Divergent metabolic substrate utilization in brain during epileptogenesis precedes chronic hypometabolism

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Abstract

Alterations in metabolism during epileptogenesis may be a therapy target. Recently, an increase in amino acid transport into the brain was proposed to play a role in epileptogenesis. We aimed to characterize alterations of substrate utilization during epileptogenesis and in chronic epilepsy. The lithium-pilocarpine post status epilepticus (SE) rat model was used. We performed longitudinal O-(2-[(18F)fluoroethyl]-L-tyrosine (¹⁸F-FET) and ¹⁸F-fluorodeoxyglucose (¹⁸F-FDG) positron emission tomography (PET) and calculated ¹⁸F-FET volume of distribution (V_t) and ¹⁸F-FDG uptake. Correlation analyses were performed with translocator protein-PET defined neuroinflammation from previously acquired data. We found reduced ¹⁸F-FET V_t at 48 h after SE (amygdala: −30.2%, $p = 0.014$), whereas ¹⁸F-FDG showed increased glucose uptake 4 and 24 h after SE (hippocampus: +43.6% and +42.5%, respectively; $p < 0.001$) returning to baseline levels thereafter. In chronic epileptic animals, we found a reduction in ¹⁸F-FET and ¹⁸F-FDG in the hippocampus. No correlation was found for ¹⁸F-FET or ¹⁸F-FDG to microglial activation at seven days post SE. Whereas metabolic alterations do not reflect higher metabolism associated to activated microglia, they might be partially driven by chronic neuronal loss. However, both metabolisms diverge during early epileptogenesis, pointing to amino acid turnover as a possible biomarker and/or therapeutic target for epileptogenesis.

Keywords

Amino acid turnover, epilepsy, fluorodeoxyglucose, fluoroethyl-L-tyrosine, positron emission tomography

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Introduction

Epilepsy is a central nervous system disease affecting about 60 million people.¹ The chronic phase of the disease, which is characterized by the reoccurrence of spontaneous seizures, is typically preceded by a so-called latent period starting at the time of the epileptogenic event being free of clinical seizures. During this time period, complex processes transform the healthy into a seizure-prone brain. The term epileptogenesis refers to these processes including several alterations in cerebral metabolism, neurotransmitter systems and neuroinflammation. In the current terminology, epileptogenesis also comprises the progression of the epilepsy after spontaneous seizures are established.^{2,3}

¹⁸F-Fluorodeoxyglucose (¹⁸F-FDG) positron emission tomography (PET) is widely used to identify epileptic focus in epilepsy patients with negative magnetic

resonance imaging (MRI) scans.⁴ In addition, glucose metabolic alterations have been demonstrated in different animal models of epileptogenesis by ¹⁸F-FDG PET.^{5–8} Recently, it has been proposed that glucose

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hypometabolism might play a key role in the development of neurodegenerative diseases, including acquired epilepsy.⁹ Furthermore, targeted metabolic therapy, such as ketogenic diet, may counteract pathological processes in various neurodegenerative diseases.^{9,10}

Importantly, metabolic changes in brain diseases are not limited to glucose metabolism. Radiotracers mimicking endogenous amino acid turnover can be used for nuclear imaging to obtain further metabolic information. For example, the PET tracer O-(2-[(18F)fluoroethyl]-l-tyrosine (¹⁸F-FET) has been previously used for tumor diagnosis in glioma patients due to its reduced brain background compared to ¹⁸F-FDG.¹¹ In metabolically active cells with increased amino acid consumption due to increased protein synthesis, the uptake of ¹⁸F-FET is increased, and it acts as a surrogate marker for changed amino acid turnover. While ¹⁸F-FET is metabolically stable and usually not incorporated into proteins, influx is mediated by active transendothelial amino acid transport by L-amino acids transporters (LATs).^{12,13} In addition to impaired glucose utilization, altered amino acid turnover has recently been described in epileptic patients.^{13,14} Hutterer et al.¹³ reported increased ¹⁸F-FET uptake and expression of LAT in seizure-affected cortex of epilepsy patients and theorized a link between LAT expression and epileptogenesis.¹³ Another important process that may lead to increased glucose and protein metabolism is neuroinflammation.^{15,16} Neuroinflammation is proposed as a key factor during epileptogenesis,¹⁷ but can also be present during chronic epilepsy.¹⁸ It is not known if regional neuroinflammation during epileptogenesis¹⁹ can be detected by increased ¹⁸F-FDG and/or ¹⁸F-FET uptake.

The objective of this study is to longitudinally characterize glucose and amino acid metabolic profile during epileptogenesis and in the chronic epilepsy phase, aiming to investigate two metabolic, potentially diagnostic biomarkers using translational PET imaging. To this aim, we performed ¹⁸F-FDG and ¹⁸F-FET PET scans before, during and after epileptogenesis in the rat lithium-pilocarpine model. Furthermore, we conducted correlation analysis with previously acquired translocator protein (TSPO)-PET data¹⁹ to evaluate the contribution of microglia activation to the metabolic changes.

Material and methods

Animals

Female Sprague Dawley rats ($n=44$) were purchased from Envigo (Udine, Italy) at a body weight of 200–220 g and housed in pairs in individually ventilated biocontainment cages (Allentown Inc., Allentown, USA)

with a 14/10-h light/dark cycle. Standard laboratory chow (Altromin 1324, Altromin Spezialfutter, Lage, Germany) and autoclaved water were freely accessible. Animals were allowed to adapt to new housing conditions and repetitive handling was performed for at least one week before start of the experiments. Experiments were conducted in accordance with European Communities Council Directives 86/609/EEC and 2010/63/EU and were formally approved by the responsible local authority (Landesamt für Verbraucherschutz und Lebensmittelsicherheit, LAVES). Experiments were reported according to ARRIVE (Animal Research: Reporting in Vivo Experiments) guidelines.

Status epilepticus induction

Status epilepticus (SE) was induced as previously reported.¹⁹ Shortly, rats ($n=44$) were pretreated with lithium chloride (127 mg/kg; Sigma-Aldrich, Darmstadt, Germany) 14–16 h before pilocarpine administration. Methyl-scopolamine (1 mg/kg; Sigma-Aldrich) was administered 30 min prior to a bolus of 30 mg/kg pilocarpine hydrochloride (Sigma-Aldrich), followed by a maximum of three injections of 10 mg/kg at 30 min intervals as needed until SE onset. Beginning of SE was characterized by the onset of repetitive generalized convulsive seizures without retrieving normal behavior between seizures. SE was interrupted after 90 min by administering diazepam (10 mg/kg i.p.; Ratiopharm, Ulm, Germany). Diazepam injection was repeated up to two times (2nd repetition 5 mg/kg) at 15 min intervals as necessary. Following SE, rats were hand-fed with mashed laboratory chow and received injections of glucose electrolyte solution (Sterofundin® HEG-5; 5 ml s.c.; B. Braun, Hessen, Germany) until pre-SE weight recovery.

After injecting 38.5 ± 9.3 mg/kg of pilocarpine, 97.1% of the animals developed an SE. Despite individual nursing, 18.2% of the animals developing SE died within 48 h after SE. Animals not developing SE were excluded from further experiments. Manifestation of spontaneous seizures was visually recorded during presence in the animal room for all animals characterized as chronic epileptic.

PET imaging procedure

Animals were scanned at baseline ($n=6$, ¹⁸F-FDG; $n=5$, ¹⁸F-FET) and at several time points after SE. For ¹⁸F-FDG experiments, animals serial PET scans were performed at 4, 24 and 48 h and 7 and 15 days post SE ($n=6$, each). ¹⁸F-FET scans were performed at 24 h ($n=5$), 48 h ($n=4$), 7 days ($n=5$) and 15 days post SE ($n=5$). The baseline time point before induction of SE (naïve rats) served as control measurement.

In addition, a group of chronic epileptic animals (12–14 weeks after SE, $n = 6$) was scanned with both ^{18}F -FDG and ^{18}F -FET. Additionally, an age-matched group of naïve animals ($n = 6$) was scanned with ^{18}F -FDG and ^{18}F -FET for comparison to the chronic epileptic animals.

All imaging procedures were carried out under iso-flurane anesthesia (1.5–2.5% in a humidified oxygen flow of 1.0 l/min). Animals were continuously warmed and monitored for heart and respiration rate (BioVet, m2m imaging, Cleveland, USA). Anesthesia levels were adjusted to maintain a stable respiration rate (45–60 breaths/min). PET images were acquired using a dedicated small animal PET camera (Inveon PET scanner, Siemens, Knoxville, USA). Rats were positioned prone in the imaging bed (Minerve, Esternay, France), with the brain in the center of the field of view.

^{18}F -FDG (24.17 ± 4.90 MBq) or ^{18}F -FET (19.21 ± 1.63 MBq) was injected via a catheter inserted into a lateral tail vein. In the case of ^{18}F -FET, parallel to the tracer injection, a dynamic 60-min PET acquisition was started. List-mode data were histogrammed to 32 frames of 5×2 s, 4×5 s, 3×10 s, 8×30 s, 5×60 s, 4×300 s, and 3×600 s. For ^{18}F -FDG experiments, static scans were performed 30–60 min after injection, with tracer uptake under anesthesia. All images were reconstructed using an iterative ordered-subset expectation maximization algorithm (16 subsets, 2 iterations, $\beta = 0.01$) followed by 18 iterations of maximum a posteriori (OSEM3D/fastMAP) applying standard corrections for decay, randoms and scatter. For attenuation correction, 20 min ^{57}Co transmission scans were used. In order to obtain anatomical information for image analysis, a standard low-dose CT scan (Inveon CT, Siemens) was performed following PET imaging.

PET image analyses

Both ^{18}F -FDG and ^{18}F -FET uptake were fused to a magnetic resonance image (MRI) template using Pmod 3.703 software (PMOD Technologies, Zurich, Switzerland) as described previously.¹⁹ Specifically, CT images were first co-registered to the MRI template and then matched to their corresponding PET images. A volume of interest (VOI) template based on Schwarz et al.²⁰ was applied to the co-registered PET images.

^{18}F -FDG tracer uptake was calculated as percentage-injected dose per cubic centimeter of tissue (%ID/cc). ^{18}F -FET fused images underwent voxel-based kinetic modeling applying a Logan-plot model and calculating the volume of distribution of the tracer (V_t) using a VOI over the carotid arteries to obtain an image-derived input function (Suppl. Figure 1).

Furthermore, ^{18}F -FDG %ID/cc and ^{18}F -FET V_t parametric images were analyzed by statistical parametric

mapping (SPM, UCL, London, UK). Voxel-wise Student's t -test comparisons were performed between each time point and the respective control group. Resulting T-maps are shown fused to an MRI template and with a minimum voxel cluster size of 100 voxels.

Statistics

Data analysis was carried out using statistical software (Graphpad Prism 7, La Jolla, USA). Student's t -test compared age-matched naïve control and chronic epileptic groups. Repeated-measures one-way ANOVA was used for ^{18}F -FDG acute time course and one-way ANOVA for ^{18}F -FET studies due to mortality in this experimental group. Dunnett's post hoc test was performed for both analyses using the baseline time point as control group. Interaction of data was investigated by Pearson's correlation analysis. To evaluate potential associations between tracers, we performed a correlation analysis for the hippocampus using individual ^{18}F -FET and ^{18}F -FDG values of the chronic and control rats. In addition, to determine the relationship between metabolic tracers uptake and neuroinflammation, we correlated group values for different regions of interest (dorsal and ventral hippocampus, amygdala, thalamus, striatum, and motor, entorhinal and piriform cortex) at seven days post SE and in chronic epileptic animals of each tracer to ^{11}C -PK11195 binding potential at seven days post SE from a previous study¹⁹ using different animals, to evaluate the contribution of microglia cells to the metabolic signal. P -value was set to 0.05. Data are presented as mean $\pm$ standard deviation (SD).

Results

^{18}F -FET PET imaging during epileptogenesis

Over the timecourse of epileptogenesis, ^{18}F -FET V_t was significantly reduced at 48 h after SE in thalamus (0.61 ± 0.08 vs. 0.85 ± 0.09 ml/cc; $p = 0.049$), amygdala (0.60 ± 0.09 vs. 0.86 ± 0.11 ml/cc; $p = 0.006$) and entorhinal cortex (0.56 ± 0.13 vs. 0.75 ± 0.04 ml/cc; $p = 0.014$) but not in the hippocampus when compared to baseline conditions (Figure 1(a) and (b)). There was a trend towards decreased ^{18}F -FET V_t in the amygdala at 7 days after SE (-20.9% ; $p = 0.053$). Statistical parametric mapping demonstrated a diffuse global ^{18}F -FET V_t reduction at 48 h after SE. One week after SE, the decrease was more restricted to epileptogenesis-related areas (Figure 1(c)).

^{18}F -FDG PET imaging during epileptogenesis

^{18}F -FDG uptake was generally elevated at 4 h after SE (Figure 2(a) and (b)), with all regions of interest

Figure 1. (a) Bar graphs showing ^{18}F -FET V_t quantification in hippocampus (HIPP), thalamus (THA), amygdala (AMY) and entorhinal cortex (ENT C) at baseline and 24, 48 h, 7 and 15 days after SE ($n=4-5$). Data are shown as mean $\pm$ SD. * $p < 0.05$ versus baseline. (b) Average ^{18}F -FET V_t parametric maps for each experimental time point fused to an MRI template. (c) T-test parametric maps of each time point compared to baseline animals and fused to an MRI template showing significantly increased (hot scale) or decreased (cold scale) voxels compared to baseline. Threshold has been set to show only statistically significant voxels ($p < 0.05$; minimum cluster size of 100 voxels).

displaying increased ^{18}F -FDG signal compared to baseline, especially in the hippocampus (1.45 ± 0.24 vs. 1.01 ± 0.14 %ID/cc; $p < 0.001$, Figure 2(a)) and the amygdala (1.41 ± 0.43 vs. 0.71 ± 0.11 %ID/cc; $p < 0.001$). This increase was maintained until 24 h after SE in most regions of interest. However, amygdala was not significantly elevated compared to baseline at 24 h after SE. The largest difference at 24 h after SE was located in the hippocampus ($+42.5\%$, $p < 0.001$) and the thalamus ($+42.0\%$; $p < 0.001$). ^{18}F -FDG uptake recovered to baseline levels at 48 h after SE in all regions and no additional differences were observed during the acute phase of epileptogenesis.

SPM analysis showed a global increase of uptake 4 and 24 h after SE (Figure 2(c)). Apart from increased uptake at day 7 after SE in the amygdala, no other

significant changes were observed during epileptogenesis when comparing to the baseline condition.

Chronic epileptic animals

In chronic epileptic animals, manifesting at least one spontaneous seizure, ^{18}F -FET scans showed a trend of reduced V_t compared to age-matched controls (Figure 3(a) and (b)) in the hippocampus (0.49 ± 0.06 vs. 0.57 ± 0.06 ml/cc; $p = 0.050$). Accordingly, SPM comparison showed clusters of voxels with significant reduction of ^{18}F -FET V_t in the hippocampus of chronic animals (Figure 3(c)). Similarly ^{18}F -FDG uptake was reduced in all analyzed regions of interest, with the greatest reduction localized to the hippocampus (0.51 ± 0.07 vs. 0.80 ± 0.05 %ID/cc; $p < 0.001$;

Figure 2. (a) Bar graphs showing ¹⁸F-FDG uptake in hippocampus (HIPP), thalamus (THA), amygdala (AMY) and entorhinal cortex (ENT C) at baseline, 4, 24, and 48 h, 7 and 15 days after SE ($n = 6$). Data are shown as mean $\pm$ SD. * $p < 0.05$. (b) Average ¹⁸F-FDG %ID/cc parametric maps for each experimental time point fused to an MRI template. (c) T-test parametric maps of each time point compared to baseline and fused to an MRI template showing significantly increased (hot scale) or decreased (cold scale) voxels compared to the baseline group. Threshold has been set to show only statistically significant voxels ($p < 0.05$; minimum cluster size of 100 voxels).

Figure 3(d) and (e)), in chronic epileptic animals. Parametric t -maps showed widespread ¹⁸F-FDG hypometabolism, with peak differences located in the hippocampal area (Figure 3(f)).

¹⁸F-FDG uptake and ¹⁸F-FET V_t in hippocampus of chronic epileptic rats and the control group were strongly correlated ($r = 0.720$; $p = 0.008$, Figure 3(g)).

Correlation analyses between metabolism and brain inflammation

On comparison of regional ¹⁸F-FDG uptake with ¹¹C-PK11195 binding potential at seven days post SE in different groups of animals, we found no correlation between glucose metabolism and TSPO expression ($r = -0.011$; $p = 0.980$). Similarly, amino acid turnover did not correlate with TSPO expression at this time point ($r = -0.617$; $p = 0.103$; Figure 4(a)). Moreover, ¹¹C-PK11195 binding potential at seven days post SE

did not correlate with ¹⁸F-FDG uptake ($r = -0.333$; $p = 0.423$) but showed a negative correlation to ¹⁸F-FET V_t ($r = -0.732$; $p = 0.039$; Figure 4(b)) in chronic epileptic rats.

Discussion

In the present study, we demonstrate alterations in amino acid and glucose metabolism during the acute phase of epileptogenesis and chronic epilepsy using translational molecular imaging with ¹⁸F-FET and ¹⁸F-FDG, respectively. Our results point to a transient metabolic imbalance during the acute phase after SE that subsequently recovers over the first week. In the chronic epileptic phase, animals showed hypometabolism for glucose and amino acid turnover in the hippocampus.

Recently, Hutterer et al.¹³ reported increased ¹⁸F-FET uptake and expression of LAT in the

Figure 3. Bar graphs showing (a) ^{18}F -FET V_t and (d) ^{18}F -FDG uptake (%ID/cc) in hippocampus (HIPP), thalamus (THA), amygdala (AMY) and entorhinal cortex (ENT C) in the same chronic epileptic ($n = 6$) and age-matched control animals ($n = 6$). Average (b) ^{18}F -FET V_t and (e) ^{18}F -FDG uptake parametric maps for control and chronic epileptic animals fused to an MRI template. T-test parametric maps of the comparison of (c) ^{18}F -FET V_t or (f) ^{18}F -FDG uptake between control and epileptic animals fused to an MRI template. Threshold has been set to show only statistically significant voxels ($p < 0.05$; minimum cluster size of 100 voxels). (g) Correlation analysis between ^{18}F -FET V_t and ^{18}F -FDG uptake in chronic epileptic and age-matched control animals in the hippocampus. Every data point represents an individual animal.

Figure 4. Correlation analysis between ^{18}F -FET V_t and ^{18}F -FDG uptake at seven days post SE (a) and in chronic epileptic animals (b) to ^{11}C -PK1195 binding potential (BP_{ND}) at seven days post SE (data taken from Brackhan et al.¹⁹). Every data point represents the averaged group value for one brain region (dorsal and ventral hippocampus, amygdala, thalamus, striatum and motor, entorhinal and piriform cortex).

seizure-affected cortex of chronic epileptic patients. They theorized that increase of LAT expression could also play a role in epileptogenesis.¹³ Our ^{18}F -FET PET data, however, suggest a reduction of amino acid turnover during the acute phase after SE. This does not support the theorized acute induction of LAT by seizures as suggested previously.¹³ There is no knowledge whether a general reduction in amino acid turnover correlates directly with pro- or anticonvulsive amino acid neurotransmitter levels. Nevertheless, both energy and amino acid metabolism seem to be interconnected with regard to anti-convulsant effects of the ketogenic diet.²¹ Interestingly, during the acute phase following pilocarpine-induced SE, the impairment of numerous other functional parameters has been described, including reduced mGluR5, mGluR1 and GABA_A receptors and markedly decreased expression of various microRNAs in the hippocampus.^{22–25} In addition, this time point corresponds to the maximum peak of blood–brain barrier impairment in the pilocarpine model.²⁶ Thus, reduced ^{18}F -FET V_t seen in this model may reflect a global down-regulation of neuroreceptors and transporters in the brain as a consequence of a severe epilepsy-inducing insult. Reduced ^{18}F -FET signal during the acute phase of epileptogenesis might be a useful marker enabling identification of individuals at higher risk to develop epilepsy, who might then undergo disease-modifying treatment. In addition, it might serve as marker for monitoring antiepileptogenic treatment response in the acute phase of epileptogenesis. All brain regions returned to baseline amino acid transport levels after seven days, suggesting recovery of the amino acid transport during the acute phase of epileptogenesis.

^{18}F -FDG PET revealed glucose hypermetabolism 4 and 24 h after SE. This increased glucose consumption may be induced by high neuronal activity during SE that persists for hours in this model when visually recorded motor seizure activity has already stopped.²⁷ It has been previously described that the epileptiform activity induced with pilocarpine continues during the night despite diazepam administration 90 min after initiation of SE.²⁷ This neuronal hyperactivity persists up to 24 h after the pilocarpine administration,²⁸ which would account for higher uptake of glucose observed in our study at this time point. In the chronic phase, we noted a lower ^{18}F -FDG uptake in different brain areas. Interestingly, the severity of this reduction corresponded to the regions in which the strongest hypermetabolism was observed at 24 h after SE, and in which neurodegeneration has been previously described.²⁹ This congruence suggests that regional hyper-excitability evokes acute increases in glucose demand but may lead to neurodegeneration. It is unclear whether excitotoxicity or energy shortage is the major factor activating a cascade culminating in neurodegeneration. In future imaging studies, the combination of ^{18}F -FDG with several other PET markers potentially related to neuronal loss would be desirable. Apart from tracers measuring GABA receptor density like ^{18}F -flumazenil, several other tracers seem to be promising. Choi et al.^{19,22} found globally reduced binding potential of the mGluR5 radioligand ^{11}C -ABP688 after pilocarpine-induced SE. Further, Yamasaki et al.^{22,25} published data that show reduced binding potential of ^{11}C -ITDM, a PET ligand for mGluR1 receptor, during epileptogenesis in the same model. Recently, radiotracers for PET imaging of synaptic

vesicle protein 2A (SV2A) representing promising markers for assessing neuronal / synaptic density have been developed and characterized *in vivo*.³⁰ However, studies with this tracer during epileptogenesis are not yet available.

Reduced ^{18}F -FDG uptake in the first week after SE has been previously reported.^{6,7,31} In the present study, we did not observe such a reduction at seven days post-SE. This might be caused by differences in the protocols between the different studies. Whereas previous studies used awake uptake of the tracer, we induced the isoflurane anesthesia before ^{18}F -FDG injection and maintained the anesthesia through the whole scanning protocol. As we performed kinetic modeling for evaluation of the ^{18}F -FET PET scans, continuous dynamic imaging under anesthesia was not avoidable. To exclude a potential source of variation, we used the same anesthesia protocol for both ^{18}F -FET and ^{18}F -FDG. Furthermore, the published ^{18}F -FDG data differ in used pilocarpine doses and SE termination as well as in the sex of the animals used in the different experiments.

Metabolism of both ^{18}F -FDG and ^{18}F -FET in the hippocampus was reduced in the chronic epileptic state compared to age-matched healthy controls. Neuronal loss has been described in this region in the chronic phase of this animal model.^{29,32,33} In addition, similar alterations have been found in epilepsy patients with ^{18}F -FDG-PET, showing a clear hypometabolism in the epileptic focus.^{34–36} However, ^{18}F -FET PET studies in epileptic patients showed an increase of uptake in areas with structural MRI changes.^{13,14} Epileptic seizures in these patients were caused by brain tumors. Some brain tumors are known to increase ^{18}F -FET uptake.¹¹ The present results in chronic epileptic rats may indicate that increased ^{18}F -FET uptake seen in epileptic patients derives from tumor physiology rather than seizure activity. The decrease in the uptake of both metabolic tracers used in this study in the hippocampus of chronic epileptic animals most likely reflects regional neuronal loss. However, ^{18}F -FDG showed hypometabolism in areas with no altered amino acid turnover. Thus, other processes other than neuronal loss may play a role in glucose metabolism in chronic epilepsy.

Lastly, metabolic processes can be increased in an inflammatory environment and it has already been described that neuroinflammation leads to increases in glucose and protein metabolism by the inflammatory cells.^{15,16} Therefore, we have compared our results with the timeline of TSPO expression, as indicative of neuroinflammation.¹⁹ Neuroinflammation was first detected with TSPO-PET 48 h after SE and peaking between 7 and 15 days. Here, neither ^{18}F -FET nor ^{18}F -FDG showed correlation with peak microglia activation at seven days after SE, i.e. the acute phase of

epileptogenesis. Thus, in the context of microglia activation during epileptogenesis, these tracers are not adequate to detect the increased metabolism of this inflammatory cell type. However, peak microglia activation during epileptogenesis correlated to regional amino acid hypometabolism in the chronic epilepsy state but not to glucose metabolism. This observation may indicate that higher inflammation during the acute phase reflects greater regional damage in the chronic phase, as measured by lower ^{18}F -FET uptake. By contrast, ^{18}F -FDG uptake is more influenced by other epileptogenesis pathways rather than neurodegeneration alone, negating correlation to acute neuroinflammation.

Some limitation of our study should be acknowledged. First, isoflurane was used as anesthesia during the whole imaging protocol as dynamic acquisitions were performed for ^{18}F -FET and to maintain similar conditions for both metabolic tracers used. Previously, it has been suggested that isoflurane can have antiepileptogenic effects.³⁷ However, this study was performed in a different animal model and seizures were observed in all the animals reaching the chronic epileptic phase in this study. Second, the observed levels of ^{18}F -FET uptake in the brain were low, being difficult to distinguish real uptake from background signal. Therefore, we performed dynamic studies in order to calculate kinetic parameters that may be more sensitive to small changes in low uptake tracers.³⁸ To investigate whether inflammation and metabolic changes might correlate in single brain regions, we selected the seven days post SE time point when a regionally increased TSPO signal is obvious.¹⁹ As the animal cohort used for the microglial activation imaging was investigated independently of the experiments with ^{18}F -FET and ^{18}F -FDG, we had to base the correlation analysis on average group values instead of individual values.

Conclusion

This study showed metabolic impairment in chronic epileptic animals for both, glucose and amino acid using translational *in vivo* imaging. However, these metabolisms diverge during the acute phase of epileptogenesis. The decrease of ^{18}F -FET V_t may be a biomarker or therapeutic target of this complex process. No clear influence of activated microglia. No clear influence of activated microglia on ^{18}F -FET and ^{18}F -FDG signals was found during epileptogenesis.

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Declaration of conflicting interests

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Authors' contributions

Design of the study: PB, MBr, IL, HK, TLR, FMB, MBa and JPB.

Data acquisition: PB, MBr, IL, HK, IJ.

Analysis of experiments: PB, MBr, IL, HK.

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Supplementary material

Supplementary material for this paper can be found at the journal website: <http://journals.sagepub.com/home/jcb>

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References

1. Baulac M, de Boer H, Elger C, et al. Epilepsy priorities in Europe: a report of the ILAE-IBE Epilepsy Advocacy Europe Task Force. *Epilepsia* 2015; 56: 1687–1695.
2. Pitkanen A and Engel J Jr. Past and present definitions of epileptogenesis and its biomarkers. *Neurotherapeutics* 2014; 11: 231–241.
3. Pitkanen A, Lukasiuk K, Dudek FE, et al. Epileptogenesis. *Cold Spring Harb Perspect Med.* 2015; 5(10). pii: a022822.
4. Liew CJ, Lim YM, Bonwetsch R, et al. 18F-FCWAY and 18F-FDG PET in MRI-negative temporal lobe epilepsy. *Epilepsia* 2009; 50: 234–239.
5. Bascuñana P, Javela J, Delgado M, et al. [(18)F]FDG PET Neuroimaging predicts pentylenetetrazole (PTZ) kindling outcome in rats. *Mol Imaging Biol* 2016; 18: 733–740.
6. Goffin K, Paesschen WV, Dupont P, et al. Longitudinal microPET imaging of brain glucose metabolism in rat lithium-pilocarpine model of epilepsy. *Exp Neurol* 2009; 217: 205–209.
7. Guo Y, Gao F, Wang S, et al. In vivo mapping of temporal-spatial changes in glucose utilization in rat brain during epileptogenesis: an 18F-fluorodeoxyglucose-small animal positron emission tomography study. *Neuroscience* 2009; 162: 972–979.
8. Jupp B, Williams J, Binns D, et al. Imaging small animal models of epileptogenesis. *Neurology Asia* 2007; 12: 51–54.
9. Zilberter Y and Zilberter M. The vicious circle of hypometabolism in neurodegenerative diseases: ways and mechanisms of metabolic correction. *J Neurosci Res* 2017; 95: 2217–2235.
10. Lusardi TA, Akula KK, Coffman SQ, et al. Ketogenic diet prevents epileptogenesis and disease progression in adult mice and rats. *Neuropharmacology* 2015; 99: 500–509.
11. Dunet V, Pomoni A, Hottinger A, et al. Performance of 18F-FET versus 18F-FDG-PET for the diagnosis and grading of brain tumors: systematic review and meta-analysis. *Neuro Oncol* 2016; 18: 426–434.
12. Pauleit D, Stoffels G, Bachofner A, et al. Comparison of (18)F-FET and (18)F-FDG PET in brain tumors. *Nucl Med Biol* 2009; 36: 779–787.
13. Hutterer M, Ebner Y, Riemenschneider MJ, et al. Epileptic activity increases cerebral amino acid transport assessed by 18F-fluoroethyl-L-tyrosine amino acid PET: a potential brain tumor mimic. *J Nucl Med* 2017; 58: 129–137.
14. Kasper BS, Struffert T, Kasper EM, et al. 18Fluoroethyl-L-tyrosine-PET in long-term epilepsy associated glioneuronal tumors. *Epilepsia* 2011; 52: 35–44.
15. Backes H, Walberer M, Ladwig A, et al. Glucose consumption of inflammatory cells masks metabolic deficits in the brain. *Neuroimage* 2016; 128: 54–62.
16. Thackeray JT, Bankstahl JP, Wang Y, et al. Targeting amino acid metabolism for molecular imaging of inflammation early after myocardial infarction. *Theranostics* 2016; 6: 1768–1779.
17. Vezzani A, Friedman A and Dingledine RJ. The role of inflammation in epileptogenesis. *Neuropharmacology* 2013; 69: 16–24.
18. de Vries EE, van den Munckhof B, Braun KP, et al. Inflammatory mediators in human epilepsy: a systematic review and meta-analysis. *Neurosci Biobehav Rev* 2016; 63: 177–190.
19. Brackhan M, Bascuñana P, Postema JM, et al. Serial quantitative TSPO-targeted PET reveals peak microglial activation up to 2 weeks after an epileptogenic brain insult. *J Nucl Med* 2016; 57: 1302–1308.
20. Schwarz AJ, Danckaert A, Reese T, et al. A stereotaxic MRI template set for the rat brain with tissue class distribution maps and co-registered anatomical atlas: application to pharmacological MRI. *Neuroimage* 2006; 32: 538–550.
21. Yudkoff M, Daikhin Y, Melo TM, et al. The ketogenic diet and brain metabolism of amino acids: relationship to the anticonvulsant effect. *Annu Rev Nutr* 2007; 27: 415–430.
22. Choi H, Kim YK, Oh SW, et al. In vivo imaging of mGluR5 changes during epileptogenesis using [11C]ABP688 PET in pilocarpine-induced epilepsy rat model. *PLoS One* 2014; 9: e92765.
23. Risbud RM and Porter BE. Changes in microRNA expression in the whole hippocampus and hippocampal synaptoneurosome fraction following pilocarpine induced status epilepticus. *PLoS One* 2013; 8: e53464.
24. Gonzalez MI, Cruz Del Angel Y and Brooks-Kayal A. Down-regulation of gephyrin and GABAA receptor

- subunits during epileptogenesis in the CA1 region of hippocampus. *Epilepsia* 2013; 54: 616–624.
25. Yamasaki T, Fujinaga M, Mori W, et al. In vivo monitoring for regional changes of metabotropic glutamate receptor subtype 1 (mGluR1) in pilocarpine-induced epileptic rat brain by small-animal PET. *Sci Rep* 2017; 7: 14945.
 26. Breuer H, Meier M, Schneefeld S, et al. Multimodality imaging of blood-brain barrier impairment during epileptogenesis. *J Cereb Blood Flow Metab* 2017; 37: 2049–2061.
 27. Brandt C, Töllner K, Klee R, et al. Effective termination of status epilepticus by rational polypharmacy in the lithium-pilocarpine model in rats: window of opportunity to prevent epilepsy and prediction of epilepsy by biomarkers. *Neurobiol Dis* 2015; 75: 78–90.
 28. Löscher W and Brandt C. Prevention or modification of epileptogenesis after brain insults: experimental approaches and translational research. *Pharmacol Rev* 2010; 62: 668–700.
 29. Peredery O, Persinger MA, Parker G, et al. Temporal changes in neuronal dropout following inductions of lithium/pilocarpine seizures in the rat. *Brain Res* 2000; 881: 9–17.
 30. Constantinescu CC, Tresse C, Zheng M, et al. Development and in vivo preclinical imaging of fluorine-18-labeled synaptic vesicle protein 2A (SV2A) PET tracers. *Mol Imaging Biol* 2018; epub ahead of print.
 31. Shiha AA, de Cristobal J, Delgado M, et al. Subacute administration of fluoxetine prevents short-term brain hypometabolism and reduces brain damage markers induced by the lithium-pilocarpine model of epilepsy in rats. *Brain Res Bull* 2015; 111: 36–47.
 32. Bankstahl M, Bankstahl JP and Löscher W. Inter-individual variation in the anticonvulsant effect of phenobarbital in the pilocarpine rat model of temporal lobe epilepsy. *Exp Neurol* 2012; 234: 70–84.
 33. Wu T, Ido K, Osada Y, et al. The neuroprotective effect of perampanel in lithium-pilocarpine rat seizure model. *Epilepsy Res* 2017; 137: 152–158.
 34. Dedeurwaerdere S, Jupp B and O'Brien TJ. Positron emission tomography in basic epilepsy research: a view of the epileptic brain. *Epilepsia*. 2007; 48 Suppl 4: 56–64.
 35. Duncan J. The current status of neuroimaging for epilepsy. *Curr Opin Neurol* 2009; 22: 179–184.
 36. O'Brien TJ, Miles K, Ware R, et al. The cost-effective use of 18F-FDG PET in the presurgical evaluation of medically refractory focal epilepsy. *J Nucl Med* 2008; 49: 931–937.
 37. Bar-Klein G, Klee R, Brandt C, et al. Isoflurane prevents acquired epilepsy in rat models of temporal lobe epilepsy. *Ann Neurol* 2016; 80: 896–908.
 38. Doot RK, Dunnwald LK, Schubert EK, et al. Dynamic and static approaches to quantifying 18F-FDG uptake for measuring cancer response to therapy, including the effect of granulocyte CSF. *J Nucl Med* 2007; 48: 920–925.