Functional Network Connectivity Imprint in Febrile Seizures

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Abstract

Complex febrile seizures (CFS), a subset of paediatric febrile seizures (FS), have been studied for their prognosis, epileptogenic potential and neurocognitive outcome. We evaluated their functional connectivity differences with simple febrile seizures (SFS) in children with recent onset FS. Resting-state fMRI (rs-fMRI) datasets of 24 children with recently diagnosed FS (SFS-n=11; CFS-n=13) were analysed. Functional connectivity (FC) was estimated using time series correlation of seed region–to-whole-brain-voxels. Regional connectivity differences were correlated with clinical characteristics (corrected $p < 0.05$). CFS patients demonstrated increased FC of the bilateral middle temporal gyri (MTG), left supplementary cortex when compared to SFS. Decreased FC of the primary sensory areas and Default mode network regions were observed in the CFS**.** Number of recurrent febrile seizures positively correlated with FC of bilateral MTG and negatively correlated with left Supplementary Motor. Duration of longest febrile seizure correlated positively with connectivity of right MTG and left supplementary motor cortex. It also negatively correlated with connectivity of bilateral post central gyrus and Precuneus. Our findings report altered connectivity in CFS proportional to the seizure recurrence and duration. Regardless of the causal/consequential nature, such observations demonstrate the imprint of these disease-defining variables of febrile seizures on the developing brain.

Introduction

Febrile seizures (FS), are defined as seizures in children between 6 months to 5 years of age, accompanied by fever, but without evidence of underlying central nervous system (CNS) infection [1]. The definition excludes seizures in children due to neuroinfection, prior afebrile seizure, or a pre-existent CNS abnormality [2]. It is the most common type of seizure in childhood, occurring in 2–5% of children [3]. A polygenic basis for febrile seizures is well elaborated with multiple candidate genes /loci described [4,5]. Febrile seizures can be subtyped as either Simple Febrile Seizures (SFS) or Complex Febrile Seizures (CFS). SFS, accounting for two-thirds of FS, are typified as a generalized convulsive episode of seizure without features suggesting a focal nature. They typically last for lesser than 15 minutes and do not recur within 24 hours. CFS usually demonstrate any of the following features: focal features; prolonged duration (characterised as lasting more than 15 minutes), seizure recurrence within 24 hours/index febrile illness; postictal neurological deficits [6,7].

The immature brain is different from the adult brain in that it is more vulnerable to seizures, notwithstanding the seizure characteristics, and therapeutic responses. Normal brain maturation is punctuated by overexpression of factors enhancing neuronal excitability. The consequent relative imbalance in excitation vs inhibition is critical to synaptic plasticity and activity-directed synaptogenesis during development [8]. A pertinent concern then is whether seizures in a growing brain can influence the neurocognitive outcome. In the context of short seizures provoked by fever (exemplified by SFS), prospective epidemiological studies have demonstrated a benign outcome, without cognitive dysfunction or subsequent epilepsy[9,10]. Most children who experience SFS do not develop (or are not associated with) structural mesial temporal abnormalities [11]. Epilepsy frequency following SFS is estimated at 1.0-2.2 %, a figure not any different from the normal population[12]. The relationship of CFS with temporal lobe epilepsy (TLE) is ridden with conflicting

data. Retrospective risk-factor analyses have revealed that patients with intractable TLE happen to report a history of prolonged febrile seizures at higher frequency (30-60%) [9,13]. CFS is associated with a heightened risk of epilepsy in 4.1-6.0 % of the cases [12]. Min Lan Tsai et al, in a study on long term neurocognitive outcomes in subjects with CFS noted significantly lower full-scale intelligence quotient (FSIQ), perceptual reasoning index, and working memory index scores than in the control group[14]. Dube et al indicated that hyperthermic seizures in the immature rat model of FS do not cause spontaneous limbic seizures during adulthood. [15]. However, “prolonged” experimental FS led to later-onset limbic (temporal lobe) epilepsy and interictal epileptiform EEG abnormalities in a significant proportion of rats[16].

Imaging literature towards understanding disease neurobiology of febrile seizures is sparse. Theodore et al., in a study of 35 subjects presenting as refractory Complex Partial Seizures [CPS] with video-EEG of temporal lobe onset found 9 patients with a prior presentation of CFS had smaller volumes of ipsilateral Hippocampal Formation [HF]. FS in the clinical history had a predictive value on the severity of HF atrophy [17]. To the best of our knowledge, there are no studies that have evaluated connectivity differences in children with a recent diagnosis of febrile seizure. With the above-cited differences in experimental, clinical and neurocognitive outcome profiles as the background, we hypothesize that there could be differences in the connectivity patterns between patients with SFS and CFS. To this end, we used functional connectivity patterns as derived from Blood Oxygen Level Dependent (BOLD) rs-fMRI in subjects with CFS and SFS.

Results

24 subjects were included in the final analysis [Simple FS - 11 and Complex FS -13] within 12 days [IQR= 8.5 -13.5] and 10 days [IQR = 9-30] after the last seizure respectively. Among the clinical variables analysed, namely mean age at onset, a number of recurrent febrile seizures, the maximum duration of febrile seizures, duration of the disease, none showed significant between-group differences. Functional connectivity (FC) was estimated using time series correlation of seed region–to-whole-brain-voxels and the brain regions showing significant between group connectivity differences were correlated with disease-defining clinical characteristics. Our results revealed that CFS group had altered temporal lobe connectivity proportional to recurrences, and duration of the seizure.

Altered brain connectivity in complex febrile seizure

Seed to Voxel analysis revealed that the patterns of connectivity of multiple seed ROI’s in patients in the complex febrile seizure group were significantly different (FDR-corrected, $P < 0.005$) from those in simple febrile seizure group (Figure.1)(Table 2). Patients with CFS demonstrated increased connectivity involving bilateral temporal lobes involving the medial and lateral temporal cortices, bilateral thalamus, bilateral accumbens, bilateral amygdala and left supplementary cortex when compared to simple febrile seizure group. On the other hand, decreased functional connectivity of the primary sensory areas and Default Mode Network (DMN) was seen involving the Posterior Cingulate Cortex (PCC), bilateral postcentral gyrus, and left occipital lobe in the CFS group.

Increased connectivity in complex febrile seizure

Bilateral middle temporal gyrus revealed increased functional connectivity with the bilateral hippocampus, bilateral parahippocampal gyrus and bilateral accumbens. Right middle temporal gyrus (Figure 1a) had additional increased connectivity with bilateral thalamus and ipsilateral amygdala and putamen. Left middle temporal gyrus (Figure 1b) demonstrated increased connectivity involving the amygdala bilaterally, ipsilateral orbitofrontal and subcallosal cortex. The left supplementary cortex revealed increased connectivity with the parahippocampal gyrus and bilateral thalamus.

Decreased connectivity in complex febrile seizure

Precuneus and posterior cingulate cortex (Figure 1f) revealed decreased connectivity with bilateral supplementary motor cortex, postcentral gyrus, bilateral caudate and right lentiform nucleus. Left supplementary motor cortex (Figure 1e), in addition, showed decreased connectivity with left inferior frontal gyrus. Bilateral postcentral gyri (Figure 1 c,d) showed additional decreased connectivity with left lingual gyrus, fusiform gyrus and intracalcarine cortex.

Altered connectivity proportional to the seizure recurrence and duration

The number of recurrences of febrile seizures was positively correlated with brain functional connectivity of bilateral middle temporal gyri to right Hippocampus [right: r = 0.6; p = 0.003; left: r = 0.54, p = 0.0086], and Left Middle Temporal gyrus with right Parahippocampus [r = 0.57, p = 0.0055] and negatively correlated with left Supplementary Motor to Precuneus [r = -.043, p = 0.041] (Figure 2; Table 3).

Furthermore, duration of the longest febrile seizures was positively correlated (Figure 3) with the functional connectivity of right Middle Temporal gyrus (MTG) to left Hippocampus [r= 0.59, p = 0.003], right Parahippocampus [r = 0.58, p = 0.004], bilateral thalami [right, r= 0.65, p =0.001; left, r= 0.59, p= 0.003], right putamen [r = 0.60, p =0.002], amygdala [r = 0.59, p =0.003], bilateral accumbens [r= 0.49, p = 0.019] and left supplementary motor cortex to right Parahippocampus [r= 0.44, p = 0.04]. Additionally, duration of longest febrile seizures was negatively correlated with connectivity of the bilateral post central gyrus to left lingual gyrus (right: r= -0.48, p = 0.023; left: r= -.55, p =0.006), Precuneus (right : r= -.45, p =0.03; left r= -0.49, p = 0.02;) and right post central gyrus to left intracalcarine cortex [r= -0.53, p = 0.01] was noted (Fi 3; Table 3).

No statistically significant correlations of age of onset of illness, time interval between imaging and last seizures with connectivity changes were noted.

Discussion

The FEBSTAT study remarked that children presenting with Febrile Status Epilepticus are at risk for acute hippocampal injury in the background of structural imaging abnormalities [24]. While the causal nature and relevance of structural imaging findings are being debated [25,26], the current study probing changes in functional connectivity adds value to the existing literature. In this study, we demonstrate, that children with complex febrile seizures have increased connectivity in the bilateral temporal lobes involving the medial and lateral temporal cortices, bilateral thalami, bilateral accumbens, bilateral amygdala and left supplementary cortex when compared to simple febrile seizure group. We also observed decreased functional connectivity

of the primary sensory areas and DMN (involving the PCC, bilateral postcentral gyrus) and left occipital lobe in the complex febrile seizure group. The increased connectivity of temporal lobes and decreased connectivity of the sensory-motor areas and DMN correlated with the number of recurrent febrile seizures, and the duration of the longest seizures. Since the temporal lobe hyperconnectivity and the sensorimotor hypoconnectivity were proportional to the seizure frequency and recurrences we suspect these changes to be interlinked. One argument we considered were postictal reversible MRI changes, since these changes are known to exist usually for 7 days [27,28]. The fact that the children were imaged around 10 days after the last seizure makes the confounding influence of post-ictal phenomenon less likely. Also, there was no correlation between the time to imaging after last seizure in our study. Owing to the paucity of in-vivo studies in this group, we can only surmise that rs-fMRI-derived functional connectivity alterations found in children with complex febrile seizures may be similar to experimental evidence of alterations of functional neuronal and network properties in early life epileptogenesis [29,30] without structural alterations such as neuronal death, genesis or altered branching [31]

The functional connectivity imprint of febrile seizures reported in the current study could represent either the cause or effect of febrile seizure. Hyperconnectivity, a probable marker of increased neural resource use, is a well-documented ubiquitous response in many neurological disruptions [32]. Notwithstanding its implications in terms of processing speed, cognitive fatigue and resource use [33], increased connectivity has nevertheless been noted as a pervasive reaction to many neurologic disturbances. Evidence suggests that this change in connectivity stems from increased spiking output from neuronal ensembles[34]. In the pathobiology of complex febrile seizures, the observed hyperconnectivity may have a role that is not necessarily compensatory. Enhanced EEG connectivity in febrile seizures has been incriminated as a seizure-prone state by Birca et al[35]. Mossy fibre plasticity and enhanced hippocampal excitability, without neither hippocampal cell loss nor altered neurogenesis, have been reported in animal models of prolonged febrile seizures[36]. Frequently described in the context of temporal lobe epilepsy, Kindling is a self-propagating reduction in the ability of the brain to limit seizures in which duration and behavioural involvement of induced seizures increase after repetitive induction of mesial temporal lobe seizures[37]. Sequential exaggerated mossy fibre invasion of the molecular and granule cell layers have been associated with the process of “kindling”. Murine models of hyperthermic seizures have documented evidence of the kindling-like phenomenon in epileptogenesis[38]. We propose that processes like these above-detailed mechanisms may operate in the genesis of hyperconnectivity in CFS as well the observed correlation with Febrile seizures’ recurrence seizure duration. .

Seizure circuitry in the context of temporal lobe epilepsy has been classified broadly as (a) the minimal-size initiating circuit(s) involving the trisynaptic circuit of the entorhinal cortex, the dentate gyrus(a "gatekeeper" resisting recruitment) and Ammon's horn of the hippocampus and (b) the pathways of seizure spread by which additional brain circuits are recruited as the seizure continues and spreads[29]. In this context, it becomes difficult to ignore the similarity of the regions described in the experimental models with those obtained from the data-driven methodology used in the current study. The hyperconnectivity between allocortical [hippocampus, amygdala, accumbens] and neocortical regions [middle temporal gyrus] of bilateral temporal lobes is similar to the regions described in seizure initiating circuit. There was also positive

connectivity of right middle temporal gyrus to bilateral thalami and bilateral thalami to the left supplementary motor cortex which in turn had increased connectivity to bilateral parahippocampal gyri, probably indicating pathways of spread.

Decreased metabolic activity is observed in the default mode network in absence seizures through multimodal electrophysiologic and neuroimaging methods, possibly reflecting the theorem of “network inhibition hypothesis” [39]. It is analogous to the activity-dependent push-pull mechanism between various circuits operating in normal physiological conditions. The observation of commensurate decreased connectivity of the DMN and sensorimotor regions observed in CFS patients in our study could then indicate this compensatory phenomenon to the increased demand in the seizure circuitry. In the absence of longitudinal studies/ follow-up for the outcome, the translational clinical relevance of the observed altered connectivity will remain obscure. We speculate that the observed altered connectivity could fall in the continuum of pathophysiology in children with complex febrile seizures.

Though the study has advanced the knowledge of the neurobiology of febrile seizures, it needs to be noted that imaging was performed in natural sleep due to ethical aspects of giving sedation in children undergoing imaging for febrile seizures. In this precarious situation, it was difficult to monitor the stages of sleep and hence the confounding effects of various stages of sleep on results cannot be eliminated. For the same reason, a lack of control group might also seem to be a limitation, but there is evidence for advantages of disease matched controls in evaluating heterogeneous diseases like epilepsy[40]. Results of our study are based on the group-level analysis between the two groups and might not be relevant to an individual patient. We lost significant data of 9 children due to uncorrectable head motion due to snoring and hence the study sample size is small and generalizability to a larger population becomes difficult. Larger samples with longitudinal observations and the clinical outcome would add further evidence to the above observations.

Conclusions

Children with recently diagnosed complex febrile seizures reveal altered connectivity in several regions including temporal lobes proportional to the frequency, and duration of the seizure. This evidence is in tune with experimental evidence in febrile seizures but will require larger longitudinal studies to ascertain clinical relevance.

Materials And Methods

The prospective study was conducted at a tertiary care referral centre for neurologic disorders in children with recent onset febrile seizures Written informed consent was obtained from the caregiver of each participant, and the study was approved by the NIMHANS Human Ethics Committee-Basic and Neurosciences Division to be performed in children without using sedation. Hence all children underwent imaging while they were naturally sleeping inside the MRI gantry. This was associated with increased scan time, due to multiple pauses and restarts and resultant loss of data in nine subjects

Study population

Thirty -three patients with febrile seizures were recruited - [Simple FS -19; Complex FS -14]. Confirmation of the diagnosis of febrile seizures was based on clinical parameters and biochemical investigations according to the American Academy of Pediatrics guidelines[18]. After exclusion of subjects with uncorrectable head motion, 24 subjects were included in the final analysis [Simple FS - 11 and Complex FS -13]. The Male: Female ratio was 10:3 and 8:3 in the CFS and SFS groups respectively(p=1.0). A positive family history of seizures was present in four and three patients in the CFS and SFS groups respectively. Time interval from the last seizure to the MR imaging for the CFS and SFS groups were 12 days [IQR= 8.5 -13.5] and 10 days [IQR = 9-30] respectively. Among the clinical variables analysed viz mean age at onset, a number of recurrent febrile seizures, the maximum duration of febrile seizures, duration of the disease, none showed significant between-group differences.

The demographic and clinical features of the patients are provided in Table 1

Image acquisition

MRI was performed in a 3 Tesla scanner (SKYRA, Siemens, Erlangen, Germany). Child was allowed to sleep in the gantry room in the hands of the parent after ensuring that they both were metal free after the room lights were dimmed. Once the child was asleep in the MR environment, trained technologist transferred the child on the table and positioned with minimum disturbance to the sleeping child wrapped in a blanket. If at any point the child woke up, the entire cycle was repeated. Head was well supported with soft pads. 32 Channel head coil was used. The resting-state fMRI acquisition (rs-fMRI) using blood oxygen level-dependent (BOLD) contrast were as follows: 200 volumes, repetition time 2030ms, 40 slices, 3 mm slice thickness, FOV 195×195 mm, matrix 64×64, refocusing pulse 90°, voxel size-3 x 3 x 3mm The total time of acquisition for rs-fMRI was 6 minutes 52 seconds. Anatomic images were acquired by using a 3D T1-weighted MPRAGE sequence in 192 sagittal sections with a TR of 1900ms, TE of 2.5ms, a TI of 900ms, a FOV of 256 x 256 and a section thickness of 1mm. Oblique Coronal T2 Fast Spin Echo (FSE) planned perpendicular to the hippocampus was also done to rule out other structural abnormalities. Structural imaging revealed that one patient with complex febrile seizure had bulky and T2 hyperintense right hippocampus. Rest of the imaging studies did not reveal any abnormality.

Image analysis

Pre-processing- Image analysis was performed using Statistical Parametric Mapping 8 (SPM8) (http://www.fil.ion.ucl.ac.uk/spm/software/spm8). Following realignment, co-registration of the T1 Images to the EPI counterparts was done. Segmentation of data to grey, white matter, CSF tissue probability maps were done. The normalization step warped each individual subject imaging space into a standard space (University of North Carolina, UNC paediatric [two years] standard space). It was done independently for both structural and functional images.

Anatomic Parcellation - The fMRI data were segmented into 90 anatomic ROIs on the basis of a University of North Carolina [UNC] paediatric [two years] atlas for whole-brain regions by using the anatomically labelled template reported by Shi et al, 2011[19].

Functional Connectivity Analysis - A seed-to-voxel–based functional connectivity analysis was performed by computing the temporal correlation between the blood oxygen level-dependent signals to create a correlation matrix showing connectivity from a seed region to all other voxels in the brain by using the functional connectivity toolbox (CONN, version 17) implemented in SPM8 (http://www.nitrc.org/projects/conn)[20]. Meanwhile source reduction of WM and CSF-related physiologic noises was carried out before connectivity estimation, by using the CompCor algorithm[21]. Bivariate correlations were analysed so as to reflect connections between the seed region to the rest of the brain voxels. Then Fisher's r-to-z transformation was used on the connectivity matrix. This was followed by a general linear model that was designed to determine those statistically significant BOLD signal correlation between the mean time series from each seed ROI and that of every other brain voxel, at the individual subjects' level (first-level analysis) [22,23].

Second-level random-effects analysis was used to create within-group statistical parameter maps for each network and to examine connectivity differences between groups. The group mean effects were estimated for both groups. Statistically significant, FDR corrected ($p < 0.05$), seed to target connectivity was calculated using 2nd level co-variate analysis. Pearson linear correlation was performed between clinical variables [number of recurrent febrile seizures, the longest duration of febrile seizure, duration of disease, age of onset and time interval between last seizures and MRI] using the effect size of statistically significant seeds to target connectivity. The correlation coefficient, r (rho) and statistical significance, p values were calculated for each of these connectivity using MATLAB. Positive correlations were designated with a plus ("+") sign and negative correlations with a minus ("-") sign.

Declarations

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Author contributions

RDB, SS, PSB designed research. JS, AKG, KR, RCM, KML recruited patients. UVA, KK, RP acquired and analysed the data. UVA, KK, RP and RDB wrote the manuscript. All authors contributed to result interpretation and editing of the manuscript.

Disclosure of Conflicts of Interest

None of the authors has any conflict of interest to disclose.

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Tables

Table 1: Demographic and clinical features of the patients

Clinical and demographic features	Complex febrile seizures (n=13)	Simple febrile seizures (n=11)	P Value
Male/female ratio, n	10:3	8:3	1.0
Family history of febrile seizures, n (%)	4/13 (30.8)	3/11 (27.3)	1.0
Mean (±SD) age at time of first imaging, m	27.9 ± 9.6	29.3 ± 12.5	.074
Mean (±SD) age at onset, m	16.6 ± 5.8	20.6 ± 8.5	0.19
Number of recurrent febrile seizures			
Mean (±SD)	3.1 ± 2.0	3.3 ± 1.7	0.548
Median (IQR)	2.0 (2-4)	3.0 (2-4)	
Time interval between last seizures and MRI, days			
Median (IQR), d	12 (8.5 -13.5)	10 (9-30)	0.64
Maximum duration of febrile seizures, minutes			
Mean (±SD), min	16.2 (10.2)	9.9 (5.5)	0.192
Median (IQR), min	10 (10-30)	10 (5-15)	
Duration of disease, months			
Mean (±SD), m	11.31 (8.35)	8.75 (9)	0.257
Median (IQR), m	8 (5.5-18)	4 (2-18)	

n – number of subjects, m – months, d – days, min - minutes, SD- Standard Deviation, IQR- Inter Quartile Range.

Table 2. Results of seed-to-voxel-based connectivity showing significant changes in Complex Febrile Seizure group compared to Simple Febrile Seizure group. The connectivity cluster was corrected with cluster size (p-FDR corrected < 0.05).

Seed ROI Region	Connectivity Regions	COG coordinates	Cluster size- Number of Voxel	Effect size	p-value (FDR)	Connectivity Pattern
R-Middle Temporal Pole	R-Hippocampus	28; -28; -06	169	1.58	0.000001	Increased
	R-putamen	28; 26; 09	162	2.47	0.000001	Increased
	R-Insula	21; 22; 10	84	1.9	0.000001	Increased
	R-Thalamus	28; -28; -06	43	2.7	0.000001	Increased
	R-Parahippocampus Gyrus	28; -26; 12	54	2.08	0.000001	Increased
	R-Amygdala	20; 14; 58	30	2.38	0.000001	Increased
	L-Hippocampus	-26; -28; -18	153	1.92	0.000213	Increased
	L-Thalamus	-20; -34; 01	59	1.87	0.000213	Increased
	L-Parahippocampus Gyrus	-26; -28; -20	31	2.17	0.000213	Increased
	R-Accumbens	-06, 22, 00	29	1.85	0.000213	Increased
	L-Accumbens	10, 14, 08	20	1.83	0.000213	Increased
L-Middle Temporal Pole	R-Hippocampus	18; -10; -14	219	1.1	0.000494	Increased
	R-Parahippocampus Gyrus	30; -04; -30	85	1.09	0.000494	Increased
	R-Amygdala	10; 20; 16	45	1.85	0.000494	Increased
	L-Hippocampus	-17; 10; 18	110	1.11	0.000001	Increased
	L-Parahippocampus Gyrus	-14; 02; 24	124	2.26	0.000001	Increased
	L-Amygdala	-02; 20; 14	91	2.26	0.000001	Increased
	L-Frontal Orbital Cortex	-24; 22; 11	173	2.5	0.000001	Increased
R-Post Central Gyrus	Precuneus	-0; -52; 1	222	-2.35	0.000001	Decreased
	L-Lingual Gyrus	-14; -52; 13	162	-3.67	0.000001	Decreased
	L-Intracalcarine Cortex	-10; -48; 10	90	-1.82	0.000001	Decreased

	L-Occipital Fusiform Gyrus	-14; -52; 15	106	-3.76	0.000001	Decreased
L-Post Central Gyrus	Precuneus	0; 52, 0	835	-2.37	0.000001	Decreased
	L-Lingual Gyrus	-12; 52, 1	766	-2.10	0.000001	Decreased
	L-Occipital Fusiform Gyrus	-12; 52, 1	415	-1.59	0.000001	Decreased
	L-Intracalcarine Cortex	-12; 52, 1	258	-1.07	0.000001	Decreased
PCC	R-Putamen	28; -04; 10	83	-1.87	0.000005	Decreased
	R-Caudate	10; 10; 12	108	-1.9	0.000005	Decreased
	L-Supplementary Motor Cortex	-2; -4; 60	84	-2.6	0.015196	Decreased
L-Supplementary Motor	R-Thalamus	10; -32; -02	152	3.24	0.000001	Increased
	R-Parahippocampus Gyrus	14; -32; -03	128	2.68	0.000001	Increased
	L-Parahippocampus Gyrus	-14; -38; -02	115	2.87	0.000164	Increased
	L-Thalamus	-10; -32; -03	79	2.83	0.000164	Increased
	PCC	-08; -56; 30	352	-2.78	0.001287	Decreased
	L-Inferior Frontal Gyrus	-48; 40; 04	215	-2.19	0.001287	Decreased
	MPFC	-02; 64; 06	320	-1.98	0.001287	Decreased

Table 3-Correlation of clinical variables [number of recurrent febrile seizures, the longest duration of febrile seizures and duration of disease] to statistically significant seed to target connectivity between complex and simple febrile seizure groups

Seed Region	Connectivity Region and connectivity	Clinical variable					
		Recurrence		Duration of Seizure		Disease Duration	
		r value	P value	r value	P value	r value	P value
L Post Central Gyrus	Precuneus (decreased)	-0.04	0.8464	**-0.49**	**0.0204**	-0.11	0.6025
	L Lingual Gyrus (decreased)	-0.08	0.7071	**-0.55**	**0.0067**	-0.08	0.7175
	L Occipital Fusiform Gyrus (decreased)	0.03	0.8689	-0.32	0.1409	-0.12	0.5704
PCC	R Caudate (decreased)	-0.35	0.1074	-0.29	0.1902	0.08	0.7108
	L Caudate (decreased)	-0.28	0.1989	-0.36	0.0914	-0.11	0.609
	R Putamen (decreased)	-0.20	0.3599	-0.28	0.1994	0.09	0.6881
	R Pallidum (decreased)	-0.15	0.4892	-0.32	0.1395	0.01	0.9329
L Supplementary Motor Cortex	L Inferior Frontal Gyrus, pars triangularis (decreased)	0.05	0.8045	-0.14	0.5146	0.18	0.422
	Precuneus, PCC (decreased)	**-0.43**	**0.0411**	-0.13	0.5476	0.18	0.4055
	L Parahippocampus Gyrus (increased)	0.13	0.5507	0.31	0.159	0.10	0.6463
	R Parahippocampus Gyrus (increased)	0.13	0.5589	**0.44**	**0.0404**	-0.06	0.7798
	L Thalamus (increased)	0.14	0.5232	0.30	0.1729	0.10	0.6395
	R Thalamus (increased)	-0.02	0.8948	0.31	0.1468	0.01	0.9692
L Middle Temporal Pole	R Hippocampus (increased)	**0.54**	**0.0086**	0.35	0.107	0.40	0.0825
	L Hippocampus (increased)	0.19	0.3837	0.36	0.0994	0.02	0.9239
	L Frontal Orbital Cortex (increased)	-0.11	0.6069	0.23	0.2976	-0.15	0.4923
	Subcallosal Cortex (increased)	0.19	0.3951	0.27	0.2165	-0.15	0.4826
	R Parahippocampus Gyrus (increased)	**0.57**	**0.0055**	0.07	0.7516	-0.01	0.965
	L Parahippocampus Gyrus and left amygdala (increased)	0.01	0.9484	**0.43**	**0.0433**	0.02	0.9238

	R Amygdala (increased)	0.19	0.3845	0.40	0.0626	0.09	0.6812
	R Accumbens (increased)	0.19	0.3951	0.27	0.2165	-0.15	0.4826
R Post Central Gyrus	Precuneus (decreased)	-0.15	0.4871	**-0.45**	**0.0322**	-0.01	0.9462
	L Lingual Gyrus (decreased)	-0.07	0.7495	**-0.48**	**0.023**	-0.03	0.8754
	L Intracalcarine Cortex (decreased)	-0.05	0.7663	**-0.53**	**0.0109**	-0.08	0.6942
	L Occipital Fusiform Gyrus (decreased)	-0.12	0.5862	**-0.48**	**0.023**	-0.04	0.8309
R Middle Temporal Pole	R Hippocampus (increased)	**0.60**	**0.0028**	0.32	0.1447	0.26	0.2325
	L Hippocampus (increased)	0.08	0.7045	**0.59**	**0.0038**	0.17	0.4441
	R Parahippocampus Gyrus (increased)	0.13	0.5433	**0.58**	**0.004**	0.15	0.4925
	L Parahippocampus Gyrus (increased)	0.07	0.7499	0.36	0.0968	0.02	0.9047
	R Putamen (increased)	-0.02	0.9291	**0.60**	**0.0026**	0.10	0.6442
	L Thalamus (increased)	0.09	0.6787	**0.59**	**0.0036**	0.16	0.4559
	R Thalamus (increased)	0.05	0.813	**0.65**	**0.001**	0.30	0.1638
	R Amygdala (increased)	-0.01	0.9465	**0.59**	**0.0036**	0.08	0.7418
	B/L Accumbens (increased)	0.01	0.9383	**0.49**	**0.0196**	0.001	0.9892
R Supplementary Motor Cortex	Precuneus, PCC (decreased)	0.25	0.2537	-0.27	0.21	0.36	0.0953

Figures

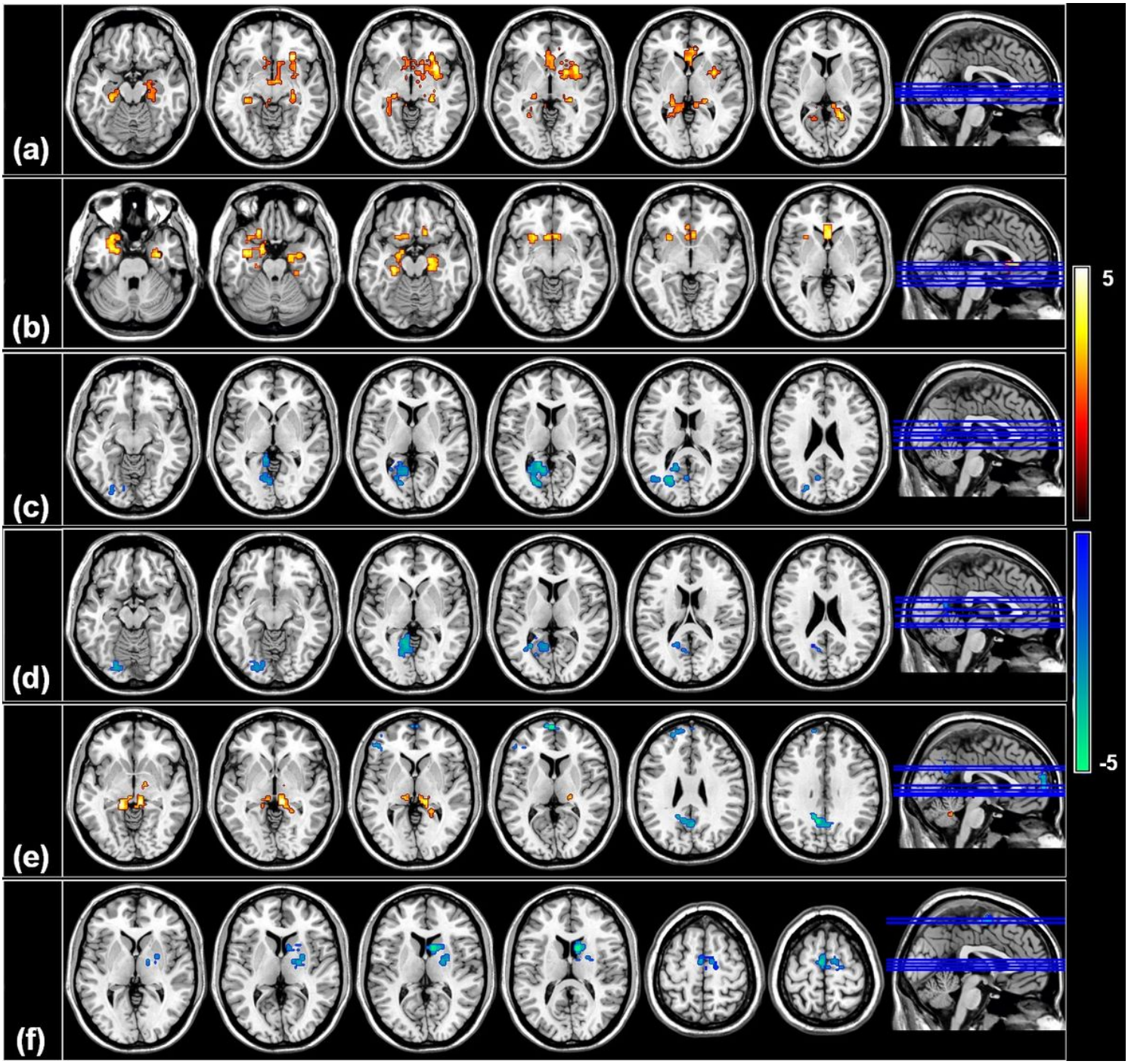

Figure 1

Seed-to-voxel-based connectivity of Complex Febrile Seizure (CFS) compared to Simple Febrile Seizure (SFS) (CFS > SFS) for (a) Right Middle Temporal Gyrus (MTG) (b) Left MTG (c) Right Post Central Gyrus (d) Left Post Central Gyrus (e) L-Supplementary Motor cortex and (f) PCC. The connectivity changes were viewed in the MNI -152 T1 structural template with multi-slice axial view. The color bars indicate the changes in connectivity strength. The connectivity cluster was corrected with cluster size -p-FDR corrected <0.05.

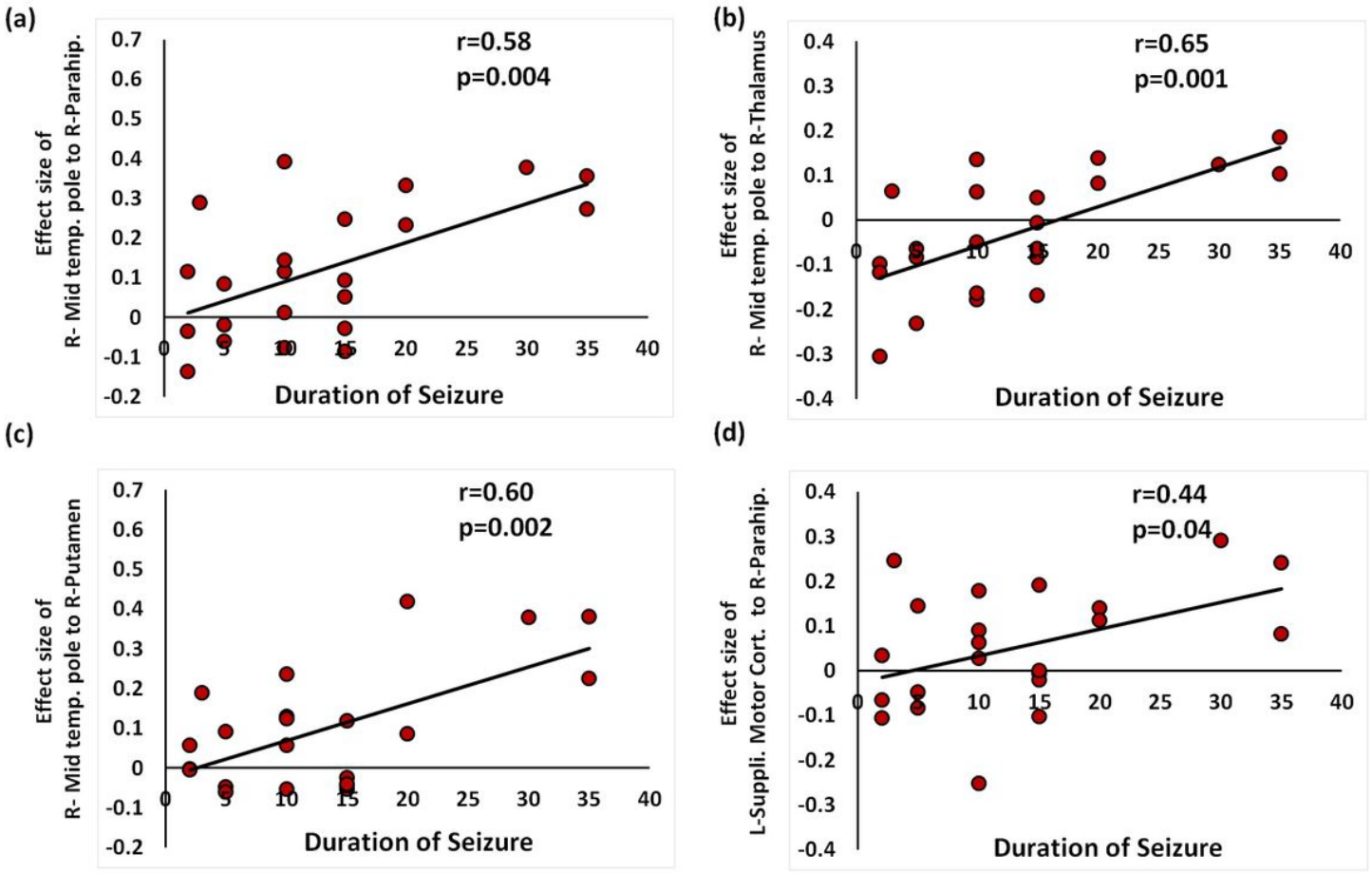

Figure 2

Correlation of the effect size of significant connectivity difference (CFS >SFS) across the seed pairs with Duration of seizure. Positive correlation of Duration of seizure with connectivity of (a) Right MTG to right Parahippocampus (b) Right MTG to right thalamus (c) Right MTG to right putamen (d) Left supplementary motor cortex to right Parahippocampus.

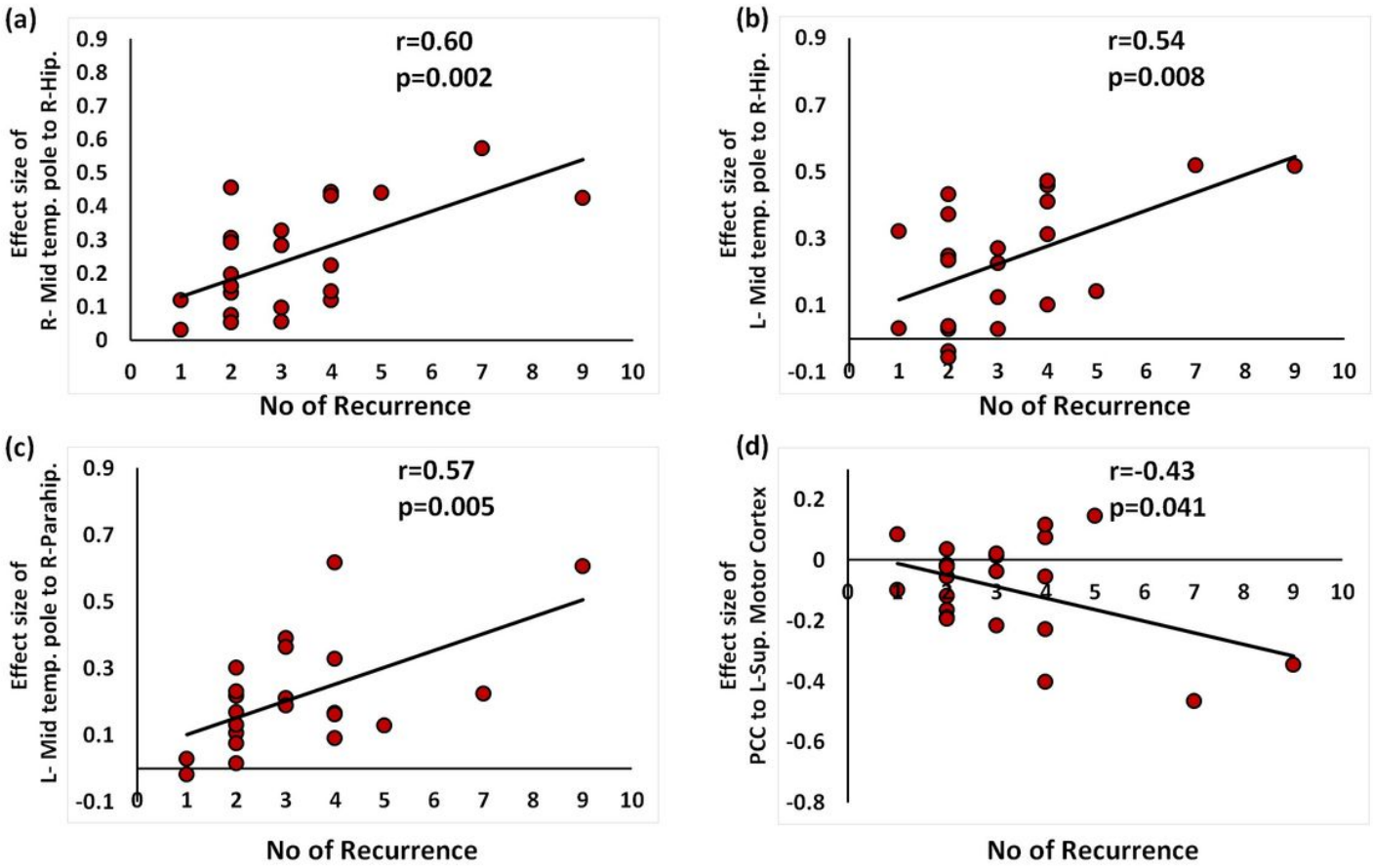

Figure 3

Correlation of the effect size of significant connectivity difference (CFS>SFS) across the seed pairs with number of recurrences. Positive correlation of number of recurrence with connectivity of (a) right middle temporal pole to right Hippocampus (b) left middle temporal pole to right thalamus (c) left middle temporal pole to right Parahippocampus and (d) Negative correlation of number of recurrences with PCC to Left supplementary motor cortex.