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Neuroinflammation as a pathophysiological factor in the development and maintenance of functional seizures: A hypothesis

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ABSTRACT

The neurobiological underpinnings of functional seizure (FS) development and maintenance represent an active research area. Recent work has focused on hardware (brain structure) and software (brain function and connectivity). However, understanding whether FS are an adaptive consequence of changes in brain structure, function, and/or connectivity is important for identifying a causative mechanism and for FS treatment and prevention. Further, investigation must also uncover what causes these structural and functional phenomena. Pioneering work in the field of psychoneuroimmunology has established a strong, consistent link between psychopathology, immune dysfunction, and brain structure/function. Based on this and recent FS biomarker findings, we propose a new etiologic model of FS pathophysiology. We hypothesize that early-life stressors cause neuroinflammatory and neuroendocrine changes that prime the brain for later FS development following secondary trauma (e.g., traumatic brain injury or psychological trauma). This framework coalesces existing knowledge regarding brain aberrations underlying FS and established neurobiological theories on the pathophysiology of underlying psychiatric disorders. We also propose brain temperature mapping as a way of indirectly visualizing neuroinflammation in patients with FS, particularly in emotion regulation, fear processing, and sensory-motor integration circuits. We offer a foundation on which future research can be built, with clear recommendations for future studies.

Introduction

Functional seizures (FS), also called psychogenic non-epileptic seizures (PNES), resemble epileptic seizures but they are phenotypically distinct from epilepsy because they are not grounded in aberrant electrical activity [1]. Patients perceive FS as involuntary. However, FS are generated by brain structures and pathways that participate in voluntary, self-generated actions, which are modulated by independent and distributed structures and connections [1-3]. In adults, the diagnosis of FS intricately overlaps and co-occurs with neurological comorbidities, psychiatric symptoms, and adverse life events [1,4]. The majority of patients with FS also report a prior history of trauma, abuse, and/or neglect [5,6]. Lastly, a traumatic brain injury (TBI) precedes the onset of FS in up to 83% of patients, making TBI an integral component of FS pathophysiology [7,8]. While the neurobiological underpinnings of FS are understudied, there is an emerging neuroimaging literature that focuses on various aspects of brain anatomy and function in patients with functional neurological disorders, including FS [3,9-11]. In the absence of uniformly confirmed pathophysiology, FS patients' comorbid and concomitant symptoms (e.g., anxiety, depression) are currently treated with psychotropic medications e.g., antidepressants and anxiolytics [12]. However, there is no clear evidence that these treatments target the underlying pathophysiology of FS [12]. Cognitive behavioral therapy (CBT) approaches have proven to be efficacious at reducing and/or eliminating FS events in many children and adults [13-15]. However, it is critical to understand the neurobiological underpinnings of FS so that we can develop treatments that are efficacious in all patients.

In the past, a critical barrier to understanding neurobiological underpinnings of FS was the lack of consensus that it was a worthwhile pursuit. Experts long regarded FS as a manifestation of psychological distress without an "organic" cause [16]. This conceptualization was not grounded in findings from empirical studies, and instead stemmed from the previously hypothesized separation between mind and body that is, at times, still reflected in modern medicine [17]. In the past, experts explained FS using a

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psychodynamic framework, which posits that FS are somatic manifestations of unconscious conflicts [18]. In contrast, behavioral learning theories conceive FS as illness behaviors stemming from maladaptive coping and communication styles [8,16,18]. Experts in the field have also proposed scaffolding models based on classical conditioning, whereby FS are hardwired from prior experiences and are automatically executed during autonomic arousal in response to threatening stimuli [16,19].

A recent review postulated that in some patients with FS, TBI may cause "dissociogenic" lesions (e.g., multifocal damage or damage to long-range axonal connections) that interrupt global metacognitive functioning and predispose individuals to dissociation, disinhibition, amnesia, and loss of volition [8]. These authors suggested that the blending and fusing of several dynamics contributes to FS development, including e.g., catastrophic illness beliefs, stress reactions to medical events, and maladaptive cognitive-behavioral processes such as symptom modelling and aversive conditioning [8]. This notion is supported by recent work suggesting that, at least in children, targeting catastrophic symptom expectation and sense of control may improve FS [12,15].

Current understanding of the functional seizure pathophysiology: The gaps

From a neurobiological or neurodevelopmental perspective, it is difficult to concede that psychological distress alone produces FS, especially since – in many patients – FS develop and persist long after the cessation of initial psychological stressors [14]. What causes a person with prior psychological trauma, recent TBI, and concurrent anxiety and depressive symptoms to develop FS? Are the depression and anxiety symptoms pre-existing (leading to) or a result of FS? If two individuals undergo the same type of early-life stress (e.g., physical or emotional trauma during brain development), what neurobiological factors cause FS onset in one but not the other? For the patient who develops FS, low resilience may underlie the psychological vulnerability that precipitates emotional dysregulation and dissociation in the face of serious life stressors. Even then, pathologically low resilience capable of generating such symptoms must be grounded in a potent underlying pathophysiology. Thus, the question is whether FS are a biopsychosocial disease modulated by emotion regulation tools, resilience, and capacity to overcome adversity. Moreover, do these events stem from an identifiable neurobiological phenomenon?

Objectives

Of the explanatory models mentioned, one theorized FS pathophysiology was grounded in aberrations caused by dissociogenic lesions following TBI [8]. However, this model does not address the mechanism that produces these post-TBI lesions in the first place. Based on the existing neuroimaging work and studies of inflammatory biomarkers, neuroinflammation may be a mechanism that contributes to the development of dissociogenic lesions. This paper fills the gap(s) of prior explanatory models of FS neuropathophysiology, with the central hypothesis that neuroinflammation is a key pathophysiological contributor to FS. We review evidence that supports our hypothesis. We also propose magnetic resonance spectroscopic imaging and thermometry (MRSI-t) as a tool for non-invasively investigating the consequences of neuroinflammatory phenomena *in vivo*.

Prior neuropathophysiologic evidence for functional seizures

Studies of brain imaging correlates and disease biomarkers in FS/FNDs are in their infancy. However, they highlight aberrations in brain structure, function, and connectivity that are important to explore in detail (see Table 1 for summary) [3,9]. Clinical imaging findings in patients with FS are typically reported to be unremarkable, though the presence of visible abnormality in some patients may be a predictor of poorer long-term outcomes, suggesting that the presence of structural abnormality is of importance for disease outcome [20]. Thus, it is important to note that when compared to healthy controls (HCs), patients with FS exhibit altered structure, function, and/or connectivity in brain regions that mediate emotion regulation, voluntary movement, and executive control [3,9,10].

Structural and functional brain changes that may be a consequence of neuroinflammation

In one study of 11 FS and 10 HCs, dissociation in the FS group was associated with greater functional connectivity between insular subregions and the inferior frontal gyrus, parietal cortex, and precentral sulcus [21]. Dissociation and emotion dysregulation in FS have also been shown to correlate with increased activation of the insula and cingulate cortices, and with decreased activation in the supplementary motor area (SMA) [22]. Functional aberrations are accompanied by altered structural connections between the medial prefrontal cortex (mPFC) and the amygdala, both of which are critical for orchestrating an autonomic response to threatening or stressful stimuli [23]. The uncinate fasciculus (UF), which connects the mPFC to limbic regions, exhibits rightward asymmetry in FS more than in HCs and the degree of asymmetry correlates with patient age at FS onset [24]. UF abnormalities are associated with emotional processing abnormalities in a number of psychiatric disorders, as well with post-TBI behavioral issues [25]. For example, microstructural damage to the UF following moderate to severe TBI has been linked to emotional and behavioral dysregulation in children [26]. Microstructural analyses revealed diminished white matter integrity of the fornix/stria terminalis (FST) in patients with a history of TBI with FS compared to patients with TBI without FS [27]. The FST is a white matter tract connecting the hippocampus, amygdala, and hypothalamus. In addition to decreased FST integrity, the FST and UF also exhibited decreased myelination, providing further evidence of microstructural alterations in FS [27]. The amygdala initiates the neuroendocrine stress response via activation of the hypothalamic pituitary adrenal (HPA) axis [28]. Based on abnormal findings in the mPFC, amygdala, FST, and UF, HPA axis dysfunction likely contributes to psychopathology in FS.

Emotion regulation and self-agency mediation centers also exhibit hypoactivity and abnormalities in structural and functional connectivity. For example, compared to healthy controls, hypometabolism of the right inferior parietal and bilateral anterior cingulate cortices was found in FS on positron emission tomography with 2-deoxy-2-[fluorine-18] fluoro-D-glucose, implicating networks supporting emotion regulation and consciousness of the self and the environment in FS pathophysiology [29]. FS-focused investigations of brain volume and surface topography have yielded inconsistent results in part because of small and heterogeneous samples involved in the studies, and in part because of varying methodologies [1]. FS patients were also compared to HCs without psychiatric symptoms, which introduced a further confound [30-32]. In the largest study to date, we investigated structural abnormalities in FS [11]. We accounted for the prevalence of brain injury and psychiatric comorbidities in FS by enrolling participants with TBI, both with and without FS. Compared to TBI-only controls, TBI-FS participants had regional atrophy in the left inferior frontal gyrus and right cerebellar lobule VIII, as well as decreased left insular sulcus depth. The TBI-FS group also exhibited reduced fractal

Table 1
Summary of findings that support neuroinflammatory pathophysiology may contribute to functional seizure (FS).

Region or Structure	Function	Key Finding	Ref
Insula	Integration of thoughts and emotions → emotional cognitive processing and motor behavior	↑ functional connectivity with IFG, parietal cortex, precentral sulcus	[21]
		↑ activation *	[22]
		↓ sulcal depth in FS-TBI < TBI-only	[11]
Inferior frontal gyrus (**IFG**)	Executive control, response inhibition	↑ functional connectivity with insula	[21]
		↓ GM volume, L-sided (i.e., atrophy) in FS-TBI < TBI-only	[11]
Temporoparietal junction **(TPJ),** R Inferior parietal	Self-agency, theory of mind	Hypometabolism (↓glucose utilization)	[29]
		↓ fractal dimensionality (↓ cortical folding) in FS-TBI < TBI-only	[11]
		↓ activity, ↓ functional connectivity with cerebellar vermis and limbic regions in patients with motor FNDs including FS	[33]
Supplementary Motor Area **(SMA)**	Voluntary motor control	↓ activation *	[22]
Cingulate cortices	Emotional responses to sensory input, role in sickness behaviors	↑ activation *	[22]
		Hypometabolism (↓ glucose utilization on PET-FDG)	[29]
R Cerebellar lobule VIII	Sensorimotor processing	↓ GM volume (i.e., atrophy) in FS-TBI < TBI-only	[11]
Amygdala	Conditioned fear and associative learning, neuroendocrine stress response	Altered connections with mPFC	[23]
		Amygdala hyporeactivity during stress is associated with low salivary-amylase levels	[38]
Medial prefrontal cortex **(mPFC)**	Emotional regulation, tempering learned fear responses	Altered structural connections with the amygdala	[23]
Uncinate fasciculus **(UF)**	Connects mPFC to limbic regions	Rightward asymmetry of UF streamlines, with correlation between degree of asymmetry and age at FS onset	[24]
		↓ myelination in FS-TBI < TBI-only	[27]
Fornix/stria terminalis **(FST)**	Fiber tracts that connect amygdala, hypothalamus, and hippocampus	↓ WM integrity and ↓ myelination in FS-TBI < TBI-only	[27]
Salivary alpha-amylase	↑ during autonomic activation	↓ in FS, linked to amygdala hypoactivation	[38]
Brain-derived neurotrophic factor (**BDNF**)	↑ neurogenesis, biomarker of psychiatric symptom resolution	↓ in FS = ↓ in Epilepsy < Healthy	[45]
TNF-related apoptosis-inducing ligand (**TRAIL**)	pro- and anti-inflammatory functions	↑ in FS > Epilepsy	[45]
Intracellular adhesion molecule-1 (**ICAM-1**)	pro- and anti-inflammatory functions	↑ in FS > Epilepsy	[45]

All findings are reported for phenomena seen in patients with functional seizures, as compared to healthy controls. It is indicated if FS were compared to a control group other than healthy participants.
Abbreviations: FS, functional seizures; IFG, inferior frontal gyrus; TPJ, temporoparietal junction; R, right; SMA, supplementary motor area; mPFC, medial prefrontal cortex; UF, uncinate fasciculus; FST, fornix/stria terminalis; GM, grey matter; WM, white matter; FS-TBI, patients with FS and history of traumatic brain injury; PET, positron emission tomography; FDG, 2-deoxy-2-[fluorine-18] fluoro-D-glucose; FNDs, functional neurological disorders.
* Finding was associated with dissociation and emotional dysregulation in FS participants

dimensionality in the temporoparietal junction (TPJ). This finding is consistent with previous reports of TPJ hypoactivity and decreased functional connectivity between the right TPJ, cerebellar vermis, and limbic regions in patients with motor FNDs including FS [33]. Based on these studies, TPJ hypoactivity and decreased interaction with regions at the sensory-motor interface are likely tied to the morphological alterations in these regions. The TPJ funnels and processes signals from the thalamus and somatosensory, visual, and auditory cortices, which foster self-agency and theory of mind (e.g., self-evaluation, perspective-taking) [33,34]. TPJ hypoactivity and associated connectivity abnormalities may drive dysfunctional feed-forward processing and thus impair self-agency in FS, leading to the perception that self-generated movements are involuntary [33,34].

Are these abnormalities in FS patients' brain structure and function byproducts of brain injury? Do they reflect structural and functional abnormalities caused by prior trauma during brain development? Or, are these changes simply a reflection of psychiatric co-morbidities? The brain's structure and function are closely linked and neural circuitry is shaped and constrained by the foundational architecture. Our findings of aberrant cortical folding offer novel insights into the timing of brain changes in FS: structural brain volume and cortical thickness can vary as a function of disease, but cortical folding begins during the 3rd trimester in utero and is stable by early childhood [11,35,36]. If the described imaging findings stem from processes initiated early in life, a full explanatory model must consider the impact of all predisposing factors in FS – especially those that initiate abnormalities early on that persist into adulthood.

Peripheral biomarkers of inflammation in functional seizures

Given the prevalence of early-life trauma and ongoing depressive and/or anxiety symptoms in FS, peripheral cortisol and salivary alpha-amylase levels first emerged as biomarkers of interest [37]. One study discovered basal hypercortisolism in patients with FS compared to HCs, specifically in patients with a history of sexual abuse [38]. However, great discrepancies emerge when reviewing the larger body of findings. Cortisol levels in FS have been found to be increased, decreased, or equivalent to epilepsy patients and healthy controls alike [39,40]. Cortisol is highly reactive to psychological and environmental stressors and is highly variable over time and in response to a range of physiological states [40]. It is thus best regarded as a general biomarker of stress rather than a sensitive or specific biomarker of FS pathophysiology. Similar inconsistencies resulted when investigating prolactin or adrenocorticotropic hormone [39]. Alpha-amylase, a salivary biomarker of autonomic activation, is elevated following psychological and physical stress, but found to be markedly low in the context of FS [37,41]. In our recent study, we documented a relationship between right amygdala activation and alpha amylase response, indicating amygdala hyporeactivity to stress in FS when compared to HCs [37].

Of the serum biomarkers investigated to date, at least three findings are worth future probing: decreased brain-derived neurotrophic factor (BDNF; FS = Epilepsy < HC), increased TNF-related apoptosis-inducing ligand (TRAIL; FS > Epilepsy), and increased intracellular adhesion molecule-1 (ICAM-1; FS > Epilepsy) [42-44]. Decreased BDNF levels in FS are consistent

with prior literature demonstrating low BDNF levels in patients with mood and depressive disorders, which rise to detectable levels following efficacious treatment [45]. Even in the absence of symptoms, low BDNF is a risk factor for depression [45]. TRAIL expression and repression balances the life and death of cells, with pro- and anti-inflammatory functions of this ligand determined by the cellular context [43]. ICAM-1 increases in FS have similar implications [43]. When considering the full body of findings in FS, increased TRAIL and ICAM-1 may signify the presence of neuroinflammation in patients with FS [44,46-48]. This is further corroborated by low serum BDNF in FS. BDNF promotes neurogenesis and synaptic reorganization following tissue repair and anti-inflammatory interventions, processes that may ground BDNF's status as a marker of psychiatric symptom resolution [49]. BDNF is also an established mediator of resilience, downstream regulator of fear memory processing, and suppressant of anxiety and depressive symptoms [50-53]. Structural abnormalities such as cortical thinning and lower white matter integrity may be, in part, the result of TRAIL/ICAM-1 protein overexpression in these patients [46-48]. The overexpression/repression of these proteins may vary based on a brain region's involvement in FS, e.g., overexpression in the amygdala and repression in the TPJ.

The hypothesis

Neuroinflammation as a potential contributor to functional seizures

Early-life stress and prior brain injury emerge as two important predisposing factors in the development of FS. The term "early-life stress" broadly encompasses an array of stressors and negative life events during childhood. This includes abuse (emotional, physical, verbal), neglect, and negative life events such as parental divorce, substance abuse, or death [54]. This also includes psychological trauma such as life-threatening events, serious injury, or sexual violence [55]. Considering the aforementioned imaging and serum biomarker findings, as well as existing psychoneuroimmunology studies, we propose a two-hit neuropathophysiological hypothesis of FS (Fig. 1):

First hit: Early-life stress causes neuroinflammatory and neuroendocrine alterations that affect (and change) brain structure and function, and increase likelihood of developing psychopathology.
Second hit: When faced with subsequent injury and/or additional trauma, the brain is less resilient and, thus, more vulnerable to developing FS (or other FNDs).

When a threat is perceived the brain activates a complex, intertwined series of pathways within the endocrine, nervous, and immune systems, collectively known as the stress response [56]. The stress response is evolutionarily adaptive in that anxiety motivates behaviors that avoid harm or injury [57-60]. However, prolonged or inappropriate activation of these cascades can be pathologic, with especially devastating effects when brain and immune system development is ongoing [57-61]. Early-life stress is associated with elevated pro-inflammatory cytokine release in animal models and observational human studies [62-64]. A body of prospective, longitudinal studies has demonstrated that childhood maltreatment and other adverse experiences in early-life are associated with inflammation that steadily rises through adolescence and persists into adulthood [65-68]. For example, in a study of 1037 participants followed from birth, childhood maltreatment predicted adult inflammation as measured by plasma C-reactive protein levels [67]. This is demonstrated by work showing that early-life stress is associated with heightened threat reactivity to acute stress during adulthood, which induces an acute rise in inflammatory biomarkers [69]. Immune activation during brain development can 1) trigger and hypersensitize HPA axis and catecholamine activation, which engage further pro-inflammatory mediators and 2) impair neurogenesis in impacted regions with corresponding aberrations in anatomy, function and connectivity [70].

Testing the hypothesis: Brain temperature mapping in functional seizures

Based on prior findings and the hypothesis outlined in Section 1.3, further evidence of neuroinflammation in FS is critical. Volumetric magnetic resonance spectroscopic imaging and thermometry (MRSI-t) can non-invasively map brain temperature, which is elevated in the context of neuroinflammation [71-74]. Brain temperature increases parallel to neuroinflammatory processes such as leukocyte extravasation and cerebral edema, and have demonstrated sensitivity for detecting low-level inflammation [73]. Thus, since we hypothesize there is neuroinflammation in the regions typically implicated in the emotion regulation and self-agency [71-74], we expect to observe higher by 0.5–1 °C brain temperature in those regions as investigated with MRSI-t.

Participants

Data are presented for three female FS participants and a subset of 12 female HCs aged 23 to 50 years. Recruitment was based on the following inclusionary criteria: 1) age 18 to 75 years, 2) no contraindications to undergoing MRI at 3-Tesla (e.g., negative pregnancy test if female of childbearing potential), 3) absence of comorbid neurological or immunological diseases with a neuroinflammatory or autoimmune etiology. All participants provided written informed consent and all study procedures were approved by the UAB Institutional Review Board.

Data collection, processing, and analysis

Participants were scanned on a 3 T Siemens Magnetom Prisma scanner using a 20-channel head coil. T1-weighted structural images were acquired using a magnetization-prepared rapid gradient echo sequence: TR = 2400 ms; TE = 2.22 ms; flip angle = 8°; 208 slices (0.8 mm thick); matrix = 256x256. Whole-brain MRSI-t data were collected using 3D echo planar sequence imaging: TR_1 = 1500-ms, TR_2 = 511 ms, TE = 17.6 ms, lipid inversion-recovery time = 198 m; FOV = 280x280x180mm; 5.6x5.6x14.4 mm voxels. Off-resonance frequency correction, saturation band placement, and both automated and manual shimming were completed before commencing MRSI-t data acquisition. Anxiety and depressive symptoms were evaluated using the Hospital Anxiety and Depression Scale (HADS) [75]. Subscale scores determined whether anxiety or depressive symptoms were classified as normal (score of 0–7), borderline abnormal (score of 8–10), or abnormal (score of 11 +) [75]. Participants also completed the Profile of Mood States (POMS), which measured distress on the basis of the total mood disturbance (TMD) score [76]. The POMS TMD is calculated by totaling the five negative subscale scores (tension-anxiety, depression, anger-hostility, fatigue, and confusion), and then subtracting the vigor subscale score [76]. Participants' concurrent diagnoses, medical history, and prior imaging results were obtained via patient self-report and medical records review.

Image reconstruction and spectral processing were completed within the Metabolite Imaging and Data Analysis System (MIDAS) software package as previously described [77,78]. Briefly, this included spatial reconstruction, frequency alignment, B_0 inhomogeneity correction, co-registration of T1-weighted images and MRSI-t data, atlas registration, lipid suppression, spectral fitting, and normalization and spectral integration with water reference

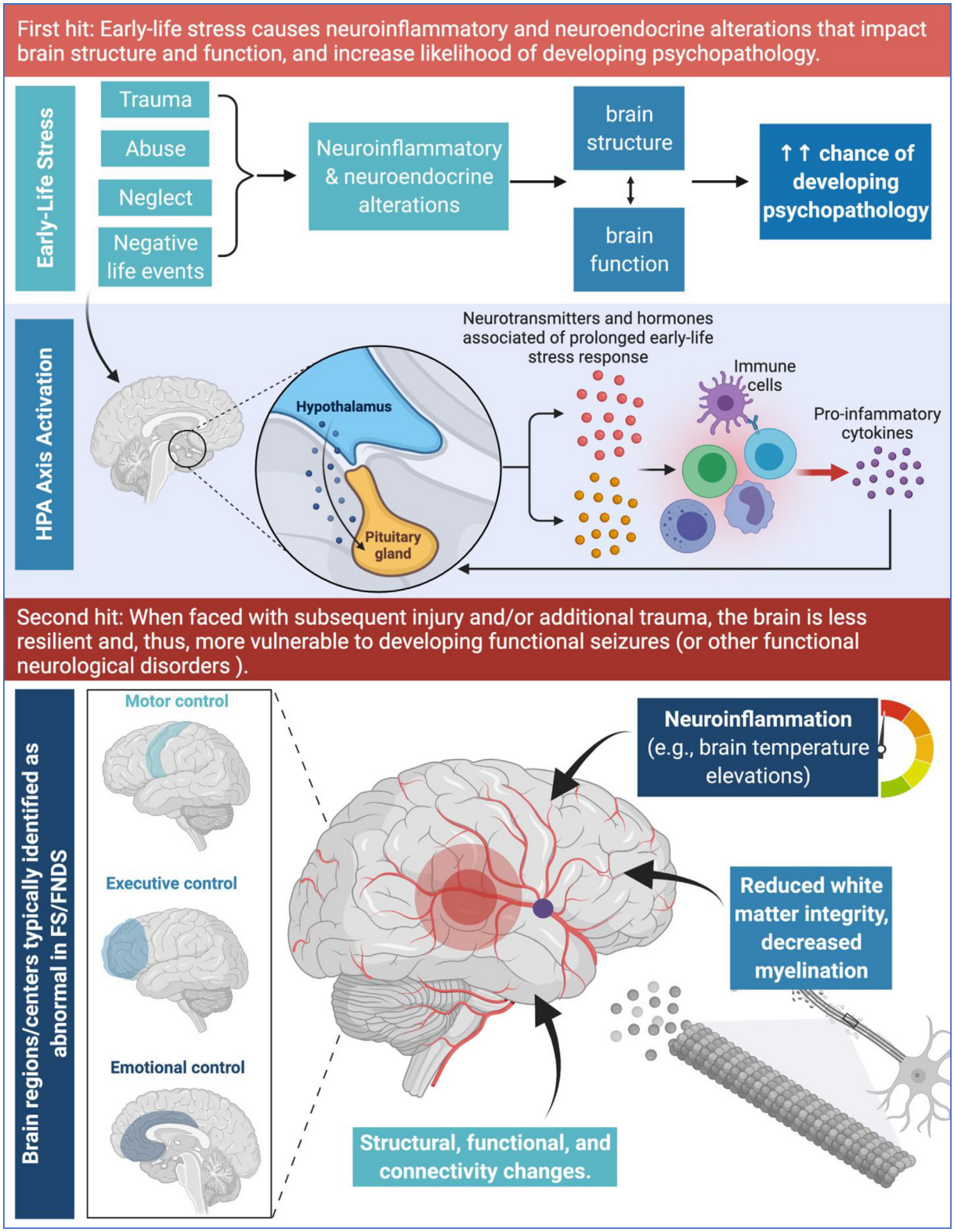

Fig. 1. Pictorial depiction of the first hit / second hit hypothesis as outlined in section 1.3. Early-life stress causes neuroinflammatory and neuroendocrine changes that can produce lifelong structural, functional, and network changes. Stress triggers the hypothalamic-pituitary axis (HPA), which can become chronically hypersensitized if threats are prolonged. Sustained immune activation during brain development also hypersensitizes the HPA. These changes, along with myriad subsequent downstream alterations, render the brain more capable of FS onset following additional injury or trauma. The figure was created using Biorender (https://biorender.com/). Abbreviations: FS, functional seizures; HPA, hypothalamic-pituitary axis; FS, functional seizures; FNDs, functional neurological disorders.

data. Voxelwise brain temperature was derived using the following equation: $T_{CRE} = -102.61(\Delta_{H2O\text{-}CRE}) + 206.1$ °C. for regions of interest (ROIs) delineated by the modified Automated Anatomical Labeling atlas [79].

FS participants' brain temperature (T_{CRE}) elevations were identified on the basis of mean HC data at the individual and group levels. Thus, data for each of the 3 FS participants were visualized relative to MRSI-t data from 4 age-matched HCs. FS patients' T_{CRE} clusters > 38 °C (or 3 SD from the HC mean) were regarded abnormal elevations. Voxelwise T_{CRE} difference maps (FS – healthy mean) were computed using Analysis of Functional Neuroimaging (AFNI) *3dcalc* and *3dclust* packages [78,80].

Results

An overall summary of participants' ages and scores on self-report assessments of mood, anxiety, and depression is provided in Table 2. Each FS participant's brain temperature maps are visualized in Fig. 2, and summarized alongside prior medical history and risk factors in Table 3. The difference map for the FS – HC in Fig. 3 is visualized using a color range of 1.5 – 3.5 °C since the maximum difference in voxelwise T_{CRE} was 3.48 °C. This is consistent with prior work indicating elevations of 1.5 – 2 °C above normal temperature are considered pathologic [73,78]. When axial and coronal views are considered together, differences are seen in the bilateral middle and inferior temporal lobe, as well as cerebellar (R≫L) regions. Right-side differences were also found in the right insula, fusiform and parahippocampal gyri, caudate, posterior orbitofrontal cortex, and the superior temporal pole.

Patient 1 – Childhood onset FS that resolved and resumed following [repeated] head injury, domestic abuse, heart valve disease likely resulting from rheumatic fever, and numerous neurological and psychiatric symptoms.

This is a 42-year-old female with FS onset during childhood. FS events briefly remitted during adolescence and resumed in early 20 s; sole FS diagnosis was confirmed via video/EEG monitoring. She suffered a head injury in 20 s, as well as probable head injury following a motor vehicle collision in 2018. She was also exposed to domestic abuse at several points throughout her life. She experiences frequent, severe migraine attacks, tachycardia, asthma, insomnia, and depressive symptoms, all of which are exacerbated during stress. Other problems include mitral valve prolapse, rheumatic fever, and vitamin D deficiency. She reported borderline abnormal anxiety symptoms, though depressive symptoms and total mood disturbance were within normal ranges. Though Patient 1′s prior video EEG and structural MRI were unremarkable except for the captured habitual FS, MRSI-t data showed pronounced T_{CRE} elevations in temporal and sensorimotor cortices (Fig. 2A). T_{CRE} was elevated in the right middle and inferior temporal gyri, with neighboring elevations in the parahippocampal and hippocampal areas. On the left, T_{CRE} elevations were localized to the fusiform, inferior temporal, and paracentral lobule regions.

Patient 2 – FS onset following recent severe head injury, history of prematurity

Patient 2 is a 22-year-old female treated for FS, GERD, migraines, and vitamin D deficiency. FS lasting 15–30 minutes began 1.5 years ago, ~5 days after enduring a severe head injury following a snowmobile crash. Diagnosis of FS was confirmed by capturing typical events via video/EEG monitoring. She had a history of prematurity and two prior concussions. She reported abnormally high anxiety, borderline abnormal depressive symptoms, and high mood disturbance. T_{CRE} was elevated in the right SMA and precentral gyrus (Fig. 2B). Bilateral T_{CRE} elevations were found in the right and left inferior temporal lobe, as well as the right parahippocampal and hippocampal regions. Brain temperature was also elevated in a small cluster within the middle temporal lobe.

Patient 3 – Post-stroke, history of abuse and developmental delays, endocrinal and cardiovascular risk factors.

Patient 3 is a 50-year-old female with onset of FS at 47 years. FS lasting 30–40 s occur 1–5 times/day, with aura preceding approximately half of the events. She had been diagnosed with several endocrine and cardiovascular conditions, including hypothyroidism, diabetes mellitus type 2, and hyperlipidemia; she also reported having suffered from MRI-negative stroke. She provided history of verbal abuse by her mother, as well as developmental delays of unclear nature during childhood. The patient reported abnormally high anxiety, depression, and mood disturbance. Structural MRI and CT did not show any evidence of chronic or acute stroke, raising the possibility of a coexistence of two FNDs – FS and functional stroke; there was generalized atrophy that appeared to be advanced for age [81]. Patient 3′s MRSI-t data were evaluated and visualized alongside data from 4 female healthy

Table 2
Participant characteristics and mood, anxiety, and depression scores.

Variable		Healthy Controls	FS
Number of participants		N = 12	N = 3
Age		28.5 ± 8.4	38.3 ± 13.9
Profile of Mood States (POMS)			
Total mood disturbance (TMD) score		6.8 ± 21.2	64.0 ± 59.6
	Tension-Anxiety	5.7 ± 7.5	18.0 ± 12.5
	Depression	4.3 ± 4.0	17.3 ± 16.6
	Anger-Hostility	4.5 ± 3.7	15.0 ± 19.5
	Vigor (higher score is better)	18.1 ± 6.3	12.7 ± 5.5
	Fatigue	7.6 ± 4.8	13.0 ± 9.0
	Confusion	2.8 ± 4.2	13.3 ± 3.1
Hospital Anxiety and Depression Scale (HADS)			
Depression score		1.3 ± 1.4	7.7 ± 3.5
	# Normal (% of group)	12 *(100%)*	1 *(33.3%)*
	# Borderline Abnormal (% of group)	0	1 *(33.3%)*
	# Abnormal (% of group)	0	1 *(33.3%)*
Anxiety score		6.1 ± 2.5	11.7 ± 4.0
	# Normal (% of group)	8 *(66.7%)*	0
	# Borderline Abnormal (% of group)	4 *(33.3%)*	1 *(33.3%)*
	# Abnormal (% of group)	0	2 *(66.7%)*

Results are presented as means ± standard deviation (SD).

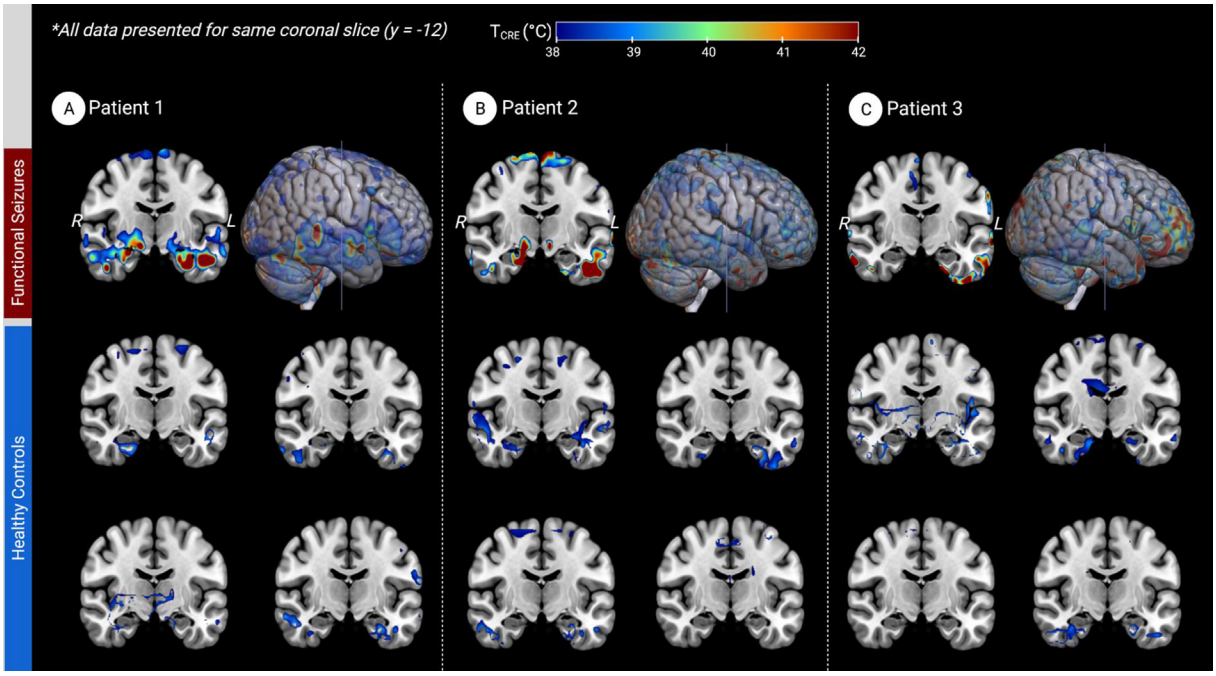

Fig. 2. Examples of brain temperature (T_{CRE}) elevations in three female patients with functional seizures (FS). Each FS patient's data were visualized in coronal section (y = -12) alongside data from 4 age-matched female healthy controls (HC). Elevations were characterized by T_{CRE} > 38 °C (or 3 SD from the HC mean), with an upper threshold of 42 °C. As indicated by the color bar (*top center*), blue to green = 38 °C ≤ T_{CRE} ≤ 40 °C and yellow to red = 40 °C ≤ T_{CRE} ≤ 42 °C. T_{CRE} maps were overlaid and visualized on the Montreal Neurological Institute single-participant template using open-source software MRIcroGL (McCausland Center for Brain Imaging, University of South Carolina; https://www.mccauslandcenter.sc.edu/mricrogl/). The figure was created using Biorender (https://biorender.com/). A. Patient 1 (female, 42 years old): Brain temperature was elevated in the right middle and inferior temporal gyri, as well as the parahippocampal gyrus and hippocampus. On the left, T_{CRE} was elevated in the fusiform, inferior temporal, and paracentral lobule areas. These data were evaluated and visualized alongside data from 4 female healthy controls ranging in age from 23 to 28 years. Of these, one healthy control had borderline abnormal anxiety on the HADS. B. Patient 2 (female, 23 years old): Brain temperature was elevated in the right supplementary motor area and precentral gyrus. Bilateral T_{CRE} elevations were found in right and left inferior temporal gyri, as well as the parahippocampal and hippocampal regions. Brain temperature was also elevated in a small cluster within the middle temporal lobe. These data were visualized alongside data from 4 female healthy controls ranging in age from 22 to 23 years. Of these, two HCs had borderline abnormal anxiety on the HADS. C. Patient 3 (female, 50 years old): Brain temperature elevations were localized to the right and left inferior temporal gyri, left parahippocampal gyrus, and a small cluster in the left middle temporal gyrus. T_{CRE} was also elevated in the left postcentral gyrus. These data were evaluated and visualized alongside data from 4 female healthy controls ranging in age from 30 to 52 years. Of these, one control participant had borderline abnormal anxiety symptoms on the HADS. Abbreviations: T_{CRE}, voxel-wise brain temperature (T_{CRE}); FS, functional seizures; R, right; L, left; HC, healthy control; SD, standard deviation; HADS, Hospital Anxiety and Depression Scale.

controls ranging in age from 30 to 52 years. Of these, one control participant had borderline abnormal anxiety symptoms ($HADS_{ANX}$ = 10). T_{CRE} was elevated in the bilateral inferior temporal areas, left parahippocampal gyrus, and left middle temporal gyrus (Fig. 2C). T_{CRE} was also elevated in the left postcentral gyrus.

Discussion

The hypothesis: Evidence from prior studies and preliminary findings

The links between inflammation, brain structure and function, and psychopathology are well-documented. Our hypothesis builds on the idea that FS are not a manifestation of psychological distress alone, but rather represent a complex, multifactorial disease associated with various types of brain pathology. The brains of patients with FS have shown structural, functional, and connectivity alterations in regions that coordinate emotion regulation, self-agency, and the neuroendocrine and autonomic arms of the stress response [9]. These findings support our speculations that neuroinflammation may be an important neuropathophysiological factor in shaping a brain network capable of producing FS. Our hypothesis is further supported by preliminary evidence of brain temperature elevations (T_{CRE}) presented here (Figs. 2-3, Tables 2-3), which demonstrate MRSI-t's promise for mapping the consequences of local neuroinflammatory milieu. Of the FS patients investigated using MRSI-t, all 3 were impacted by early life stressors and/or risk factors and presented with a history of head injury, high anxiety, and several co-morbid conditions. Each FS patients' brain temperature elevations converged in four key regions: inferior and middle temporal gyri, parahippocampal gyrus, and hippocampus. These findings reinforce the notion that, despite the heterogeneity of patients with FS, aberrations in specific brain regions may drive disease features common to all patients. These findings highlight the need for future studies of FS biomarkers of neuroinflammation. While the presented data are sufficient for hypothesizing neuroinflammation, the data are restricted to the first 3 FS patients of our on-going study, and thus group analyses are not possible. The small sample also barred the ability to statistically control for other conditions that may confound findings and/or factors that could increase brain temperature by non-neuroinflammatory means. Thus, while our findings open an exciting avenue for future research, they may not be representative of all patients with FS. Further, it is not clear whether the temperature elevations (T_{CRE}) are a feature of FS or indicative of neuroinflammatory psychopathology observed in patients with mental health disorders in general. The need to untangle these and other issues related to psychiatric and immune functioning has resulted in the development of a new field of study termed psychoneuroimmunology [66-68,82-85].

Suggestions for future research

Generally, future studies must enroll larger sample sizes that capture a range of demographic, developmental, and clinical vari-

Table 3
Summary of FS patients' prior medical history and preliminary MRSI-t findings. Diagnosis of FS was confirmed in all patients via recording of at least one habitual seizure during video/EEG monitoring.

Medical History		Patient 1	Patient 2	Patient 3
Age (years)		42 years	22 years	50 years
Onset of FS		Childhood	20 years of age	47 years of age
Risk factors		Lifelong history of domestic abuse	History of prematurity	History of verbal abuse by mother, childhood developmental delays of unclear nature
Head injury/insult		Probable head injury following motor vehicle collision (2018)	Recent severe head injury following snowmobile crash, 2 prior concussions	Reported having suffered from MRI-negative stroke
Other symptoms & conditions		Frequent, severe migraine attacks Tachycardia Asthma Heart valve disease (mitral valve prolapse), likely consequence of rheumatic fever Vitamin D deficiency	Migraines GERD Vitamin D deficiency	Hypothyroidism Diabetes mellitus, type 2 Hyperlipidemia
Prior imaging		EEG and structural MRI unremarkable	EEG unremarkable	EEG showed generalized theta slowing No evidence of stroke on structural MRI or CT, though generalized atrophy was apparent
Mood and emotion				
HADS	Anxiety	Borderline abnormal	Abnormally high	Abnormally high
	Depression	Normal	Borderline abnormal	Abnormally high
POMS	TMD	Normal	High mood disturbance	High mood disturbance
Brain temperature elevations *(MRSI-t T_{CRE})*				
Bilateral elevations		Inferior temporal gyri	Inferior temporal and parahippocampal gyri, hippocampi	Inferior temporal gyri
	R-sided elevations	Middle temporal and parahippocampal gyri, hippocampus	Supplementary motor area, precentral gyrus	
	L-sided elevations	Fusiform and paracentral lobule areas	Middle temporal gyrus	Parahippocampal, postcentral, and middle temporal gyri

Abbreviations: FS, functional seizures; GERD, gastroesophageal reflux disease; EEG, Electroencephalography; MRI, magnetic resonance imaging; CT, computerized tomography; HADS, Hospital Anxiety and Depression Scale; POMS, Profile of Mood States; TMD, Total Mood Disturbance on the POMS; MRSI-t, magnetic resonance spectroscopic imaging and thermometry; T_{CRE}, brain temperature derived from MRSI-t; R, right; L, left.

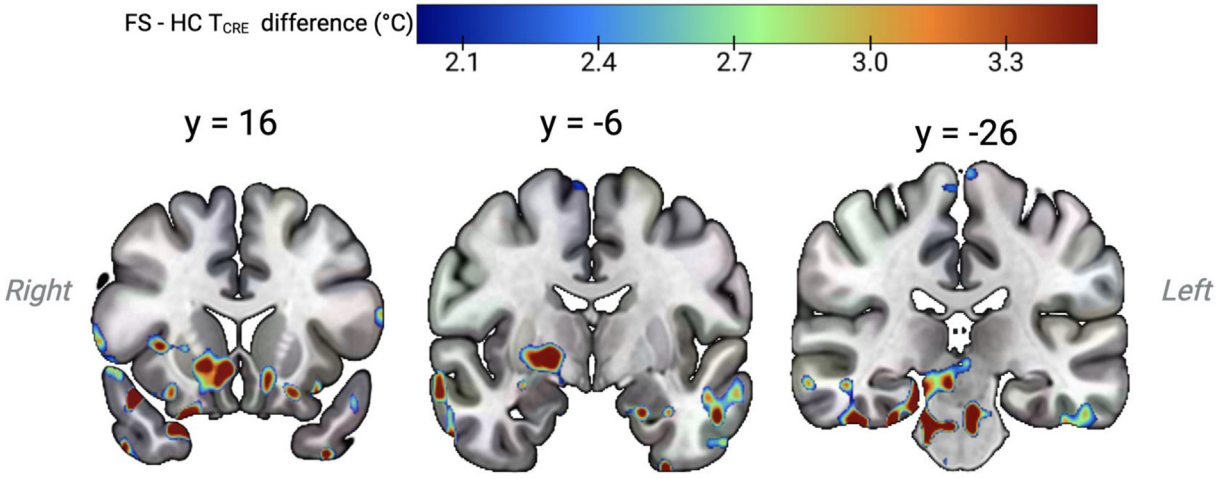

Fig. 3. Regions where patients with functional seizures (FS) had higher mean brain temperature (T_{CRE}) than healthy controls (HC). Data are visualized in coronal section, with red clusters representing the greatest temperature difference between groups (FS > HC). Since patients with FS demonstrated voxelwise brain temperature elevations (see Fig. 2) in several brain regions, this group difference map was computed as follows: $(\text{Mean } T_{CRE})_{FS} - (\text{Mean } T_{CRE})_{HC}$. Mean T_{CRE} maps were generated for each group before difference map computation. Since the maximum T_{CRE} difference between groups was 3.48 °C, data were visualized using a color range of 1.5 – 3.5 °C (see top color bar). As indicated by large red clusters, groups' mean T_{CRE} deviated in the cerebellum and portions of inferior and middle temporal lobe (R≫L). Right-sided differences (FS > HC) were localized to the insula, fusiform and parahippocampal gyri, caudate, posterior orbitofrontal cortex, and the superior temporal pole. Abbreviations: T_{CRE}, voxel-wise brain temperature (T_{CRE}); FS, functional seizures.

ables [67,68,82-85]. This includes mental health variables that may be associated with various degrees and types of neuroinflammatory responses. As with our study of brain morphometry in FS, relevant comparison groups (e.g., TBI-only) must be included to allow a better distinction between the likely associations of FS itself and of concurrent psychopathology, prior trauma/abuse, and psychiatric comorbidities. Careful study design will also help account for FS development in the considerable portion of FS patients who do not report prior trauma. Neurocognitive assessments and psychiatric examination should be conducted in controls and FS participants.

Future studies must delineate the time course of brain temperature elevations in FS, e.g., whether temperature elevations sparked by FS remain elevated or resolve following successful treatment. Prospective studies are needed to resolve whether neuroinflammation precedes FS onset, and its temporal link to

traumatic events. It will also be critical to determine whether brain temperature elevations can be married with other markers of peripheral (e.g., alpha amylase, TRAIL or ICAM-1) or central (e.g., diffusion MRI or PET) inflammation. If the broadly defined early-life stress is the primary instigator, proper identification and development of interventions targeting post-trauma neuroinflammation may serve a preventative function. The history of abuse, trauma, and adverse life events that intersect with FS semiology may be intimately tied to the phenotypic expression of each individual's FS [86].

Further, the neuropathophysiological correlates of FS cannot be understood with the use of a single imaging modality. Multimodal imaging of FS will be critical for mapping the concordance between implicated brain regions' function, connectivity, and structural integrity. Longitudinal data must be collected before and after treatment to better address the role of interventions in FS and whether changes in cortical thickness represent a predisposition to, or consequence of FS. This includes prospective studies that allow characterizing the location and time course of structural, functional, and biochemical changes, especially in regards to FS diagnosed after brain insult. Insights into these aspects may allow pinpointing when changes develop and whether cortical folding aberrations precede or follow adverse life events and/or trauma during childhood. Studies must investigate the interplay of neuroinflammation, prior medical history, and psychological and cognitive symptoms with imaging- and serum-based biomarkers of neuroinflammation. The impact of stress, prior abuse, and trauma should be evaluated with objective and subjective measures, especially with regards to the impact these factors have on magnitude and extent of brain abnormalities. For example, the subjective appraisal of a putative traumatic experience, rather than the nature of the event itself, likely predicts the magnitude of the stress response and may also impact resultant brain changes [87-89].

Conclusions

In this hypothesis manuscript, we discuss the possibility of neuroinflammation as a contributor to the development and maintenance of FS and, possibly, to the other comorbid FNDs that co-occur in many patients [81]. These speculations are based on prior evidence, with converging findings from our preliminary evidence of brain temperature elevations in FS patients using MRSI-t. While it remains unclear how and to what extent neuroinflammation contributes to the FS process, we believe there is enough data regarding peripheral and central neuroinflammation to support further investigation of this hypothesis. It is our expectation that our hypothesis will spark further discussion in the field, and open new avenues for studying biomarkers of neuroinflammatory pathophysiology in FS. Even if the underlying pathophysiology is not neuroinflammatory in basis, this framework brings us closer to uncovering the pathophysiological processes that must be targeted for more efficacious treatment of FS.

Ethical statement

We confirm that any aspect of the work covered in this manuscript that has involved human patients or their protected health information has been conducted with the ethical approval of all relevant bodies and that such approvals are acknowledged within the manuscript.

The University of Alabama at Birmingham Institutional Review Board approved all study procedures. All participants were screened for MR compatibility. Written informed consent was obtained from all participants before initiating the protocol.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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