth electrodes and peak frequencies of the signals were compared to visualize the similarities between MEG and dEEG. MEG can be very useful to study the activity in the hippocampus at the group level, because the peak frequencies of virtual electrodes were positively correlated with the peak frequencies measured in the hippocampus with dEEG HM. This kind of comparison between the microscopically and macroscopically recorded hippocampal activity in multiple epileptic patients has not been done before. Simultaneous recordings of MEG and intracerebral stereotaxic EEG have been studied before, and significant correlations have been revealed[7,8]. Still, these recordings were done simultaneously and electrodes were not anatomically matched. Additionally, the quality of the non-invasive signals was lower, because of the usage of other older spatial scanning programs then the beamformer technique. Also, when recording simultaneously, brain-measuring tools could influence the quality of the MEG signals. Our study found correlations between signals measured at different time points. In some patients, the time in between the recordings is more than a year. This could bias the results, because medications and the presence of epileptic rhythms could be different. However, the fact that different time-points were considered, could also be seen as strength of this study. The fact that correlations at different time points are

confirmed in the MEG data shows the reliability of the beamformer technique. Additionally, MEG was indicated as being sensitive to specific regions, mostly cortical, which is opposite to our finding[8]. As the comparable correlations coefficients in the additional analysis of comparing deeper and superficial electrodes indicate, the used beamformer is reliable even to deeper sources in the brain, like the hippocampus.

To further investigate the revealed correlation between MEG and dEEG HM, the correlation coefficients were analyzed at individual level. These correlations have been shown to be positive and significant in four out of nine patients. The non-significant and negative correlations can be explained by visually checking the recorded signals in the hippocampus for MEG and dEEG HM. In patients with low correlation coefficients, either in dEEG signals or MEG signals, interictal epileptic peaks or aberrant rhythms were found. The fact that, even when physiological rhythms were present in the signals, correlations between MEG and dEEG were revealed, is a valid finding, which can be built on in the future. Patients with signals showing lower epileptic activity, revealed higher correlation coefficients. If only using clean non-noisy signals, the correlations between MEG and dEEG could be higher. This study only analyzed hippocampal trajectories, which were relevant trajectories for the patients to study for clinical reasons. Studying more trajectories could help finding an indicator for localizing the SOZ based on non-invasive measurements. Also, a more reliable measurement than peak frequency should be used to classify the signals, for example epileptic spikes, high frequency oscillations or network connectivity measurements should be measured and analyzed.

Summarized, this study shows an interesting opportunity for investigating the brain with virtual electrodes reconstructed out of MEG sensor signals with the beamformer. Although this technique shows to be promising for future clinical application, further studies with more patients and different settings are needed. It could be helpful, not only for epilepsy investigations, also for other diseases, which could be studied in deeper sources non-invasively using the beamformer technique on a MEG scan. Ultimately, the intensive dEEG recordings should be replaced with a non-invasive method for pre-surgical work-up studies in epilepsy patients, whereby MEG could be a reliable prospective tool.

ROLE OF THE STUDENT

The idea of MEG beamforming being a possible future alternative for dEEG already existed. During the three-months internship, the student designed how and why these two modalities could be compared, prepared the data for analyzing, processed the results and drew the conclusions.

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