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Citation for published version (APA):

Yaakub, S. N., Tangwiriyasakul, C., Abela, E., Koutroumanidis, M., Elwes, R. D. C., Barker, G., & Richardson, M. (in press). Heritability of alpha and sensorimotor network changes in temporal lobe epilepsy. *Annals of Clinical and Translational Neurology*.

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1	Heritability of alpha and sensorimotor network changes in
2	temporal lobe epilepsy
3	
4 5	Running head: Alpha-related imaging endophenotypes for mTLE
6	Siti Nurbaya Yaakub PhD <sup>1,2</sup> , Chayanin Tangwiriyasakul PhD <sup>1</sup> , Eugenio Abela MD <sup>1,3</sup> , Michalis
7	Koutroumanidis MD <sup>4</sup> , Robert D. C. Elwes MD, FRCP <sup>3</sup> , Gareth J. Barker PhD <sup>5</sup> and Mark P. Richardson
8	FRCP, PhD <sup>1,3</sup>
9	
10	<sup>1</sup> Department of Basic & Clinical Neuroscience, Institute of Psychiatry, Psychology & Neuroscience,
11	King's College London, London, United Kingdom
12	<sup>2</sup> King's College London & Guy's and St Thomas' PET Centre, School of Biomedical Engineering &
13	Imaging Sciences, King's College London, London, United Kingdom
14	<sup>3</sup> Department of Clinical Neurophysiology, King's College Hospital NHS Foundation Trust, London,
15	United Kingdom
16	<sup>4</sup> Department of Clinical Neurophysiology and Epilepsies, Guy's and St. Thomas' NHS Foundation
17	Trust, St. Thomas' Hospital, London, United Kingdom
18	<sup>5</sup> Department of Neuroimaging, Institute of Psychiatry, Psychology & Neuroscience, King's College
19	London, London, United Kingdom
20	
21	Corresponding Author:
22	Professor Mark P. Richardson (mark.richardson@kcl.ac.uk)
23	Department of Basic & Clinical Neuroscience,
24	Maurice Wohl Clinical Neuroscience Institute,
25	Institute of Psychiatry, Psychology & Neuroscience,
26	King's College London,
27	125 Coldharbour Lane, London SE5 9NU
28	
29	Character Count: Title (80); Running Head (45)
30	Word Count: Abstract (249); Body of Manuscript (3380)
31	Number of Figures: 3 (including 3 colour figures)
32	Number of Tables: 1
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### 35 Abstract

**Objective:** Electroencephalography features in the alpha band have been shown to differ between people with epilepsy and healthy controls. Here, in a group of patients with mesial temporal lobe epilepsy, we seek to confirm these electroencephalography features, and using simultaneous functional magnetic resonance imaging, we investigate whether brain networks related to the alpha rhythm differ between patients and healthy controls. Additionally, we investigate whether alpha abnormalities are found as an inherited endophenotype in asymptomatic relatives.

42

43 Methods: We acquired scalp electroencephalography and simultaneous electroencephalography 44 and functional magnetic resonance imaging in 24 unrelated patients with unilateral mesial temporal 45 lobe epilepsy, 23 asymptomatic first-degree relatives of patients with mesial temporal lobe epilepsy, 46 32 healthy controls. We compared peak alpha power and and frequency from 47 electroencephalographic data in patients and relatives to healthy controls. We identified brain 48 networks associated with alpha oscillations and compared these networks in patients and relatives to 49 healthy controls.

50

51 **Results:** Patients had significantly reduced peak alpha frequency across all electrodes. 52 Asymptomatic relatives also had significantly reduced peak alpha frequency primarily over central 53 electrodes. Both patients and asymptomatic relatives showed a combination of increased activation 54 and a failure of deactivation in relation to alpha oscillations compared to healthy controls in the 55 sensorimotor network.

56

57 Interpretation: Genetic factors may contribute to the shift in peak alpha frequency and alterations in 58 brain networks related to alpha oscillations. These may not entirely be a consequence of anti-epileptic 59 drugs, seizures or hippocampal sclerosis and deserve further investigation as mechanistic 60 contributors to mesial temporal lobe epilepsy.

61

### 62 Introduction

63 Mesial temporal lobe epilepsy (mTLE) is the most common type of medically refractory focal epilepsy 64 in adults. Most adults with mTLE present with hippocampal atrophy or hippocampal sclerosis, which 65 may be amenable to surgical treatment<sup>1</sup>. Abnormalities in patients with hippocampal sclerosis extend 66 beyond the hippocampus and are thought to involve a network of regions including temporal, thalamic 67 and limbic regions<sup>2</sup>; evidence for whether abnormalities precede seizure onset or are a result of long-68 term seizures is scarce and mixed<sup>3,4</sup>. While mTLE is generally thought of as an acquired disorder, 69 there is emerging evidence for a genetic link in sporadic mTLE<sup>5,6</sup>, and studies have found alterations 70 in structural brain morphology in asymptomatic relatives of patients with HS<sup>7,8</sup>. This structural 71 alteration in asymptomatic relatives suggests an inherited trait that precedes seizure onset<sup>9</sup>.

72

73 Alpha is one of the main background electroencephalography (EEG) physiological rhythms observed 74 primarily over the bilateral posterior (occipital) areas when subjects are awake and relaxed, and their 75 eyes are closed. Its frequency, from late childhood through adulthood is within the 8 to 13 Hz range. 76 Alpha activity is typically attenuated (or blocked) by both visual and non-visual stimuli, and mental 77 tasks<sup>10</sup>. Recent studies have shown evidence for active involvement of alpha activity in cognitive 78 processes<sup>11–13</sup>, and an emerging theory is that the alpha-rhythm governs neural excitability through 79 top-down modulated cognitive control networks<sup>14</sup>. The neural substrates underpinning alpha activity 80 are still not well understood but are thought to be thalamo-cortical in origin<sup>15</sup>, based on animal models<sup>16–19</sup> and neuroimaging studies in humans<sup>20–24</sup>. 81

82

83 It is known that there are alterations in alpha activity in patients with epilepsy, but these alterations 84 are rarely reported or described<sup>25</sup>. While it is known that alpha activity is influenced by, among other 85 things, anti-epileptic drugs<sup>26,27</sup>, reductions in peak alpha frequency (PAF) have been shown to be 86 epilepsy-specific<sup>25</sup> and to distinguish between focal and generalised epilepsy<sup>28</sup> and changes in both 87 frequency and power of alpha activity are related to the severity and type of seizures after accounting 88 for anti-epileptic drug load<sup>28,29</sup>.

89

Alpha power and peak frequency have been shown to be highly reproducible within individuals<sup>30,31</sup>, and highly heritable<sup>32,33</sup>. There exists a large amount of inter-individual variation related to age, memory and cognition, and neurological conditions<sup>34–38</sup>, which suggest that alpha activity measures may be too unspecific to be considered biomarkers or endophenotypes<sup>14</sup>. However, there is some evidence for a brain network endophenotype for idiopathic generalised epilepsy derived from the "low alpha" frequency band<sup>39</sup>. It is so far unknown whether there may be functional or structural network alterations related to alpha activity in relatives of patients with mTLE.

97

98 The present study seeks to characterise EEG alpha band power and peak frequency in patients with 99 mTLE and asymptomatic relatives compared to healthy controls and investigate whether the 100 functional brain networks related to alpha oscillations differ in patients with mTLE and asymptomatic 101 relatives compared to healthy controls.

102

## 103 Materials and methods

### 104 **Participants**

105 The study was performed at the National Institute for Health Research/Wellcome Trust King's Clinical 106 Research Facility at King's College Hospital, London, United Kingdom. All experimental procedures 107 were reviewed and approved by the London – Bromley National Research Ethics Service. Written 108 informed consent was obtained from each participant after all procedures were fully explained.

109

110 Twenty-four unrelated patients with mTLE were recruited from outpatient epilepsy and neurology 111 clinics in hospitals in south London. The diagnosis of mTLE was made on the basis of clinical 112 evaluation including history, seizure semiology, scalp EEG, and conventional clinical MRI reported by 113 experienced neuroradiologists. Patients who had other pathologies, for example malformations of 114 cortical development or tumours, who had undergone surgical resection of the affected temporal lobe,

- or who had recent invasive brain investigations (including depth electrode recordings) were excludedfrom the study.
- 117

118 Twenty-three asymptomatic first-degree relatives were recruited either through patients included in 119 the study or through patients who had a diagnosis of mTLE but were themselves excluded from the 120 study due to a history of surgical resection or recent invasive brain investigations. (Note that recruiting 121 in this way means that while we refer to them as "relatives", some members of this group have no 122 patient to whom they are related in the patient group). Thorough clinical interview of these relatives 123 revealed no current or previous diagnosis of neurological disorders, and no history of symptoms or 124 clinical events suggestive of epileptic seizures. Scalp EEG, carried out as part of the study, showed 125 no epileptiform discharges in any relative.

126

127 Thirty-two healthy control participants with no current or previously diagnosed personal or family 128 history of neurological disorders were recruited for comparison (Table 1A).

129

Full clinical information for patients is given in Supplementary Table 1 and details of relatives andclinical information of their associated probands given in Supplementary Table 2.

132

#### 133 Data acquisition

#### 134 Study design

Participants had a 20-minute EEG recording outside the MRI scanner, a high-resolution structural T1weighted MRI scan and a 10-minute simultaneous EEG and functional MRI (fMRI) recording in the scanner. During EEG and fMRI recordings, participants were instructed to stay awake and relax with their eyes closed.

139

- 140 To increase power to detect pathological differences, imaging and EEG data from patients with right-
- sided mTLE (n = 7; 38.9%) and relatives of patients with right-sided mTLE (n = 5; 41.7%) were left-

to-right flipped so that the ipsilateral side is on the left. All changes were considered as ipsilateral or
contralateral to the pathological hippocampus in patients. For consistency, a similar proportion of
healthy control data (n = 12; 38.7%) was randomly chosen to be side flipped.

145

Not all participants completed the simultaneous EEG and fMRI investigations due to reasons including
claustrophobia and equipment problems. The subset of participants with complete data is described
in Table 1B.

149

#### 150 EEG data

EEG data were recorded using a 64-channel MRI-compatible EEG system (Brain Products GmbH, Munich, Germany). All participants were fitted with a BrainCap MR EEG cap with 63 Ag/AgCI electrodes arranged according to the extended international 10-20 system with the reference electrode placed between Fz and Cz and the ground between Fz and Fpz. The electrocardiogram (ECG) was recorded at a sampling frequency of 5kHz using the BrainVision Recorder software (Brain Products). EEG recordings outside the scanner were performed in an electrically shielded Faraday cage room.

158

#### 159 MRI data

160 MRI was performed on a General Electric 3T MR750 scanner (GE Healthcare Systems, Chicago, 161 USA) using the body coil for radiofrequency transmission and a 12-channel head coil for signal 162 reception. Resting-state fMRI data were acquired using a gradient echo echo-planar imaging 163 sequence in a plane parallel to the AC-PC line, 2160ms repetition time (TR), 25ms echo time, 75° flip 164 angle, 36 interleaved slices of 64×64 matrix size, giving a 211×211mm field of view with a voxel size 165 of 3.3×3.3×3.3mm. Simultaneous EEG was recorded during the fMRI scan at a sampling frequency 166 of 5kHz with the SyncBox device (BrainProducts) used to synchronise EEG and fMRI acquisition. A 167 three-dimensional inversion recovery-prepared spoiled gradient-echo image was acquired in the 168 sagittal plane with 270mm field of view, 256×256 matrix (resulting in an in-plane voxel size of

- 169 1.05×1.05mm), 196 sagittal slices, 1.2mm slice thickness, 7.312ms repetition time, 400ms inversion
  170 time, 3.016ms echo time and 11° excitation flip angle.
- 171

### 172 Data analysis

#### 173 EEG power spectral analysis

EEG data acquired outside the scanner was used for the power spectral analysis conducted in MATLAB (R2015b, The MathWorks Inc., Natick, MA, 2015) using tools from the FieldTrip EEG software toolbox<sup>40</sup>. In previous work, we have shown that the choice of segment does not affect the analysis<sup>28</sup>. We conducted the analysis on the first 5 minutes of EEG recording to exclude any possible confounding effect of the choice and length of segments. Data were re-referenced to the average of all channels except the Fp1, Fp2 and ECG electrodes, and de-trended. Segments were visually inspected for artefacts and the presence of interictal discharges in patients.

181

182 Data were bandpass filtered between 0.5 and 70Hz and the power spectral density for each 183 participant's segment was estimated using Welch's method with a window length of 4s and 50% 184 overlap, giving a frequency resolution of 0.25Hz. The relative power at each frequency resolution 185 point was computed as a fraction of the total power between 0.5 and 70Hz. Data were band-passed 186 within the alpha frequency band (6-13Hz) with the lower boundary of the alpha band modified to 187 include "low alpha" frequencies<sup>41</sup>, since previous work has shown alterations in the low alpha band in 188 epilepsy<sup>28,39</sup>. The peak power (maximum power) and peak frequency (frequency at which the 189 maximum power occurs) were computed for each subject. Statistical group comparisons of peak 190 power and peak frequency were restricted to parietal and occipital channels, where the alpha rhythm 191 is most prominently expressed. We used one-tailed two-sample t-tests (with the hypothesis that 192 patients and relatives would show reduced alpha power and frequency compared to healthy controls) 193 and controlled the false discovery rate (FDR; over 17 parietal and occipital channels) using the 194 Benjamini-Yekutieli procedure with  $\alpha$  = 0.05.

196 We performed three sub-group analyses to investigate the effects of medication, seizure control and 197 relatedness. Since carbamezepine is known to cause slowing of the alpha frequency, we split the 198 patient group into patients taking carbamazepine (n = 8) and patients who were not (n = 16). To 199 examine the effect of seizure control, we split the patient group into patients with good (n = 4) and 200 poor (n = 20) seizure control (with the threshold for poor seizure control at  $\geq$  4 seizures/year, as 201 defined in <sup>28</sup>). We had seven pairs of related patients and relatives in this analysis. In the final sub-202 group analysis, to exclude any confounding effects of relatedness, we excluded four patients and 203 three relatives so that all patients and relatives remaining in the analysis were unrelated. In each of 204 these sub-group analyses, peak power and frequency in the alpha band were compared to healthy 205 controls as described above.

206

### 207 Simultaneous EEG-fMRI

208 EEG data recorded in the scanner were pre-processed using BrainVision Analyzer (version 2.0, Brain 209 Products) to remove MR gradient and ballistocardiogram artefacts from the simultaneous EEG-fMRI 210 data. A sliding average template of MRI scanner artefacts using the average of 21 TR intervals 211 identified by the gradient onset markers was subtracted from the EEG signal to correct for MR gradient 212 artefacts. Data were downsampled to 250Hz. The peaks of the R-waves were identified from the ECG 213 signal in a semi-automated manner using a template pulse wave, and subsequently visually checked 214 and adjusted. Ballistocardiogram artefacts were corrected by subtracting a sliding average template 215 for the R-waves from the EEG.

216

The alpha power time-series was extracted from the pre-processed in-scanner EEG data using MATLAB. EEG data were averaged over the three occipital electrodes (O1, O2 and Oz) and a shorttime Fourier transform was applied to compute the spectrogram using windows equal to the fMRI data sampling TR of 2.16s with no overlap. To account for intra-subject variation of peak alpha frequency, the alpha power time series was computed as the mean alpha power over a narrow band of ± 2Hz around the peak alpha frequency for each subject. Outliers in the mean alpha power time series were identified as data points where the amplitude exceeded the standard boxplot outlier definition, Q3 +
1.5 × IQR, and replaced by linearly interpolated values. Each alpha power time series was then
truncated to exclude the first 5 and last 5 TR of data to exclude end effects from average template
correction and scaled between 0 and 1.

227

228 FMRI data were pre-processed using FSL's FEAT software (v6.0)<sup>42</sup>. FMRI data pre-processing 229 involved motion correction, spatial smoothing with a 6mm full width at half maximum Gaussian filter, 230 and bandpass filtering between 0.01Hz and 0.12Hz. Each subject's native space data were co-231 registered to the Montreal Neurological Institute (MNI) standard space image by way of a linear transform to the subject's high-resolution T1-weighted image and then a non-linear registration to 232 233 standard space. To model and exclude effects of noise within the data, several nuisance regressors 234 were used as variables of non-interest in the subsequent general linear model analysis. These were 235 the average signal each from the white matter and cerebrospinal fluid, and the 6 motion parameters.

236

237 For each subject, a voxel-wise whole-brain general linear model analysis was implemented with FEAT 238 with the alpha power time series as the variable of interest and the nuisance regressors as variables 239 of non-interest. The alpha power time series was convolved with the canonical double-gamma 240 hemodynamic response function to take into account the delay associated with the blood-oxygen-241 level-dependent fMRI (BOLD-fMRI) response. Group analyses were performed in FEAT with a mixed-242 effects analysis of covariance model with age and sex as covariates. Contrasts for the main effect of 243 group and group differences between healthy controls and each of the patient and relative groups 244 were set up and significant effects were identified with a cluster threshold of p < 0.05, FWE-corrected.

245

We used the normalised alpha power estimated from the O1, O2 and Oz electrodes as a measure of vigilance during the simultaneous EEG-fMRI recording<sup>43</sup>. For each subject, we estimated the normalised alpha power averaged across the three occipital electrodes using 10 second sliding windows across the in-scanner EEG data (after discarding the first and last 5 TR as above). We then

estimated the slope of the normalised alpha power for each subject and compared this betweengroups using a one-way analysis of variance.

252

### 253 **Results**

The participant groups were not significantly different in age or gender for any of the datasets when assessed using one-way analyses of variance and  $\chi^2$  tests.

256

### 257 Alterations in the EEG power spectrum

Peak power and peak frequency within the alpha frequency band were averaged across group, and patients with mTLE and asymptomatic relatives were compared to healthy controls. No significant differences in peak power in either patients or relatives from controls were present (Fig 1A). PAF was significantly reduced in patients with mTLE compared to healthy controls across all parietal and occipital channels (p < 0.05, FDR-corrected; Fig 1B). Asymptomatic relatives also showed a reduction in PAF compared to healthy controls in 14 of the 17 parietal and occipital channels (p < 0.05, FDRcorrected; Fig 1B).

265

Peak alpha frequency remained significantly reduced compared to healthy controls in the sub-group who were not on carbamazepine (Supplementary Figure 1), in the sub-group of patients with poor seizure control (Supplementary Figure 2) and in both sub-groups of unrelated patients and relatives (Supplementary Figure 3).

270

### 271 EEG alpha correlates of BOLD-fMRI network

272 Regions showing positive and negative correlations of BOLD-fMRI activity and EEG alpha oscillations 273 across all subjects are shown in Fig 2A (p < 0.05, FWE-corrected). Positive correlations were primarily 274 found in the bilateral thalamus and parahippocampal gyrus, brainstem and subcallosal cortex. 275 Negative correlations were primarily seen in bilateral cortical regions in the dorsal attention network, including the middle and inferior frontal gyri, superior parietal lobes, lateral occipital cortices andinferior frontal gyri.

278

279 Compared to healthy controls, patients showed significantly higher BOLD signal correlations with 280 alpha activity in regions of the sensorimotor network, including the bilateral pre- and post-central gyri 281 extending into the supplementary motor area as well as in regions of the cingulo-opercular/insular 282 network, including the anterior cingulate, bilateral insulae and frontal and parietal opercula (p < 0.05, 283 FWE-corrected; Fig 2B). Relatives also showed significantly higher BOLD signal correlations with 284 alpha activity compared to healthy controls in regions of the sensorimotor network, including the 285 bilateral pre- and post-central gyri and anterior cingulate, in addition to the occipital cortex (p < 0.05, 286 FWE-corrected; Fig 2C).

287

Both patients and relatives appeared to show a combination of higher activation (or higher correlation with alpha oscillations) and a failure of deactivation (i.e. a failure to decouple with alpha oscillations) compared to healthy controls who mainly showed reduced correlation with alpha in these regions (Fig 3). There were no significantly lower BOLD correlations with alpha in either the patients or relatives compared to healthy controls.

293

The slope of the peak alpha power was not significantly different between groups: F(2,62) = 1.361, *p* = 0.264, indicating that the level of vigilance between groups was not significantly different across the EEG-fMRI recording.

297

# 298 Discussion

The present study identified EEG peak alpha frequency reductions and functional brain network alterations associated with alpha oscillations in both patients with mTLE and asymptomatic relatives compared to healthy controls. Analysis of the EEG power spectrum revealed a shift of the alpha rhythm towards lower frequencies in both patients with mTLE and asymptomatic relatives. The PAF 303 shift was observed across all EEG channels in patients, while in relatives it was seen primarily over 304 fronto-central channels. With simultaneous EEG-fMRI, we showed that cortical regions in the 305 sensorimotor network failed to deactivate in relation to alpha oscillations in both patients and 306 asymptomatic relatives compared to healthy controls. In addition, patients also showed this pattern of 307 increased fMRI activation and a failure to deactivate with alpha oscillations in the cingulo-308 opercular/insular network. Reduced PAF and altered topographical distribution of EEG alpha power 309 in patients with epilepsy has been reported previously, and we replicate this finding in independent 310 data here. We show here, for the first time, that brain activity related to the alpha rhythm differs 311 between patients with epilepsy and healthy controls. We also show, for the first time, that reduced 312 PAF and altered brain activity related to the alpha rhythm, differ between asymptomatic relatives and 313 healthy controls. These findings in relatives suggest that alterations in alpha activity in patients with 314 epilepsy are not necessarily related to AEDs or seizures, and are an inherited endophenotypic 315 predisposition to epilepsy, which is currently mechanistically unexplained.

316

### 317 Peak alpha frequency shift

318 The shift in PAF toward lower frequencies in patients has been demonstrated previously in both 319 patients with focal and generalized epilepsy, and is thought to be linked to poor seizure control<sup>28</sup>. We 320 also found evidence for a fronto-central PAF decrease in asymptomatic relatives. Importantly, the 321 asymptomatic relatives in this study were unmedicated, hence the reduced PAF cannot be attributed 322 to effects of antiepileptic drugs. The slowing of the alpha rhythm has also been reported in several 323 other neurological and psychiatric disorders including depression and Alzheimer's disease<sup>19,44</sup>. This 324 suggests that the PAF on its own may not be a specific enough measure to serve as an 325 endophenotype for mTLE and may instead point to a more general indicator of susceptibility to 326 abnormal brain function.

327

#### 328 Brain network alterations related to alpha oscillations

Across the whole group of participants, we show positive correlations in the thalamus and negative correlations in the dorsal attention network. The pattern of positive and negative correlations of brain fMRI activity with alpha oscillations is largely in line with the literature in this field<sup>14</sup>.

332

Group comparisons showed higher correlation of alpha oscillations with regions of the sensorimotor network in both patients with mTLE and asymptomatic relatives compared to healthy controls. In healthy controls, cortical regions mainly showed decreased activation with alpha oscillations. In patients, there was a combination of increased activation and a failure to deactivate in regions of the sensorimotor network and the cingulo-opercular/insular network. Interestingly, in the sensorimotor network, relatives also showed the same pattern of increased brain activation and a failure to deactivate in relation to alpha oscillations.

340

Alpha oscillations are thought to govern cortical excitability, or a rhythmic inhibition, where increases in alpha power generally result in an increase in inhibition and hence a decrease in cortical excitability<sup>14,45,46</sup>. The increased correlation between alpha oscillations and sensorimotor network activity observed in patients and relatives could suggest that alpha oscillations have a reduced effect of inhibition over sensorimotor network activity.

346

Compared to healthy controls, patients with mTLE also had higher correlations with alpha in the cingulo-opercular/insular network. It is unclear what the link is between this network and alpha oscillations in mTLE. The cingulo-opercular/insular network is thought to underpin "tonic alertness" through the modulation of alpha oscillations<sup>47</sup>. The insular cortex has also been implicated in ictogenicity in mTLE<sup>48,49</sup>. As one of the few regions with direct connections to the cholinergic basal forebrain, it has been suggested that the link between the alpha rhythm and cholinergic basal forebrain activity is modulated by the insula<sup>50</sup>.

354

This study represents the first investigation of functional brain changes related to the altered alpha rhythm in patients with mTLE and asymptomatic relatives. The findings suggest that the shift in PAF

and alterations in brain function related to the alpha rhythm may deserve further investigation as an
 endophenotype for mTLE and may not entirely be a consequence of anti-epileptic drugs, seizures or
 hippocampal sclerosis.

360

## 361 Acknowledgements

This study was supported by a Medical Research Council (MRC) Programme Grant number MR/K013998/1. MPR is supported in part by the National Institute for Health Research (NIHR) Biomedical Research Centre at the South London and Maudsley Hospital NHS Foundation Trust and King's College London; by the Engineering and Physical Sciences Research Council (EPSRC) Centre in Predictive Modelling in Healthcare grant number EP/N014391/1; and by the MRC Centre for Neurodevelopmental Disorders grant number MR/N026063/1.

368

This study represents independent research supported by the National Institute for Health Research (NIHR)/Wellcome Trust King's Clinical Research Facility and the NIHR Biomedical Research Centre and Dementia Unit at South London and Maudsley NHS Foundation Trust and King's College London. The views expressed are those of the author(s) and not necessarily those of the NHS, the NIHR or the Department of Health.

374

## 375 Author Contributions

MPR, GJB and SNY conceived and designed the study. MK and RDCE contributed primary patient
referrals and clinical data. SNY, CT and EA acquired the study data and conducted the analyses.
SNY drafted the manuscript. All authors reviewed the paper.

379

# 380 Conflicts of Interest

381 The authors report no conflicts of interest relevant to this work.

382

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501

## 503 Figure Legends

**Figure 1. EEG topographical plots for the alpha band.** Group-averaged EEG topographical plots of (A) peak power and (B) peak frequency in the alpha frequency band. In the patients' and relatives' plots, channels that show a significant group difference from healthy controls are indicated by pink dots (p < 0.05, FDR-corrected across parietal and occipital channels only). "L" indicates the left or ipsilateral side. Pat: patients with mTLE, Rel: asymptomatic relatives, Con: healthy controls.

509

510 Figure 2. BOLD fMRI correlates of EEG alpha oscillations. (A) Regions showing positive and 511 negative fMRI correlations with EEG alpha oscillations across all subjects. (B) Regions showing 512 higher correlations with alpha oscillations in (B) patients and (C) asymptomatic relatives compared to 513 healthy controls. Voxels in the sensorimotor region that were significantly different from controls in 514 the relatives group had high overlap with voxels found significantly different from controls in the patient 515 group. Images show Z-statistics at a cluster threshold of p < 0.05 (FWE-corrected). Positive values 516 are shown in red/yellow and negative values in blue. MNI coordinates are shown above each slice. 517 "L" represents the left or ipsilateral side.

518

519 Figure 3. Mean group difference in correlation with alpha oscillation. (A) Clusters showing 520 significantly higher correlation with alpha oscillation in patients compared to healthy controls. (B) 521 Clusters showing significantly higher correlation with alpha oscillation in relatives compared to healthy 522 controls. The mean parameter estimates are shown on the upper axes and the mean group 523 differences shown on the lower axes as bootstrap sampling distributions. Mean differences are 524 depicted as dots; 95% confidence intervals are indicated by the ends of the vertical error bars. Pat: 525 patients with mTLE, Rel: asymptomatic relatives, Con: healthy controls. Figures created on 526 www.estimationstats.com.

527

Supplementary Figure 1. EEG topographical plots for the alpha band in the sub-group analysis
 investigating effect of carbamazepine therapy. Group-averaged EEG topographical plots of (A)

peak power and (B) peak frequency in the alpha frequency band. In the patients' plots, channels that show a significant group difference from healthy controls are indicated by pink dots (p < 0.05, FDRcorrected across parietal and occipital channels only). "L" indicates the left or ipsilateral side. Pat (no CAR): patients with mTLE who are not taking carbamazepine, Pat (CAR): patients with mTLE who are taking carbamazepine, Con: healthy controls.

535

Supplementary Figure 2. EEG topographical plots for the alpha band in the sub-group analysis investigating effect of seizure control. Group-averaged EEG topographical plots of (A) peak power and (B) peak frequency in the alpha frequency band. In the patients' plots, channels that show a significant group difference from healthy controls are indicated by pink dots (p < 0.05, FDR-corrected across parietal and occipital channels only). "L" indicates the left or ipsilateral side. Pat (poor control): patients with mTLE who have  $\ge 4$  seizure per year, Pat (good control): patients with mTLE who have < 4 seizures per year, Con: healthy controls.

543

544 Supplementary Figure 3. EEG topographical plots for the alpha band in the sub-group analysis 545 excluding the effect of relatedness. Group-averaged EEG topographical plots of (A) peak power 546 and (B) peak frequency in the alpha frequency band. In the patients' plots and relatives' plots, 547 channels that show a significant group difference from healthy controls are indicated by pink dots (p 548 < 0.05, FDR-corrected across parietal and occipital channels only). "L" indicates the left or ipsilateral 549 side. Pat: patients with mTLE (unrelated to relatives included in this analysis), Rel: asymptomatic 550 relatives of patients with mTLE (unrelated to patients included in this analysis), Con: healthy controls.

# 552 Tables

## 553 **Table 1. Demographic information for all participants and clinical information for patients.**

	Patients	Relatives	Controls							
(A) Participants with EEG data only										
Number	24	23	32							
Age (years)	40.2 ± 11.9	36.7 ± 13.0	36.9 ± 10.8							
Sex (male/female)	13/11	10/13	16/16							
mTLE side <sup>a</sup> (right/left)	10/14	9/14	-							
Epilepsy onset age (years)	21.8 ± 9.9	-	-							
Duration of epilepsy (years)	18.0 ± 14.0	-	-							
Seizure frequency (/month) <sup>b</sup>	$5.6 \pm 6.3$	-	-							
(B) Participants with simultane	eous EEG-fMRI da	ata								
Number	22	18	25							
Age (years)	39.3 ± 12.7	35.9 ± 13.3	34.8 ± 7.9							
Sex (male/female)	11/11	8/10	13/12							
mTLE side <sup>a</sup> (right/left)	9/13	8/10	-							
Epilepsy onset age (years)	23.1 ± 9.1	-	-							
Duration of epilepsy (years)	15.6 ± 13.7	-	-							
Seizure frequency (/month) <sup>b</sup>	5.8 ± 6.6	-	-							

554 Data are means ± standard deviations.

<sup>a</sup> Refers to side of seizure onset in Patients group, and of the proband in Relatives group.

556 <sup>b</sup> Data unavailable for one patient.



EEG topographical plots for the alpha band. Group-averaged EEG topographical plots of (A) peak power and (B) peak frequency in the alpha frequency band. In the patients' and relatives' plots, channels that show a significant group difference from healthy controls are indicated by pink dots (p < 0.05, FDR-corrected across parietal and occipital channels only). "L" indicates the left or ipsilateral side. Pat: patients with mTLE, Rel: asymptomatic relatives, Con: healthy controls.

80x68mm (300 x 300 DPI)



BOLD fMRI correlates of EEG alpha oscillations. (A) Regions showing positive and negative fMRI correlations with EEG alpha oscillations across all subjects. (B) Regions showing higher correlations with alpha oscillations in (B) patients and (C) asymptomatic relatives compared to healthy controls. Voxels in the sensorimotor region that were significantly different from controls in the relatives group had high overlap with voxels found significantly different from controls in the patient group. Images show Z-statistics at a cluster threshold of p < 0.05 (FWE-corrected). Positive values are shown in red/yellow and negative values in blue. MNI coordinates are shown above each slice. "L" represents the left or ipsilateral side.</li>

180x119mm (300 x 300 DPI)



Mean group difference in correlation with alpha oscillation. (A) Clusters showing significantly higher correlation with alpha oscillation in patients compared to healthy controls. (B) Clusters showing significantly higher correlation with alpha oscillation in relatives compared to healthy controls. The mean parameter estimates are shown on the upper axes and the mean group differences shown on the lower axes as bootstrap sampling distributions. Mean differences are depicted as dots; 95% confidence intervals are indicated by the ends of the vertical error bars. Pat: patients with mTLE, Rel: asymptomatic relatives, Con: healthy controls. Figures created on www.estimationstats.com.

180x193mm (300 x 300 DPI)

ID	Age	Sex	Onset	Localization features	Seizure	Medication	Febrile	EEG?	EEG-
			age		frequency	(daily dose)	seizures?		fMRI?
PAT01	41	F	24	Right sided HS on MRI.	9-10	LMT (550);	*	Yes	Yes
						LEV (1500);			
						PER (4); CLB			
						(60)			
PAT02	35	M	15	Left sided HS on MRI.	2	LMT (400);	*	Yes	No
						VPA (900);			
						CLB (10)			
PAT03	43	F	20	Left sided HS on MRI. Bitemporal seizure onset on intra-	3-7	CAR (1400);	No	Yes	Yes
				cranial EEG.		LMT (200)			
PAT04	57	F	5	Left sided HS on MRI. Left temporal discharges on EEG.	6-7	LEV (875); CIT	No	Yes	Yes
						(30)			
PAT05	22	M	16	Left sided HS on MRI.	20-24	CAR (1200)	No	Yes	Yes
PAT06	34	M	11	Right sided HS on MRI. Right anterior temporal spike and	2-3	PHB (60); VPA	No	Yes	Yes
				wave epileptiform discharges on EEG.		(1200); OLA			
						(7.5); CIT(*)			
PAT07	52	F	15	Loss of digitation in the left hippocampal head. Left	4	LAB (150); CIT	No	Yes	Yes
				temporal hypometabolism on FDG PET. Left temporal		(50); LOR (2)			
				discharges on EEG.					
PAT08	51	F	31	Left sided HS on MRI.	<1	LAC (400)	*	Yes	Yes
PAT09	31	M	25	Right sided HS on MRI.	16-20	CAR (800);	*	Yes	Yes
						LEV (200);			
						CLB (10)			
PAT10	48	M	33	Right sided HS on MRI.	<1	LEV (3000);	Yes	Yes	Yes
						TOP (100)			
PAT11	31	M	21	Right sided HS on MRI.	3-4	LEV (3000);	No	Yes	Yes
						ZON (200);			
						CLN (2)			

# **Supplementary Table 1.** Further clinical details of patients in the study.

PAT12	58	F	47	Left sided HS on MRI.	3-4	TOP (200);	*	Yes	Yes
						CLB (10)			
PAT13	47	M	40	Right sided HS on MRI	<1	LEV (400)	*	Yes	No
PAT14	24	М	22	Ectopic grey matter lateral to body of right hippocampus.	3-4	CAR (400)	Uncertain	Yes	Yes
				Focal temporal lobe seizures and right temporal					
				discharges on EEG.					
PAT15	25	M	23	MRI normal. Left temporal focal seizures on EEG.	3-4	VPA (800);	No	Yes	Yes
				Seizure semiology suggestive of left TLE onset.		TOP (300)			
PAT16	43	F	40	Left sided HS on MRI	4	CAR (800)	No	Yes	Yes
PAT17	23	M	22	Right sided HS on MRI	<1	ZON (200)	Yes	Yes	Yes
PAT18	47	М	15	Normal MRI. Left temporal focal seizures on EEG.	1-2	CAR (600)	No	Yes	Yes
PAT19	24	F	22	Left sided HS on MRI	*	*	No	No	Yes
PAT20	57	М	25	Right sided HS on MRI	<1	LMT (300)	*	Yes	Yes
PAT21	37	F	27	Left sided HS on MRI	12	CAR (400)	Yes	Yes	Yes
PAT22	31	F	22	Left sided HS on MRI	3	LEV (3000)	No	Yes	Yes
PAT23	44	F	1	Left sided HS on MRI.	4	CAR (1200);	Uncertain	Yes	No
						CLB (10)			
PAT24	22	F	18	Left sided HS on MRI. Seizure semiology: posturing of	1-2	None at time of	No	Yes	Yes
				right hand.		scan			
PAT25	52	М	25	Right sided HS on MRI.	15	LMT (750);	*	Yes	Yes
						PER (8)			

\* indicates missing information; HS = Hippocampal Sclerosis; FS = Febrile Seizures. Seizure frequency is the approximate number of seizures per month. Drug abbreviations: CAR = Carbamazepine, CIT = Citalopram, CLB = Clobazam, CLN = Clonazepam, LAC = Lacosamide, LEV = Levetiracetam, LMT = Lamotrigine, LOR = Lorazepam, OLA = Olanzapine, PER = Perampanel, PHB = Phenobarbitone, PHE = Phenytoin, TOP = Toparimate, VPA = Valproate, ZON = Zonisamide.

ID	Age	Sex	Relationship	Proband mTLE details	Proband	EEG?	EEG-
			to proband		in study		fMRI?
REL01	49	М	Son of female	Age of onset 48. Left HS on MRI. Patient has well controlled seizures on medication and	No	Yes	Yes
			patient	has not undergone surgery.			
REL02	24	F	Daughter of	See Supplementary Table 1.	PAT07	Yes	Yes
			female patient				
REL03	34	М	Twin brother	See Supplementary Table 1.	PAT06	Yes	Yes
			of male patient				
REL04	37	F	Daughter of	See Supplementary Table 1.	PAT04	Yes	No
			female patient				
REL05	60	F	Mother of	See Supplementary Table 1.	PAT11	Yes	Yes
			male patient				
REL06	31	F	Daughter of	Age of onset 40. Right HS on MRI. No history of febrile convulsions. Patient has had	No	Yes	Yes
			male patient	right temporal lobectomy and amygdalohippocampectomy and is currently seizure free			
				on medication.			
REL07	17	Μ	Son of female	Age of onset 10-15 years. Left HS on MRI. No history of febrile seizures. Patient has	No	Yes	Yes
			patient	had amygdalohippocampectomy and was seizure free for 7 years, but seizures have			
				recently recurred. Updated telemetry showed left sided abnormalities and independent			
				right sided abnormalities, with some evidence for right sided hypometabolism on PET.			
REL08	25	Μ	Brother of	Age of onset 19. Right HS on MRI. No febrile seizures. Patient has had right temporal	No	Yes	Yes
			female patient	hippocampectomy and was seizure free for a period before a recurrence of nocturnal			
				seizures. Pathology confirmed hippocampal sclerosis.			
REL09	30	Μ	Son of female	Age of onset 10. Left HS on MRI. Patient has had left temporal hippocampectomy.	No	Yes	Yes
			patient	Pathology confirmed hippocampal sclerosis plus FCD type 2b. Seizures remain post-			
				surgery.			
REL10	44	Μ	Brother of	Early age of onset. Right HS on MRI. Patient has not undergone surgery.	No	Yes	Yes
			male patient				
REL11	25	М	Brother of	See Supplementary Table 1.	PAT13	Yes	Yes
			male patient				

**Supplementary Table 2.** Further details of relatives and clinical details of associated probands.

REL12	54	F	Mother of	See Supplementary Table 1.	PAT19	Yes	Yes
			female patient				
REL13	24	F	Daughter of	Early age of onset. Right HS on MRI. Patient has not undergone surgery.	No	Yes	Yes
			female patient				
REL14	18	F	Sister of male	See Supplementary Table 1.	PAT15	Yes	No
			patient				
REL15	40	F	Sister of	Early age of onset. Bilateral HS on MRI. Patient has not undergone surgery.	No	Yes	Yes
			female patient				
REL16	40	F	Sister of	Early age of onset. Bilateral HS on MRI. Patient has not undergone surgery.	No	Yes	Yes
			female patient				
REL17	20	F	Daughter of	Age of onset 33. Left HS on MRI. Patient has had left temporal lobectomy. Pathology	No	Yes	Yes
			female patient	confirmed hippocampal sclerosis. Seizures remain post-surgery.			
REL18	60	F	Mother of	Age of onset 5. Left HS on MRI showing volume loss and hyperintensity on T2-	No	Yes	Yes
			female patient	weighted MRI. Prolonged febrile seizures at 8 months. Patient has had left temporal			
			_	lobectomy and is now seizure free.			
REL19	28	М	Brother of	Age of onset 5. Left HS on MRI showing volume loss and hyperintensity on T2-	No	Yes	Yes
			female patient	weighted MRI. Prolonged febrile seizures at 8 months. Patient has had left temporal			
				lobectomy and is now seizure free.			
REL20	31	F	Sister of	Early age of onset. Right HS on MRI. Patient has not undergone surgery.	No	Yes	Yes
			female patient				
REL21	49	М	Brother of	Early age of onset. Left HS on MRI. Patient has not undergone surgery.	No	Yes	No
			female patient				
REL22	47	М	Son of female	Early age of onset. Left HS on MRI. Patient has not undergone surgery.	No	Yes	No
			patient				
REL23	44	F	Daughter of	Early age of onset. Right HS on MRI. Patient has not undergone surgery.	No	Yes	No
			male patient				

Supplementary Figure 1. EEG topographical plots for the alpha band in the sub-group analysis investigating effect of carbamazepine therapy. Group-averaged EEG topographical plots of (A) peak power and (B) peak frequency in the alpha frequency band. In the patients' plots, channels that show a significant group difference from healthy controls are indicated by pink dots (p < 0.05, FDR-corrected across parietal and occipital channels only). "L" indicates the left or ipsilateral side. Pat (no CAR): patients with mTLE who are not taking carbamazepine, Pat (CAR): patients with mTLE who are taking carbamazepine, Pat (CAR): patients with mTLE who are taking carbamazepine, Con: healthy controls.



Supplementary Figure 2. EEG topographical plots for the alpha band in the sub-group analysis investigating effect of seizure control. Group-averaged EEG topographical plots of (A) peak power and (B) peak frequency in the alpha frequency band. In the patients' plots, channels that show a significant group difference from healthy controls are indicated by pink dots (p < 0.05, FDR-corrected across parietal and occipital channels only). "L" indicates the left or ipsilateral side. Pat (poor control): patients with mTLE who have  $\geq 4$  seizure per year, Pat (good control): patients with mTLE who have  $\leq 4$  seizure per year, Pat (good control): patients with mTLE who have  $\leq 4$  seizures per year, Con: healthy controls.



Supplementary Figure 3. EEG topographical plots for the alpha band in the sub-group analysis excluding the effect of relatedness. Group-averaged EEG topographical plots of (A) peak power and (B) peak frequency in the alpha frequency band. In the patients' plots and relatives' plots, channels that show a significant group difference from healthy controls are indicated by pink dots (p < 0.05, FDR-corrected across parietal and occipital channels only). "L" indicates the left or ipsilateral side. Pat: patients with mTLE (unrelated to relatives included in this analysis), Rel: asymptomatic relatives of patients with mTLE (unrelated to patients included in this analysis), Con: healthy controls.

