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Title

Metabolic Alterations of the Dorsolateral Prefrontal Cortex in Sleep-Related Hypermotor Epilepsy: A Proton Magnetic Resonance Spectroscopy Study

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Abbreviated title

MRS Study of Sleep-Related Hypermotor Epilepsy

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Declaration of conflicting interests

The authors declare no conflict of interest.

Author contributions

QY and QG conceived this evaluation. WW collected the imaging data. XS, QT, and HS designed and maintained the database. WW and XW did data analysis and drafted the manuscript. LL provided statistical support and data interpretation. GJK and QY contributed to data interpretation and critically revised the manuscript. DZ, XW, HG and WL obtained clinical data. SZ and XY did data interpretation and editing. All approved the final version.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Abstract

Sleep-related hypermotor epilepsy (SHE) is a focal epilepsy whose neurobiological underpinnings remain poorly understood. The present study aimed to identify possible neurochemical alterations in the dorsolateral prefrontal cortex (DLPFC) in participants with SHE using proton magnetic resonance spectroscopy (^{1}H MRS). Thirty-nine participants with SHE (mean age, 30.7 years ± 11.3 [standard deviation], 24 men) and 59 controls (mean age, 29.4 years ± 10.4, 29 men) were consecutively and prospectively recruited and performed brain magnetic resonance imaging and ^{1}H MRS in the bilateral DLPFC. Brain concentrations, including N-acetyl aspartate (NAA), *myo*-inositol (mI), choline, creatine, the sum of glutamate and glutamine, glutathione (GSH) and γ-aminobutyric acid, were estimated with LCModel and corrected for the partial volume effect of cerebrospinal fluid using tissue segmentation. ANCOVA analyses revealed lower concentration of NAA in the left DLPFC in participants with SHE compared with controls. A significant difference of NAA concentration between DLPFC in the two hemispheres (left > right) was observed only in the control group. We further confirmed a higher GSH concentration in men than in women in SHE participants which probably indicates that men are more susceptible to this disease. The mI concentration in the right DLPFC was negatively correlated with epilepsy duration. This study demonstrates that DLPFC is an important brain region involved in the pathophysiology of SHE, in which both neurons and astrocytes appear impaired, and the elevated GSH level may suggest an abnormality related to oxidative stress.

Significance

Recent evidence suggests that the sleep-wake circuitry depends on neurotransmitters such as glutamate and GABA and plays a pivotal role in seizure precipitation in sleep-related hyper motor epilepsy (SHE).

However, the in vivo neurochemical changes of this disease have not been explored. Our results reveal metabolic abnormalities in the dorsolateral prefrontal cortex, indicating this brain region involved in the pathophysiology of SHE. These findings shed light on some of the neurobiochemical mechanisms of SHE and enrich our knowledge about MRI-negative SHE patients.

Keywords

sleep-related hypermotor epilepsy; epilepsy; magnetic resonance spectroscopy; dorsolateral prefrontal cortex; GABA.

1 Introduction

Sleep-related hypermotor epilepsy (SHE), formerly known as nocturnal frontal lobe epilepsy (NFLE), is a focal epilepsy with a prevalence of at least 1.8/100,000 (Vignatelli et al., 2015), characterized by vigorous hyperkinetic or asymmetric tonic-dystonic seizures occurring mainly during non-rapid eye movement sleep (Tinuper & Bisulli, 2017; Tinuper et al., 2016). Diagnosis is mostly based on clinical history and semiology. Video-electroencephalography (VEEG), alone or in combination with polysomnography, is the recommended gold standard (Tinuper et al., 2016). However, onset during sleep and usually uninformative ictal and interictal EEG make it a difficult condition to diagnose, especially when seizures originate from the deep cortex (Nobili et al., 2007; Provini et al., 1999). Other objective evaluation methods would, therefore, be useful to provide support for the diagnosis.

The etiology of SHE remains unknown. Genetic findings are inconsistent between familial cases and sporadic cases. In familial forms, autosomal dominant SHE (ADSHE), the syndrome is related to mutations in the genes which code for the subunits of the neuronal acetylcholine nicotinic receptor (nAChRs) (Aridon et al., 2006; De Fusco et al., 2000; Steinlein et al., 1995), and mutations in other genes (Heron et al., 2012; Korenke et al., 2016; Picard et al., 2014). However, most patients with SHE are sporadic cases, in whom similar genetic anomalies are rarely found (Phillips et al., 2000; Sansoni et al., 2012). Neuronal AChRs can regulate the delivery of acetylcholine or other fast neurotransmitters such as

γ-aminobutyric acid (GABA) and glutamate (Glu) (Alkondon et al., 2000). The high density of nAChRs has been found in the thalamus which may be overactivated through brainstem ascending cholinergic pathway (Aridon et al., 2006; Picard et al., 2006). Earlier models believe that wake-sleep regulation was controlled by cholinergic arousal systems (Saper et al., 2005), but recent evidence indicates that the sleep-wake circuitry, including brainstem, thalamus, and frontal lobe, depends on neurotransmitters such as Glu and GABA rather than acetylcholine (Saper & Fuller, 2017) and plays a pivotal role in seizure precipitation in ADSHE (Marini & Guerrini, 2007). The finding of a decrease nAChRs density in the dorsolateral prefrontal cortex (DLPFC) accords with focal epilepsy with the involvement of frontal lobe in patients with ADSHE (Picard et al., 2006). Furthermore, vigorous hyperkinetic or asymmetric tonic-dystonic seizures are also linked to the involvement of the frontal regions despite heterogeneous seizures origin (Proserpio et al., 2011; Tinuper et al., 2016).

The *in vivo* neurochemical changes of sporadic SHE has rarely been explored, although its prevalence is much higher than ADSHE. Proton magnetic resonance spectroscopy (^{1}H MRS) can measure brain neurochemical metabolites, which are potentially relevant to epileptogenesis, such as N-acetyl aspartate (NAA) and *myo*-inositol (mI), as well as the excitatory neurotransmitter Glu and the inhibitory neurotransmitter GABA. A few studies have found decreased NAA in the frontal lobe (Krsek et al., 2007), but it has not been studied in sporadic SHE, where the only MRS study so far has found decreased NAA/Creatine in the cingulate cortex and thalamus (Naldi et al., 2017).

The present study aimed to investigate the neurochemical alterations in bilateral DLPFC in order to understand the underlying pathophysiology of SHE. We focused on DLPFC because a previous positron emission tomography study reported that nAChR density decreased in the right DLPFC (Picard et al., 2006). We explored bilateral DLPFCs because of their rich interhemispheric interconnectivity, and EEG findings in SHE were usually bilateral (Scheffer et al., 1995). We hypothesized that there may be neurochemical abnormalities in the DLPFC of SHE participants, which, if related to the symptom severity, would be an additional helpful tool in the clinical evaluation of SHE.

2 Materials and Methods

2.1 Participants

The prospective study was authorized by the local ethical committee of West China Hospital of Sichuan University and written informed consent was obtained from all participants. Clinical investigation has been performed according to the Declaration of Helsinki. SHE participants were consecutively enrolled from the Epilepsy Center of West China Hospital (X.W., D.Z.; neurologists and epilepsy experts with 10 and 35 years of experience, respectively) between December 2013 and December 2017. Primary diagnosis is based on the clinical history and semiology even in the absence of positive EEG findings. Final diagnosis was validated by video recording of hypermotor events during sleep, preferably by long-term VEEG. All cases were sporadic and did not perform genetic tests. Healthy controls were enrolled from the local area by poster advertisements. Exclusion criteria for both groups included comorbid neurologic or psychiatric diseases, major trauma or cranial surgery, age above 60 years or less than 16 years, and standard contraindications to MRI. Healthy controls with family history for epilepsy were also excluded. Participants who had any visible abnormality on MRI as judged by a neuroradiologist with >20 years of experience (Q.Y.) were excluded from the study. All of the participants were tested for the handness by observing their performance according to the Annett's handedness questionnaire (Annett, 1994).

2.2 MRI and MRS acquisition

All the participants underwent high-resolution T1-weighted MRI and ^{1}H MRS examinations using a 3T scanner (Siemens, Trio Tim, Erlangen, Germany) with an 8-channel phased-array head coil. Sponge pads were used to minimize head motion and mute earplugs were used to protect patients from scanner noise. High-resolution T1-weighted images were obtained with a three-dimensional Magnetization Prepared Rapid Gradient Echo (MPRAGE) sequence covering the whole brain with repetition time/echo time (TR/TE) 2250/2.6ms, flip angle 9°, slice thickness 1mm, total 192 contiguous sagittal slices, matrix 256×256, and field of view 256×256 mm^2.

Single voxel ^{1}H MRS acquisitions for general metabolites were conducted using Point-Resolved Spectroscopy Sequence (PRESS) with TR/TE 2000/30ms and spectral bandwidth 1200Hz, collecting 128 water-suppressed and 16 water-unsuppressed averages. In a standard PRESS, GABA is overlapped and obscured by other metabolites, and so to detect this we used Mescher-Garwood PRESS (MEGA-PRESS), which allows a well-defined GABA signal at 3.0 ppm to be separated from overlapping signals by using the J-coupling (Kaiser et al., 2008; Shungu et al., 2016): parameters were TR/TE=2000/68ms and spectral bandwidth=1200Hz, 128 water-suppressed (EDIT ON scan) and 16 water-unsuppressed averages (EDIT OFF scan) and editing pulse frequency 1.9 ppm. ^{1}H MRS voxels (12 mL = 4.0 × 2.0 × 1.5 cm^3) were placed in bilateral DLPFC, as shown in Figure 1. Pre-saturation bands were placed around the VOIs to avoid lipid contamination from scalp. All spectra data were acquired by the same investigator (W.W.) to

keep the localization consistent. Fastmap shimming was conducted to ensure width at half maximum (FWHM) of water resonance <12 Hz. Spectra were both acquired with and without water suppression. The water signal was used for eddy-current correction and also as the internal reference in absolute metabolite quantification (see below). The total time of the examination was 36 minutes per participant.

2.3 MRS Data Processing

All MRS data acquired by PRESS and MEGA-PRESS were both processed using linear combination model (LCModel) (version 6.3-1H, http://s-provencher.com/lcmodel.shtml). It is automated for baseline and phase correction, and it can also obtain the absolute metabolite concentrations and uncertainty estimations (e.g. Cramer-Rao lower bounds (CRLB)). For MEGA-PRESS，we did eddy-current correction and water-scaling correction before calculating the difference spectrum. An example of PRESS and MEGA-PRESS spectra is illustrated in Figure 2. Neurometabolites of interest included NAA, mI, choline (Cho), creatine (Cr), glutathione (GSH), the sum of glutamate and glutamine (Glx), and GABA. Although GSH overlaps with peaks from other metabolites and could in principle be better approached using spectral editing, it is possible to detect the GSH contribution even when not all individual peaks are visible (Wood et al., 2010). The quality of all spectra and the fitting results were checked since sometimes low CRLB can occur in spectra with poor quality or artifacts. Those data meeting the criteria (full width at half maximum (FWHM) $\leqslant$ 0.08 ppm, signal-noise ratio (SNR) $\geqslant$ 15, and CRLB $\leqslant$ 15%

processed by PRESS, and CRLB $\leqslant$ 30% processed by MEGA-PRESS) were included for statistical analysis (see Table 2).

Quantification of absolute concentrations was performed using both the suppressed and unsuppressed water signal (the later one as an internal reference), and they were reported as mmol/kg·wet·weight. Calculations of metabolite concentrations may be biased by inclusion in the volume of interest (VOI) of varying amounts of cerebrospinal fluid (CSF), and by variations in the amounts of gray matter and white matter (which may contain the metabolites of interest in very different concentrations). To allow for this the VOI fractions of different tissue types were calculated by segmenting each participant's three-dimensional T1-weighted images using the cortical thickness processing pipeline of Advanced Normalization Tools (ANTs) (http://stnava.github.io/ANTs/) (Tustison et al., 2014). The proportion of CSF was determined by creating a mask of the VOI on the segmented images according to its location, using Gannet (www.gabamrs.com). This was used directly to correct each metabolite concentration for CSF dilution (Hammen et al., 2008) : $C_{correct} = C_{raw} \times [V_{Total}/(V_{Total} - V_{CSF})]$, where C_{raw} is the uncorrected estimate for the whole voxel and V_{total} and V_{CSF} represent the total volume of the voxel and the volume of CSF within the voxel, respectively. The gray and white matter fractions were not directly used to correct the estimated concentrations, but in the event of group differences being detected, it is important to exclude the possible contribution of different gray/white matter fractions.

2.4 Statistics

All statistical tests were carried out at a two-sided 0.05 level of significance using the open source R package v3.6.3. We compared the age and education years using the independent-samples *t* test and using chi-square test for sex. The spectrum quality indices (FWHM and SNR) and the voxel tissue fractions (%) were also tested by independent-samples *t* test between the two groups. The Shapiro-Wilk test was used to assess normality of metabolite concentrations. We conducted analyses via analysis of covariance (ANCOVA) to test group differences of metabolites with age, sex and education years as covariates. The threshold of ANCOVA *P* was set at 0.05, after Bonferroni correction for multiple comparisons with seven metabolites.

We analyzed metabolic differences between left and right DLPFC using pared *t* test. To test the effect of sex, metabolic differences between men and women were tested by using the ANCOVA in control's group with age, sex and education years as covariates. For the SHE group, the effect of sex was also tested by using the ANCOVA with additional covariates, including seizure frequency and disease duration.

We performed partial correlations between each metabolite concentration and age at onset and illness duration after controlling the age, sex and education years. Spearman correlations were performed between each metabolite concentration and seizures frequency ('low frequency' here means seizure-free over 6 months before MR scan, or seizures no more frequent than 1 per month; 'high frequency' means seizures per night or week, or at least more than 1 per month).

3 Results

3.1 Participants

We studied 39 participants with SHE (24 males and 15 females; age 17-57 years, mean 30.7 years) and 59 healthy controls (29 males and 30 females; age 20-60 years, mean 29.4 years). All participants were right-handed. For the participants with SHE the mean age at onset was 24.2 years and mean illness duration was 6.5 years. At the time of study 21 (54%) were in the 'low frequency' seizure category and 18 (46%) were 'high frequency'. All but 8 were on antiepileptic mono- or polytherapy. The seizure type was secondary generalized tonic-clonic seizures for all but 3 participants. Interictal and ictal EEG was abnormal in 19 participants. Table 1 summarizes the demographic and clinical data. Participants with SHE and controls did not differ in terms of age ($t_{(96)} = 0.580$, $P = 0.563$) or sex ($\chi^2(1, n = 98) = 1.450$, $P = 0.228$). There is significant group difference in years of education ($P = 0.001$). Compared with controls, SHE patients had fewer years of education.

3.2 MRS Results

Table 2 summarize the neurometabolic concentrations in the two groups. It is worth noting that the number of participants varied in different metabolites. Because we collected bilateral DLPFCs' MRS data and either side meeting the quality standards would be included in this study. After controlling for age, sex and education years, ANCOVA analyses showed that NAA concentration in the left DLPFC and mI

concentrations in the bilateral DLPFCs were lower in the SHE group compared with controls (NAA, $F_{(1, 86)}$ = 7.98, n = 90, P = 0.006; mI in the left DLPFC, $F_{(1, 84)}$ = 5.56, n = 88, P = 0.021; mI in the right DLPFC, $F_{(1, 68)}$ = 4.28, n = 72, P = 0.042), while GSH and Glx concentrations were higher in the right DLPFC (GSH, $F_{(1, 66)}$ = 6.76, n = 70, P = 0.012; Glx, $F_{(1, 67)}$ = 5.04, n = 71, P = 0.028). Among these changes, only the NAA change in the left DLPFC passed Bonferroni correction (P' = 0.042). There were no significant between-group differences in other metabolites (Figure 3). Table 2 also summarizes spectral quality (FWHM and SNR) and mean CRLB, which did not differ significantly between the SHE group and control group. Table 3 shows the tissue fractions in the spectroscopy VOIs, but no significant difference was found between the groups (P > 0.05).

Table 4 shows the left-to-right DLPFC differences in metabolite concentrations: the only significant difference was in the control group, wherein a paired t test analysis of NAA level in the left hemisphere was higher than the right (P = 0.041). The metabolic comparisons between men and women are shown in Table 5. The GSH concentrations in the right DLPFC were statistically significantly higher in men than in women in participants with SHE (P = 0.001). No significant difference was found in other metabolites between men and women in both SHE group and control group.

3.3 Correlation between clinical data and metabolites

The mI concentration in the right DLPFC was negatively correlated with epilepsy duration (r_{28} =

-0.501, P = 0.011). We did not find any other significant correlations between other metabolites and illness duration of SHE, age at onset or seizure frequency (P > 0.05).

4 Discussion

In the present study concentration of NAA in the left DLPFC and mI concentrations in the bilateral DLPFCs were lower in participants with SHE than in controls, while GSH and Glx concentrations in the right were higher than in controls. Except for NAA, other metabolites must be interpreted with caution because they cannot pass the Bonferroni correction. There was an asymmetry of NAA concentration (left > right) in healthy controls but no difference in the SHE group. Besides, the mI concentration in the right DLPFC was negatively correlated with epilepsy duration. Even though seizures may be derived from various brain areas, they will propagate to frontal lobe to generate a similar seizure semiology by a final common path (Tassinari et al., 2005). Such a pattern of metabolic changes may represent a common frontal metabolic alteration in SHE. We discuss these abnormalities, and some of the negative findings, below.

4.1 Decreased NAA concentration in the left DLPFC

NAA is thought to reflect neuroaxonal integrity and density, being found almost exclusively in neurons, axons and dendrites (Moffett et al., 2007), and a decreased NAA is generally interpreted as a loss of

neurons or neuronal dysfunction. Reduction of NAA levels has often been reported in the epileptogenic zone or epileptic network (Fojtikova et al., 2006; Heron et al., 2012; Lundbom et al., 2001; Naldi et al., 2017; Savic et al., 2004). Such alteration has been found in temporal lobe epilepsy and it can recover after surgery (Cendes et al., 1997; Fountas et al., 2012), which might indicate it was not caused by loss of neurons. NAA is synthesized in neuronal mitochondria, whose dysfunction or increased energy requirement might lead to a reduction of NAA levels (Signoretti et al., 2001). Our main finding of lower NAA may reflect neuronal energetic impairment of the DLPFC as a chronic consequence of epileptic seizure (Hetherington et al., 2002).

We also found, but only in controls, that NAA level in the left DLPFC was higher than the right. Similar neurochemical asymmetry has been noted before: a study related to healthy right-handed subjects existed a left-right metabolic asymmetry in the temporal lobe (Bernard et al., 1996). Anatomical asymmetry has also been reported especially in the frontal lobe, related to handedness (note that all our participants were right-handed) (Amunts et al., 2000). We hypothesize that epileptic seizures may affect both frontal lobes through the rich interconnectivity between the two hemispheres, but that the left hemisphere may be more vulnerable than the right. Although the mechanisms are of course very different, a similarly increased vulnerability of the left hemisphere to distributed pathophysiological processes has been reported in Alzheimer's disease (Loewenstein et al., 1989; Thompson et al., 2003). In addition, developmental factors may explain this phenomenon, because the left hemisphere matures later and more

slowly than the right hemisphere and it may be more affected by early insults from early-onset seizures (Kemmotsu et al., 2011).

4.2 Decreased myo-inositol (mI) concentrations in the bilateral DLPFCs

This is in line with studies reporting lower mI in an extratemporal region (frontal lobe) in TLE patients (Tan et al., 2018). Myo-inositol is taken as a glial cell marker located in astrocytes and it is a physiologically important osmolyte (Fisher et al., 2002). In one animal study, oxidative stress induced the failure of osmotic control in astrocytes, together with a massive loss of myo-inositol (Brand et al., 1999). It is tempting to suggest a similar mechanism here, as we discuss next.

Furthermore, we found negative correlation between the mI concentration and epileptic duration in the right DLPFC. Perhaps more significant mI difference might be found in the right DLPFC in the future in a study with longer duration, due to less vulnerability of the right hemisphere and the progressive effects of seizures mentioned before.

4.3 Increased glutathione (GSH) concentration in the right DLPFC

GSH (a glutamate, cysteine and glycine tripeptide), the most abundant cellular thiol, is the brain's main antioxidant primarily produced in astrocytes because they can utilize various extracellular precursors to produce the three amino acids (Schmidt & Dringen, 2012). There is evidence that epileptic seizures are associated with an enormous production of reactive oxygen (Frantseva et al., 2000), which would be

expected to decreased GSH acutely, and potentially between seizures if recovery processes are insufficient or overwhelmed. There is one report of lower GSH in the parietooccipital area of focal epilepsy (Mueller et al., 2001). In contrast, we found a significantly higher GSH in the right DLPFC in SHE. Increased GSH might reflect a compensatory response to oxidative stress, as appears to be the case in a rat model after status epilepticus (Filibian et al., 2012). and in the first-episode psychosis (Wood et al., 2009). A possible explanation for the increased GSH concentration in the right DLPFC alone is that compensatory GSH synthesis might increase bilaterally, but the oxidative stress might be greater on the left and thus offset the increase of GSH though more consumption. The high vulnerability of left hemisphere might support this speculation again.

Otherwise, we further confirmed a higher GSH concentration in men than in women in SHE participants after eliminating the effects of seizure frequency, disease duration, et al. It probably indicates that men are more susceptible to this disease than women, which is in accord with a epidemiologic study showing that SHE predominates in men (7:3) (Menghi et al., 2018). Our recruited patients and another study both show a higher prevalence in men (Provini et al., 1999).

4.4 Increased the sum of glutamate and glutamine (Glx) concentration in the right DLPFC

Glutamate is the dominant central excitatory neurotransmitter (Magistretti et al., 1999), and in vivo (Petroff et al., 1995) and in vitro (Sherwin et al., 1988) studies implicate that increased glutamate

concentration is the pathophysiology of epilepsy. The trend towards increased Glx concentrations in SHE patients versus healthy controls are in line with previous epilepsy research, including idiopathic generalized epilepsy (Doelken et al., 2010; Helms et al., 2006; Simister et al., 2003) and temporal lobe epilepsy (Woermann et al., 1999). A larger sample size may contribute to verify this finding.

4.5 No alteration in GABA concentration

GABA is the major central inhibitory neurotransmitter (Olsen & Avoli, 1997) and plays an important role in epilepsy: impairment of GABA function produces seizures, whereas antiepileptic drugs (AEDs), to which SHE patients respond well, enhance GABA-mediated mechanisms (Olsen & Avoli, 1997). In the event, we did not find any significant alteration in GABA. This may be because 79% of participants with SHE were taking antiepileptic medications.

4.6 Limitations

Our study had some limitations. First, limited time precluded acquiring spectra in the thalamus, orbitofrontal cortex and brainstem. Second, AEDs use may be a confounding factor. They may have complex effects, perhaps helping to restore low NAA and prevent the loss/dysfunction of neuronal and axonal (Westman et al., 2007), but also confusing the picture for GABA. Third, sleep deprivation caused by frequent epileptic seizures in SHE is reported to impair executive function (Menghi et al., 2018), which is under DLPFC control. However, we did not perform psychological and cognitive evaluations of

the two groups, and this should be part of future studies. Fourth, although we only included GSH values with Cramer-Rao lower bounds of less than 15%, a special MEGA-PRESS that can detect the GSH signal and remove other overlapping resonances or macromolecules would be preferable to standard PRESS for the detection of GSH (Shukla et al., 2018). Additional spectra in other brain regions, such as orbitofrontal cortex and brainstem, were not acquired, due to the limited acquisition time and susceptibility artifacts. We did not acquire spectra in thalamus and anterior cingulate cortex because they have been studied (Naldi et al., 2017).

5 Conclusion

This study demonstrates that DLPFC is an important brain region involved in the pathophysiology of SHE, in which both neurons and astrocytes are impaired, and the elevated glutathione level may suggest an abnormality related to oxidative stress. These findings may shed light on the neurobiochemical mechanisms of SHE and enrich our knowledge about MRI-negative SHE patients.

References

Alkondon, M., Pereira, E. F., Eisenberg, H. M., & Albuquerque, E. X. (2000). Nicotinic receptor activation in human cerebral cortical interneurons: a mechanism for inhibition and disinhibition of neuronal networks. *J Neurosci*, *20*(1), 66-75. https://doi.org/10.1523/JNEUROSCI.20-01-00066.2000

Amunts, K., Jancke, L., Mohlberg, H., Steinmetz, H., & Zilles, K. (2000). Interhemispheric asymmetry of the human motor cortex related to handedness and gender. *Neuropsychologia*, *38*(3), 304-312. https://doi.org/10.1016/s0028-3932(99)00075-5

Annett, M. (1994). Handedness as a continuous variable with dextral shift: sex, generation, and family handedness in subgroups of left- and right-handers. *Behav Genet*, *24*(1), 51-63. https://doi.org/10.1007/bf01067928

Aridon, P., Marini, C., Di Resta, C., Brilli, E., De Fusco, M., Politi, F., . . . Casari, G. (2006). Increased sensitivity of the neuronal nicotinic receptor alpha 2 subunit causes familial epilepsy with nocturnal wandering and ictal fear. *Am J Hum Genet*, *79*(2), 342-350. https://doi.org/10.1086/506459

Bernard, D., Walker, P. M., Baudouin-Poisson, N., Giroud, M., Fayolle, H., Dumas, R., . . . Brunotte, F. (1996). Asymmetric metabolic profile in mesial temporal lobes: localized H-1 MR spectroscopy in healthy right-handed and non-right-handed subjects. *Radiology*, *199*(2), 381-389. https://doi.org/10.1148/radiology.199.2.8668782

Brand, A., Leibfritz, D., & Richter-Landsberg, C. (1999). Oxidative stress-induced metabolic alterations in rat brain astrocytes studied by multinuclear NMR spectroscopy. *J Neurosci Res*, *58*(4), 576-585.

Cendes, F., Andermann, F., Dubeau, F., Matthews, P. M., & Arnold, D. L. (1997). Normalization of neuronal metabolic dysfunction after surgery for temporal lobe epilepsy. Evidence from proton MR spectroscopic imaging. *Neurology*, *49*(6), 1525-1533. https://doi.org/10.1212/wnl.49.6.1525

De Fusco, M., Becchetti, A., Patrignani, A., Annesi, G., Gambardella, A., Quattrone, A., . . . Casari, G. (2000). The nicotinic receptor beta 2 subunit is mutant in nocturnal frontal lobe epilepsy. *Nat Genet*, *26*(3), 275-276. https://doi.org/10.1038/81566

Doelken, M. T., Mennecke, A., Stadlbauer, A., Kecskeméti, L., Kasper, B. S., Struffert, T., . . . Hammen, T. (2010). Multi-voxel magnetic resonance spectroscopy at 3 T in patients with idiopathic generalised epilepsy. *Seizure*, *19*(8), 485-492. https://doi.org/10.1016/j.seizure.2010.07.005

Filibian, M., Frasca, A., Maggioni, D., Micotti, E., Vezzani, A., & Ravizza, T. (2012). In vivo imaging of glia activation using 1H-magnetic resonance spectroscopy to detect putative biomarkers of tissue epileptogenicity. *Epilepsia*, *53*(11), 1907-1916. https://doi.org/10.1111/j.1528-1167.2012.03685.x

Fisher, S. K., Novak, J. E., & Agranoff, B. W. (2002). Inositol and higher inositol phosphates in neural tissues: homeostasis, metabolism and functional significance. *J Neurochem, 82*(4), 736-754. https://doi.org/10.1046/j.1471-4159.2002.01041.x

Fojtikova, D., Brazdil, M., Horky, J., Mikl, M., Kuba, R., Krupa, P., & Rektor, I. (2006). Magnetic resonance spectroscopy of the thalamus in patients with typical absence epilepsy. *Seizure, 15*(7), 533-540. https://doi.org/10.1016/j.seizure.2006.06.007

Fountas, K. N., Tsougos, I., Gotsis, E. D., Giannakodimos, S., Smith, J. R., & Kapsalaki, E. Z. (2012). Temporal pole proton preoperative magnetic resonance spectroscopy in patients undergoing surgery for mesial temporal sclerosis. *Neurosurgical focus, 32*(3), E3. https://doi.org/10.3171/2012.1.FOCUS11327

Frantseva, M. V., Perez Velazquez, J. L., Tsoraklidis, G., Mendonca, A. J., Adamchik, Y., Mills, L. R., . . . Burnham, M. W. (2000). Oxidative stress is involved in seizure-induced neurodegeneration in the kindling model of epilepsy. *Neuroscience, 97*(3), 431-435. https://doi.org/10.1016/s0306-4522(00)00041-5

Hammen, T., Hildebrandt, M., Stadlbauer, A., Doelken, M., Engelhorn, T., Kerling, F., . . . Stefan, H. (2008). Non-invasive detection of hippocampal sclerosis: correlation between metabolite alterations detected by (1)H-MRS and neuropathology. *NMR Biomed, 21*(6), 545-552. https://doi.org/10.1002/nbm.1222

Helms, G., Ciumas, C., Kyaga, S., & Savic, I. (2006). Increased thalamus levels of glutamate and glutamine (Glx) in patients with idiopathic generalised epilepsy. *J Neurol Neurosurg Psychiatry, 77*(4), 489-494. https://doi.org/10.1136/jnnp.2005.074682

Heron, S. E., Smith, K. R., Bahlo, M., Nobili, L., Kahana, E., Licchetta, L., . . . Dibbens, L. M. (2012). Missense mutations in the sodium-gated potassium channel gene KCNT1 cause severe autosomal dominant nocturnal frontal lobe epilepsy. *Nat Genet, 44*(11), 1188-1190. https://doi.org/10.1038/ng.2440

Hetherington, H. P., Pan, J. W., & Spencer, D. D. (2002). 1H and 31P spectroscopy and bioenergetics in the lateralization of seizures in temporal lobe epilepsy. *J Magn Reson Imaging, 16*(4), 477-483. https://doi.org/10.1002/jmri.10177

Kaiser, L. G., Young, K., Meyerhoff, D. J., Mueller, S. G., & Matson, G. B. (2008). A detailed analysis of localized J-difference GABA editing: theoretical and experimental study at 4 T. *NMR Biomed, 21*(1), 22-32. https://doi.org/10.1002/nbm.1150

Kemmotsu, N., Girard, H. M., Bernhardt, B. C., Bonilha, L., Lin, J. J., Tecoma, E. S., . . . McDonald, C. R. (2011). MRI analysis in temporal lobe epilepsy: cortical thinning and white matter disruptions are related to side of seizure onset. *Epilepsia, 52*(12), 2257-2266. https://doi.org/10.1111/j.1528-1167.2011.03278.x

Korenke, G. C., Eggert, M., Thiele, H., Nurnberg, P., Sander, T., & Steinlein, O. K. (2016). Nocturnal frontal lobe epilepsy caused by a mutation in the GATOR1 complex gene NPRL3. *Epilepsia*, *57*(3), e60-63. https://doi.org/10.1111/epi.13307

Krsek, P., Hajek, M., Dezortova, M., Jiru, F., Skoch, A., Marusic, P., . . . Komarek, V. (2007). (1)H MR spectroscopic imaging in patients with MRI-negative extratemporal epilepsy: correlation with ictal onset zone and histopathology. *Eur Radiol*, *17*(8), 2126-2135. https://doi.org/10.1007/s00330-007-0594-1

Loewenstein, D. A., Barker, W. W., Chang, J. Y., Apicella, A., Yoshii, F., Kothari, P., . . . Duara, R. (1989). Predominant left hemisphere metabolic dysfunction in dementia. *Arch Neurol*, *46*(2), 146-152. https://doi.org/10.1001/archneur.1989.00520380046012

Lundbom, N., Gaily, E., Vuori, K., Paetau, R., Liukkonen, E., Rajapakse, J. C., . . . Granstrom, M. L. (2001). Proton spectroscopic imaging shows abnormalities in glial and neuronal cell pools in frontal lobe epilepsy. *Epilepsia*, *42*(12), 1507-1514. https://doi.org/10.1046/j.1528-1157.2001.15301.x

Magistretti, P. J., Pellerin, L., Rothman, D. L., & Shulman, R. G. (1999). Energy on demand. *Science*, *283*(5401), 496-497. https://doi.org/10.1126/science.283.5401.496

Marini, C., & Guerrini, R. (2007). The role of the nicotinic acetylcholine receptors in sleep-related epilepsy. *Biochem Pharmacol*, *74*(8), 1308-1314. https://doi.org/10.1016/j.bcp.2007.06.030

Menghi, V., Bisulli, F., Tinuper, P., & Nobili, L. (2018). Sleep-related hypermotor epilepsy: prevalence, impact and management strategies. *Nat Sci Sleep*, *10*, 317-326. https://doi.org/10.2147/NSS.S152624

Moffett, J. R., Ross, B., Arun, P., Madhavarao, C. N., & Namboodiri, A. M. (2007). N-Acetylaspartate in the CNS: from neurodiagnostics to neurobiology. *Prog Neurobiol*, *81*(2), 89-131. https://doi.org/10.1016/j.pneurobio.2006.12.003

Mueller, S. G., Trabesinger, A. H., Boesiger, P., & Wieser, H. G. (2001). Brain glutathione levels in patients with epilepsy measured by in vivo (1)H-MRS. *Neurology*, *57*(8), 1422-1427. https://doi.org/10.1212/wnl.57.8.1422

Naldi, I., Bisulli, F., Testa, C., Rizzo, G., Ferri, L., Gramegna, L. L., . . . Tinuper, P. (2017). Proton MR Spectroscopy in Patients With Sleep-Related Hypermotor Epilepsy (SHE): Evidence of Altered Cingulate Cortex Metabolism. *Sleep*, *40*(9). https://doi.org/10.1093/sleep/zsx115

Nobili, L., Francione, S., Mai, R., Cardinale, F., Castana, L., Tassi, L., . . . Cossu, M. (2007). Surgical treatment of drug-resistant nocturnal frontal lobe epilepsy. *Brain*, *130*(Pt 2), 561-573. https://doi.org/10.1093/brain/awl322

Olsen, R. W., & Avoli, M. (1997). GABA and epileptogenesis. *Epilepsia*, *38*(4), 399-407. https://doi.org/10.1111/j.1528-1157.1997.tb01728.x

Petroff, O. A., Pleban, L. A., & Spencer, D. D. (1995). Symbiosis between in vivo and in vitro NMR spectroscopy: the creatine, N-acetylaspartate, glutamate, and GABA content of the epileptic human brain. *Magn Reson Imaging, 13*(8), 1197-1211. https://doi.org/10.1016/0730-725x(95)02033-p

Phillips, H. A., Marini, C., Scheffer, I. E., Sutherland, G. R., Mulley, J. C., & Berkovic, S. F. (2000). A de novo mutation in sporadic nocturnal frontal lobe epilepsy. *Ann Neurol, 48*(2), 264-267.

Picard, F., Bruel, D., Servent, D., Saba, W., Fruchart-Gaillard, C., Schollhorn-Peyronneau, M. A., . . . Bottlaender, M. (2006). Alteration of the in vivo nicotinic receptor density in ADNFLE patients: a PET study. *Brain, 129*(Pt 8), 2047-2060. https://doi.org/10.1093/brain/awl156

Picard, F., Makrythanasis, P., Navarro, V., Ishida, S., de Bellescize, J., Ville, D., . . . Baulac, S. (2014). DEPDC5 mutations in families presenting as autosomal dominant nocturnal frontal lobe epilepsy. *Neurology, 82*(23), 2101-2106. https://doi.org/10.1212/wnl.0000000000000488

Proserpio, P., Cossu, M., Francione, S., Tassi, L., Mai, R., Didato, G., . . . Nobili, L. (2011). Insular-opercular seizures manifesting with sleep-related paroxysmal motor behaviors: a stereo-EEG study. *Epilepsia, 52*(10), 1781-1791. https://doi.org/10.1111/j.1528-1167.2011.03254.x

Provini, F., Plazzi, G., Tinuper, P., Vandi, S., Lugaresi, E., & Montagna, P. (1999). Nocturnal frontal lobe epilepsy. A clinical and polygraphic overview of 100 consecutive cases. *Brain, 122*, 1017-1031. https://doi.org/10.1093/brain/122.6.1017

Sansoni, V., Nobili, L., Proserpio, P., Ferini-Strambi, L., & Combi, R. (2012). A de novo mutation in an Italian sporadic patient affected by nocturnal frontal lobe epilepsy. *J Sleep Res, 21*(3), 352-353. https://doi.org/10.1111/j.1365-2869.2011.00986.x

Saper, C. B., & Fuller, P. M. (2017). Wake-sleep circuitry: an overview. *Curr Opin Neurobiol, 44*, 186-192. https://doi.org/10.1016/j.conb.2017.03.021

Saper, C. B., Scammell, T. E., & Lu, J. (2005). Hypothalamic regulation of sleep and circadian rhythms. *Nature, 437*(7063), 1257-1263. https://doi.org/10.1038/nature04284

Savic, I., Osterman, Y., & Helms, G. (2004). MRS shows syndrome differentiated metabolite changes in human-generalized epilepsies. *NeuroImage, 21*(1), 163-172. https://doi.org/10.1016/j.neuroimage.2003.08.034

Scheffer, I. E., Bhatia, K. P., Lopes-Cendes, I., Fish, D. R., Marsden, C. D., Andermann, E., . . . et al. (1995). Autosomal dominant nocturnal frontal lobe epilepsy. A distinctive clinical disorder. *Brain, 118 (Pt 1)*, 61-73. https://doi.org/10.1093/brain/118.1.61

Schmidt, M. M., & Dringen, R. (2012). *Neural Metabolism In Vivo* (I. Y. Choi & R. Gruetter Eds.). New York: Springer.

Sherwin, A., Robitaille, Y., Quesney, F., Olivier, A., Villemure, J., Leblanc, R., . . . et al. (1988).

Excitatory amino acids are elevated in human epileptic cerebral cortex. *Neurology, 38*(6), 920-923. https://doi.org/10.1212/wnl.38.6.920

Shukla, D., Mandal, P. K., Ersland, L., Gruner, E. R., Tripathi, M., Raghunathan, P., . . . Splaine, C. (2018). A Multi-Center Study on Human Brain Glutathione Conformation using Magnetic Resonance Spectroscopy. *Journal of Alzheimer's Disease, 66*(2), 517-532. https://doi.org/10.3233/JAD-180648

Shungu, D. C., Mao, X., Gonzales, R., Soones, T. N., Dyke, J. P., van der Veen, J. W., & Kegeles, L. S. (2016). Brain γ-aminobutyric acid (GABA) detection in vivo with the J-editing (1) H MRS technique: a comprehensive methodological evaluation of sensitivity enhancement, macromolecule contamination and test-retest reliability. *NMR Biomed, 29*(7), 932-942. https://doi.org/10.1002/nbm.3539

Signoretti, S., Marmarou, A., Tavazzi, B., Lazzarino, G., Beaumont, A., & Vagnozzi, R. (2001). N-Acetylaspartate reduction as a measure of injury severity and mitochondrial dysfunction following diffuse traumatic brain injury. *J Neurotrauma, 18*(10), 977-991. https://doi.org/10.1089/08977150152693683

Simister, R. J., McLean, M. A., Barker, G. J., & Duncan, J. S. (2003). Proton MRS reveals frontal lobe metabolite abnormalities in idiopathic generalized epilepsy. *Neurology, 61*(7), 897-902.

Steinlein, O. K., Mulley, J. C., Propping, P., Wallace, R. H., Phillips, H. A., Sutherland, G. R., . . . Berkovic, S. F. (1995). A missense mutation in the neuronal nicotinic acetylcholine receptor alpha 4 subunit is associated with autosomal dominant nocturnal frontal lobe epilepsy. *Nat Genet, 11*(2), 201-203. https://doi.org/10.1038/ng1095-201

Tan, Q., Sun, H., Wang, W., Wu, X., Hao, N., Su, X., . . . Gong, Q. (2018). Quantitative MR spectroscopy reveals metabolic changes in the dorsolateral prefrontal cortex of patients with temporal lobe epilepsy. *European Radiology, 28*(11), 4496-4503. https://doi.org/10.1007/s00330-018-5443-x

Tassinari, C. A., Rubboli, G., Gardella, E., Cantalupo, G., Calandra-Buonaura, G., Vedovello, M., . . . Meletti, S. (2005). Central pattern generators for a common semiology in fronto-limbic seizures and in parasomnias. A neuroethologic approach. *Neurol Sci, 26*, s225-232. https://doi.org/10.1007/s10072-005-0492-8

Thompson, P. M., Hayashi, K. M., de Zubicaray, G., Janke, A. L., Rose, S. E., Semple, J., . . . Toga, A. W. (2003). Dynamics of gray matter loss in Alzheimer's disease. *J Neurosci, 23*(3), 994-1005. https://doi.org/10.1523/JNEUROSCI.23-03-00994.2003

Tinuper, P., & Bisulli, F. (2017). From nocturnal frontal lobe epilepsy to Sleep-Related Hypermotor Epilepsy: A 35-year diagnostic challenge. *Seizure, 44*, 87-92. https://doi.org/10.1016/j.seizure.2016.11.023

Tinuper, P., Bisulli, F., Cross, J. H., Hesdorffer, D., Kahane, P., Nobili, L., . . . Ottman, R. (2016).

Definition and diagnostic criteria of sleep-related hypermotor epilepsy. *Neurology, 86*(19), 1834-1842. https://doi.org/10.1212/WNL.0000000000002666

Tustison, N. J., Cook, P. A., Klein, A., Song, G., Das, S. R., Duda, J. T., . . . Avants, B. B. (2014). Large-scale evaluation of ANTs and FreeSurfer cortical thickness measurements. *NeuroImage, 99*, 166-179. https://doi.org/10.1016/j.neuroimage.2014.05.044

Vignatelli, L., Bisulli, F., Giovannini, G., Licchetta, L., Naldi, I., Mostacci, B., . . . Meletti, S. (2015). Prevalence of nocturnal frontal lobe epilepsy in the adult population of Bologna and Modena, Emilia-Romagna region, Italy. *Sleep, 38*(3), 479-485. https://doi.org/10.5665/sleep.4514

Westman, E., Spenger, C., Wahlund, L. O., & Lavebratt, C. (2007). Carbamazepine treatment recovered low N-acetylaspartate+N-acetylaspartylglutamate (tNAA) levels in the megencephaly mouse BALB/cByJ-Kv1.1(mceph/mceph). *Neurobiol Dis, 26*(1), 221-228. https://doi.org/10.1016/j.nbd.2006.12.012

Woermann, F. G., McLean, M. A., Bartlett, P. A., Parker, G. J., Barker, G. J., & Duncan, J. S. (1999). Short echo time single-voxel 1H magnetic resonance spectroscopy in magnetic resonance imaging-negative temporal lobe epilepsy: different biochemical profile compared with hippocampal sclerosis. *Ann Neurol, 45*(3), 369-376. https://doi.org/10.1002/1531-8249(199903)45:3<369::aid-ana13>3.0.co;2-q

Wood, A. G., Saling, M. M., Fedi, M., Berkovic, S. F., Scheffer, I. E., Benjamin, C., & Reutens, D. C. (2010). Neuropsychological function in patients with a single gene mutation associated with autosomal dominant nocturnal frontal lobe epilepsy. *Epilepsy Behav, 17*(4), 531-535. https://doi.org/10.1016/j.yebeh.2010.01.168

Wood, S. J., Berger, G. E., Wellard, R. M., Proffitt, T. M., McConchie, M., Berk, M., . . . Pantelis, C. (2009). Medial temporal lobe glutathione concentration in first episode psychosis: a 1H-MRS investigation. *Neurobiol Dis, 33*(3), 354-357. https://doi.org/10.1016/j.nbd.2008.11.018

Figure Legends

Figure 1. Sagittal, coronal and axial view of the VOI (voxel size: 12 mL = 4.0 × 2.0 × 1.5 cm^3) positioned within the left and right DLPFCs. The proportion of cerebrospinal fluid within the voxels were determined by overlaying them on the segmented T1-weighted images. A = arterial, DLFPC = dorsolateral prefrontal cortex, L = left, P = posterior, R = right, VOI = volume of interest.

Figure 2. Examples for general metabolites (A) and GABA (B) processed by LCModel.

Cho = choline, Cr = creatine, GABA = *γ*-aminobutyric acid, Glx = glutamate/glutamine, GSH = glutathione, mI = *myo*-inositol, NAA = N-acetyl aspartate.

Figure 3. Metabolic differences acquired by PRESS in participants with SHE compared with healthy controls.

Cho = choline, Cr = creatine, Glx = glutamate/glutamine, GSH = glutathione, L = left, mI = myo-inositol, NAA = N-acetyl aspartate, PRESS = Point-Resolved Spectroscopy Sequence, R = right, SHE = sleep-related hypermotor epilepsy. *: $p < 0.05$. **: $p' < 0.05$.