Epilepsia, **(*):1–9, 2012
doi: 10.1111/epi.12066

FULL-LENGTH ORIGINAL RESEARCH

Altered regional homogeneity in patients with mesial temporal lobe epilepsy and hippocampal sclerosis

*†Hongwu Zeng, ‡Ricardo Pizarro, *Veena A. Nair, *§Christian La, and *§Vivek Prabhakaran

*Department of Radiology, University of Wisconsin School of Medicine and Public Health, Madison, Wisconsin, U.S.A.; †Department of Radiology, Shenzhen Children's Hospital, Shenzhen, Guangdong, China; ‡Department of Biomedical Engineering, University of Wisconsin-Madison, Madison, Wisconsin, U.S.A.; and §Neurosciences Training Program, University of Wisconsin-Madison, Madison, Wisconsin, U.S.A.

Summary

Purpose: The purpose of the present study was to identify abnormal areas of regional synchronization in patients with mesial temporal lobe epilepsy and hippocampus sclerosis (mTLE-HS) compared to healthy controls, by applying a relatively novel method, the Regional Homogeneity (ReHo) method to resting state fMRI (RS-fMRI) data.

Methods: Eyes closed RS-fMRI data were acquired from 10 mTLE-HS patients (four right-side, six left-side) and 15 age- and gender-matched healthy subjects, and were analyzed by using ReHo. For group analysis, four right-side MTLE-HS patients' functional images were flipped, in order to make a homogeneous left MTLE-HS group (10 cases) and increase the sample size.

Key Findings: Compared to the healthy control group, patients showed significantly increased ReHo in ipsilateral parahippocampal gyrus, midbrain, insula, corpus callosum, bilateral sensorimotor cortex, and frontoparietal subcortical structures, whereas decreased ReHo was observed mainly in default mode network (DMN) (including precuneus and posterior cingulate gyrus, bilateral inferior lateral parietal, and mesial prefrontal cortex) and cerebellum in patients relative to the control group.

Significance: This study identified that ReHo pattern in mTLE-HS patients was altered compared to healthy controls. We consider decreased ReHo in DMN to be responsible for wide functional impairments in cognitive processes. We propose that the increased ReHo in specific regions may form a network that might be responsible for seizure genesis and propagation.

KEY WORDS: Regional homogeneity, Resting state, Functional magnetic resonance image, Epilepsy, Hippocampal sclerosis.

Epilepsy is a common neurologic disorder, characterized by hypersynchronous neuronal activity as shown from electrophysiologic recordings (Schevon et al., 2007; Ortega et al., 2008). Mesial temporal lobe epilepsy (mTLE) is the most common type of focal epilepsy in adults, and it is frequently caused by hippocampal sclerosis (HS) (Berg, 2008).Specific regions in the temporal cortex have been observed to undergo increased synchronization in patients with TLE, which is believed to be involved in the generation of interictal activity (Ortega et al., 2008). Intracranial electroencephalography (EEG) studies have demonstrated increased local coherence in mTLE (Ponten et al., 2007).

Currently, EEG is the most commonly used and basic approach for detecting epileptic activity. Although EEG has high temporal resolution and sensitivity, it lacks spatial resolution and is not sensitive to activity deep within the brain. With better spatial resolution, the noninvasive blood oxygenation level–dependent functional magnetic resonance image (BOLD-fMRI) method has been widely used as an effective technique for epilepsy investigation (Detre, 2006). Recently, simultaneous EEG-fMRI has been used in epilepsy study as it employs the advantages of both EEG and fMRI (Di Bonaventura et al., 2006). In this method, simultaneous EEG provides time points of interictal epileptiform data for fMRI data analysis to explore activation and deactivation in epilepsy. However, the use of simultaneous EEG-fMRI is limited because of practical issues such as high cost, complicated EEG data analysis, and time-consuming preparation.

Resting-state fMRI (RS-fMRI), which was first reported by Biswal et al. (1995), is widely used in brain research and is believed to reflect spontaneous neuronal synchronization and endogenous neurophysiologic process of the human brain (Fox & Raichle, 2007). Several data-driven methods have been developed for RS-fMRI data analysis. For instance, independent component analysis (ICA) can separate the resting state network into subset of networks

Accepted October 31, 2012.
Address correspondence to Vivek Prabhakaran, Assistant Professor, Director of Functional Neuroimaging in Radiology, Department of Radiology, UW Health UW Hospital and Clinics, 600 Highland Avenue, Madison, WI 53792, U.S.A. E-mail: vprabhakaran@uwhealth.org

(Beckmann et al., 2005; Smith et al., 2009). Morgan used two-dimensional (2D) temporal clustering analysis (2D-TCA) to investigate the alteration of BOLD signal in TLE and showed its potential capability to localize the seizure (Morgan et al., 2007, 2010). The limitations of this method include extraneous regions that may not be involved in epilepsy, leading to uncertainty in the results (Khatamian et al., 2011).

Another novel data-driven method, regional homogeneity (ReHo), explores regional brain activity during rest by examining the degree of regional coherence of fMRI time courses, and is able to measure the synchronization of activity in different brain regions (Zang et al., 2004). This method has been successfully used to investigate the functional modulations in the resting state in the patients with attention-deficit/hyperactivity disorder (ADHD), Alzheimers disease (AD), autism spectrum disorders (ASDs), and Parkinsons disease (PD), (Cao et al., 2006; He et al., 2007; Wu et al., 2009; Paakki et al., 2010). It has also been used in generalized tonic–clonic seizures in adults and nonlesion temporal lobe epilepsy in pediatric population, and successfully detected abnormal epileptic synchronization (Mankinen et al., 2011; Zhong et al., 2011). However, to the best of our knowledge, no study has observed the alteration of synchrony in mTLE adult patients with HS using the ReHo method. The purpose of the present study is to identify the abnormal pattern of regional synchronization in mTLE-HS patients compared to healthy controls, by applying the ReHo method to resting state fMRI data, and to characterize the underlying potential network differences.

3 Materials and Methods

Participants

Patients

Ten right-handed patients (mean age ± standard deviation [SD]34.4 ± 10.6 years) observed to have unilateral mesial temporal lobe epilepsy with hippocampal sclerosis (mTLE-HS) participated in the present study. Of all the patients, four had right-sided mTLE (four females), whereas six had left-sided mTLE (four females). All patients were from University of Wisconsin Madison Hospital and were scanned using fMRI between August 2010 and August 2011. According to the classification of the International League Against Epilepsy (Berg et al., 2010), the diagnosis of mTLE-HS was based on clinical, electroencephalographic, and MRI findings. All patients underwent a comprehensive clinical evaluation with the following inclusion criteria:

1 All had one or more typical symptoms of mTLE. All patients had complex partial seizures. In addition, some patients also had simple partial seizures and/or secondary generalized tonic–clonic seizures.
2 MRI manifestations of the HS, unilateral hippocampal atrophy on T1 image with associated hyperintensity on T2 fluid- attenuated inversion recovery (FLAIR) image. There was no identifiable structural MRI abnormality other than the HS in the patients' brain.
3 EEG findings, predominantly left- or right-sided interictal epileptic discharges shown by scalp EEG.

Controls

Fifteen right-handed healthy subjects were recruited in this study, which were equivalent in age and gender (mean age ± SD 35.5 ± 14.6 years, 12 female) to our seizure patient group. All were healthy and free of any neurologic or psychiatric disorders at the time of the study.

This study was approved by the University of Wisconsin Madison Institutional Review Board, and written informed consent was obtained from each participant.

Data acquisition

The participants lay supine with their head snugly held by straps and foam pads to minimize head movement. During resting-state scanning, participants were instructed to keep as motionless as possible with eyes closed, not to think of anything in particular, and not to fall asleep. Images were acquired using a 3.0-T scanner (GE MRI 750, Milwaukee, U.S.A.) in University of Wisconsin Hospital and Clinics, Madison. The resting-state functional data were acquired using an echo-planar imaging sequence with the following parameters: 28 axial slices, ~~TR~~ = 2,000 msec, ~~TE~~ = 30 ms, flip angle = 90 degrees, thickness/gap = 4.0/0.0 mm, FOV = 24 × 24 cm, matrix = 64 × 64, 150 volumes. A high resolution 3D T1-weighted anatomic image was acquired in an axial orientation using a spoiled gradient-recalled sequence covering the entire brain.

Data processing

Data preprocessing

The first 10 volumes of each subjects rest data were discarded to allow longitudinal magnetization to reach a steady state and for participants to get used to the scanning environment. Preprocessing of the fMRI datasets included standard slice timing, realignment, normalization (voxel size [3, 3, 3]), smoothing (full-width half maximum, FWHM [4, 4, 4]), archived by using DPARSF based on SPM8 and REST (Chao-Gan & Yu-Feng, 2010; Song et al., 2011) (http://www.restfmri.net, http://www.fil.ion.ucl.ac.uk/spm/). Participants with head motion greater than 3 mm or 3 degrees in any of the six parameters (x, y, z, pitch, roll, yaw) were excluded.

Regional homogeneity measurement. ReHo analysis was performed for each subject by calculating Kendall coefficient of concordance (KCC) of the time series of a given voxel with those of its nearest neighbors in all directions on

a voxel-wise basis (here, 26 voxels). The KCC is calculated in a voxel-wise manner as follows (Zang et al., 2004):

$$W = \frac{\sum (R_i)^2 - n(\bar{R})^2}{\frac{1}{12}K^2(n^3 - n)}$$

where is KCC among time series of given voxels, ranging from 0 to 1; is the sum rank of the time point; where $\bar{R} = ((n+1)K)/2$ is the mean of the R_is; K is the number of time series within a measured cluster and n is the number of ranks (our study had 150 total volumes in which 10 were discarded, making n = 140). This procedure was automatically implemented by DPARSF.

Group analyses of ReHo

All images from the four right mTLE were flipped (using the spm_flip utility from SPM2) to be left mTLE allowing for observing results from group ReHo changes to be explained as either ipsilateral or contralateral to left mTLE. This is possible because this group of patients had no identifiable structural MRI abnormality other than the hippocampal sclerosis The advantages of the approach include both maximizing sample size and examining unilateral changes with smaller sample size (left side mTLE, n = 10; Blumenfeld et al., 2009).

One-sample t-test. To observe which brain regions have significantly higher ReHo value than global mean in each group, one-sided and one-sample *t*-tests were performed to generate the T maps in both patients and healthy control groups separately.

Two-sample t-test. Voxelwise two sample *t*-tests were employed to compare the ReHo results between patients and controls, using the statistical analysis tool in REST software based on SPM8.

For both one-sample and two-sample *t*-tests, a cluster of >10 voxels with p-value < 0.001 (corrected) was considered significant.

Results

Within group

Results from one-sample *t*-test showed a specific spatial pattern and intensity of significant increased ReHo for both healthy controls and patients with mTLE, as shown in Fig. 1. In the healthy control group, significantly increased ReHo was found mainly in the default mode network (DMN) (including precuneus, posterior cingulate gyrus [PCC], bilateral inferior lateral parietal, and mesial prefrontal cortex [MPFC]), bilateral visual cortex, and cerebellum.

In the mTLE patient group, increased ReHo was observed mainly in the DMN and the visual cortex. However, compared to the healthy controls, patients showed a decrease in size and intensity of ReHo in these regions. As compared to the healthy controls, the most prominent difference observed was that in the patient group ReHo was significantly decreased in the cerebellar region and no significant increased ReHo was seen in the MPFC.

Between groups

A two-sample *t*-test was performed to examine differences between patients with mTLE and healthy controls as can be seen in Fig. 2 and Tables 1 and 2. Patients showed robust and increased ReHo in comparison to healthy controls in the ipsilateral (left side) parahippocampal gyrus (Fig. 3), midbrain, insula (mainly anterior division), rolandic operculum, putamen, and corpus callosum. Significant increased ReHo was also seen in bilateral parietal and frontal subcortical white matter and bilateral precentral and postcentral gyri. Significantly decreased ReHo was seen mainly in DMN (prominently in PCC, precuneus, MPFC) and cerebellum (prominently in the right side).

Discussion

The present study demonstrated the alterations of regional synchronization in patients with mTLE-HS using ReHo analysis during the interictal period. The main findings are as follows: Significant differences were observed in the spatial pattern and intensity of ReHo in the two groups; compared to the healthy control group, ReHo increased significantly in patients in the ipsilateral (left side) parahippocampal gyrus, midbrain, insula, corpus callosum, bilateral sensorimotor cortex, and frontoparietal subcortical structure, whereas decreased ReHo was observed in DMN and cerebellum in patients' group. These findings are discussed further below.

Large numbers of studies have suggested that low frequency fluctuations of BOLD signal in resting state reflect spontaneous fluctuations in brain physiology and metabolism at baseline (Fox & Raichle, 2007). Resting state BOLD fMRI is considered as an imaging marker of brain function in the absence of any external stimuli. ReHo, which measures the synchrony of different brain regions, is one of such indices of RS-fMRI, which could be considered as an imaging marker of brain function.

Increased ReHo

Growing evidence, from both animal model and human studies, suggests that there is an increased synchronization in the epileptogenic zone during seizures and interictal state (Bettus et al., 2008; David et al., 2008). Intracranial EEG studies have also shown increased regional synchronization of electrophysiologic signals during interictal mesial temporal lobe seizures (Ponten et al., 2007; Ortega et al., 2008). In our study, the most robust increased ReHo was observed to be in the parahippocampus, suggesting this region has the highest synchronization and leading us to infer that the parahippocampus is an epileptogenic zone in the mTLE. Of

COLOR

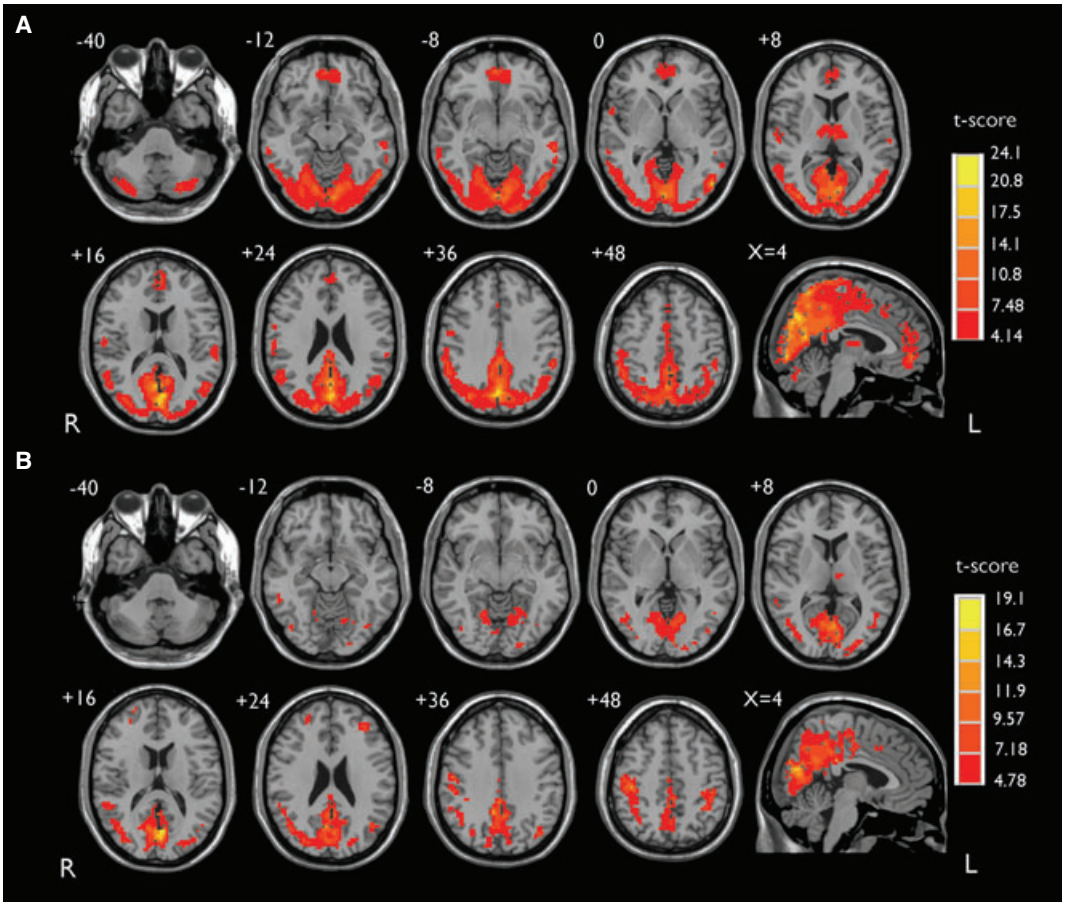


Figure 1.
Results of ReHo map across the (**A**) healthy controls and (**B**) patients with mTLE (One sample *t*-test; $p < 0.001$, voxels >10, with FWE correction). Color scale indicates increased ReHo value.
Epilepsia © ILAE

11

interest, Morgan et al. (2010) used 2D-TCA to analyze 14 ~~Rs-fMRI~~ data of the same type of patients and also found strong activation in the parahippocampus, similar to the observed activity in our study. The above findings suggest that the ReHo method may have the potential to detect the epileptogenic zone.

In the present study, the second area of significant increased ReHo was observed in the midbrain. Increased cerebral blood flow (CBF) was noted in midbrain in patients with mTLE-HS >90 s after onset of temporal lobe complex seizures in a study utilizing single photon emission computed tomography (SPECT) (Blumenfeld et al., 2004). Another SPECT study also found increased CBF in midbrain in postictal phase, and suggested that midbrain was one of the important nodes of the epileptic network (Blumenfeld et al., 2009). The results of our study demonstrated increased synchronization in midbrain, which provides further evidence that midbrain is one of the important nodes in the mTLE network. To further examine the role of the mid-brain in the seizure network, we arranged the significant regions identified in this study based on their ReHo values. The midbrain with intermediate ReHo values occupies an intermediate position in the hierarchy of regions, suggesting the midbrain may play the role of an "intermediate station" in epilepsy propagation.

Results also showed increased ReHo in the ipsilateral insula (mainly anterior division). Several neuroimaging studies have reported activity in the insula in patients with epilepsy (e.g., Bouilleret et al., 2002; Morgan et al., 2010). Moreover, positron emission tomography (PET) study also showed the ipsilateral insula involved in mTLE (Bouilleret et al., 2002). The insula is a complex structure, which has been implicated in several higher order cognitive functions such as in saliency, switching, attention, and control network, including regions that subserve motor and somatosensory functions (Menon & Uddin, 2010). Evidence from these studies collectively suggests that the insular cortex plays an essential role in seizure propagation in patients with TLE.

COLOR

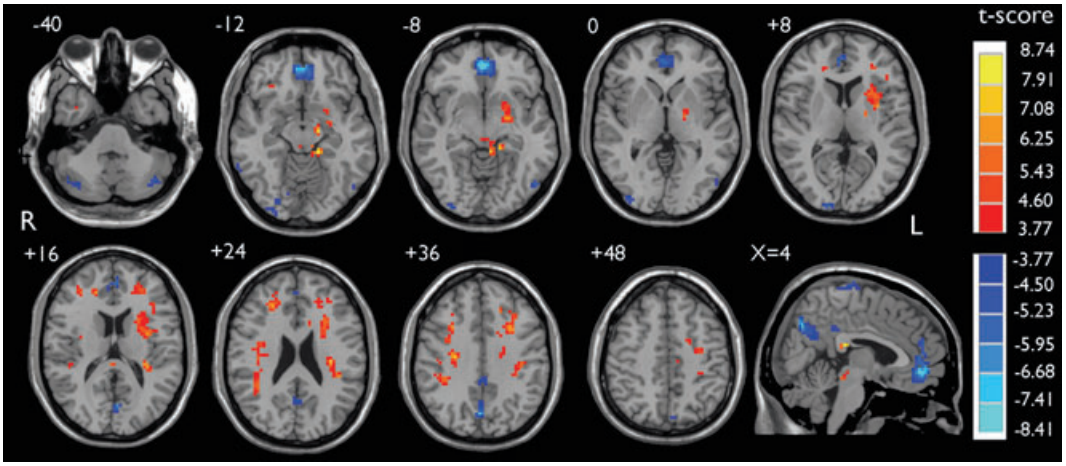


Figure 2.
Statistic *t*-map showing the difference between the mTLE group and healthy control (two sample *t*-test, $p < 0.001$, voxel >10). Warm colors indicate mTLE >healthy control, whereas cool colors indicate healthy >mTLE.
Epilepsia © ILAE

A significant increased ReHo cluster was found in the corpus callosum. Corpus callosum connects the two hemispheres of the brain, thereby allowing for interhemispheric communication, but also contributes to the spread of seizure impulses from one side of the brain to the other. A corpus callosotomy interrupts the spread of seizures from one hemisphere to the other hemisphere, and eventually helps improve the patients quality of life (Cukiert et al., 2009; Liang et al., 2010). Although BOLD signal associated with neuronal activation is thought to be mainly gray-matter dependent, there is evidence from other studies that increased ReHo can also be seen in white matter (Paakki et al., 2010; Zhong et al., 2011). Increased ReHo reflects increased synchronization, which presumably may occur in both gray and white matter based on these studies. ReHo shows increased synchrony in corpus callosum, which also suggests that it is part of the epileptogenic network. Because the ReHo value of corpus callosum, which reflects the synchrony, is between the insula, rolandic operculum, and frontal subgyral white matter (submotor cortex), we infer that corpus callosum might be an intermediate node for epilepsy propagation between insula, rolandic operculum, and frontal subgyral white matter.

Table 1. Significant increased ReHo cluster between patients with mTLE and healthy controls (patients-HC), $p < 0.001$, voxel >10

Brain region	H	Peak MNI coordinate X	Y	Z	Cluster voxels	Peak *t* value
Parahippocampal	L	−15	−36	−12	47	6.19
Midbrain	L	−15	−15	−12	69	6.02
Insula, putamen, middle frontal gyrus	L	−27	27	30	410	5.91
Rolandic operculum	L	−33	−33	18	76	5.87
Corpus callosum splenium	R	3	−30	18	27	578
Frontal subgyral white matter	R	27	33	27	106	5.57
12 Sub-pos-precentral gyral white matter	L	−30	−33	42	35	5.50
Pallidum	L	−20	−6	0	20	5.49
Frontal subgyral white matter	R	27	−15	36	35	5.39
Subfrontal/parietal white matter	R	42	−45	27	236	5.37
Paracentral_Lobule/Supp_Motor_Area	L	−9	−18	66	17	5.06
Middle and superior temporal gyri	R	39	3	−36	14	5.04
Postcentral gyrus	L	−33	−30	44	22	5.43
Precentral gyrus	R	30	−12	57	13	4.69
Postcentral gyrus	R	27	−30	66	16	4.61
Precentral gyrus	L	−31	−14	46	18	4.53

H, hemisphere; mTLE, mesial temporal lobe epilepsy.

Table 2. Significant decreased ReHo cluster between patients with mTLE and healthy controls (patients-HC), p < 0.001, voxel >10

Brian region	H	Peak MNI coordinate			Cluster voxels	Peak *t* value
		X	Y	Z		
MPFC	R/L	3	48	−9	262	−8.14
Precuneus, PCC	R/L	3	−72	36	125	−7.51
Inferior occipital gyrus	L	−51	−72	−6	23	−6.22
Cuneus	L	−3	−75	15	17	−6.05
Cerebellum posterior lobe	R	36	−66	−24	120	−5.97
Cuneus	R	15	−100	6	16	−5.91
Precuneus	L	−3	−66	69	28	−5.74
Cerebellum posterior lobe	R	48	−51	−54	31	−5.53
Cerebellum posterior lobe	L	−39	−72	−39	26	−5.11
Inferior temporal gyrus	R	66	−45	−18	11	−4.76

H, hemisphere; mTLE, mesial temporal lobe epilepsy; MPFC, mesial prefrontal cortex; PCC, posterior cingulate cortex.

Both parietal and frontal subcortical white matter and ipsilateral putamen are involved with increased ReHo. The emerging evidences suggest that subcortical structures may play a critical role in the propagation and behavioral manifestations of human epileptogenic seizures (Norden & Blumenfeld, 2002). Spencer summarized that subcortical structures are key to the manifestation of partial seizures, supporting the contention that specific subcortical regions are part of specific epileptogenic networks (Spencer, 2002). It is generally accepted that the thalamus is an important transfer station for seizure propagation, especially in generalized epilepsy (Tyvaert et al., 2009). However, both our study and Mankinens studies did not find significant increased ReHo in bilateral thalami in TLE compared to healthy controls (Mankinen et al., 2011). Of interest, Wang et al. (2011) reported increased ReHo in bilateral thalami in generalized tonic–clonic seizures. The difference between our result and that of Wang et al. may be due to the different type of epilepsy patients. The lack of increased ReHo in this region in our study and Mankinens study suggests that in patients with mTLE, the thalamus may not be an important node for this type of seizure propagation. Our study showed increased ReHo in bilateral precentral and postcentral gyri. Electrophysiologic evidence from animal models of epilepsy demonstrates increased synchronization in these regions, supporting our findings (David et al., 2008).

COLOR

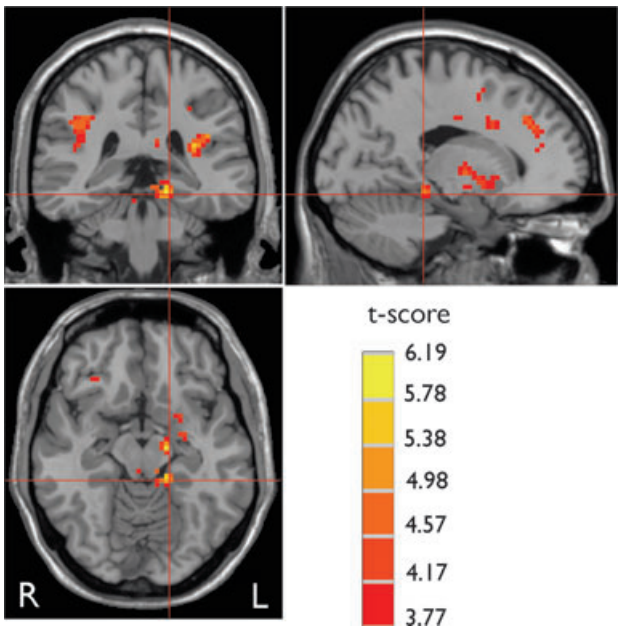


Figure 3.
Statistic *t*-map show the strongest increased of ReHo in parahippocampus (two sample *t*-test, p < 0.001, voxel >10). Warm color indicates increased ReHo in mTLE.
Epilepsia © ILAE

Compared to the healthy controls, the result of one sample *t*-test showed reduced area of ReHo in visual cortex in mTLE group. Of interest, Yan et al. (2011) found reduction of ReHo in visual cortex in older adult group comparison with young adults. Reductions of ReHo have been reported in older adult subjects and patients with Parkinsons and Alzheimers disease (He et al., 2007; Wu et al., 2007, 2009). This suggests that reduced synchronized activity in the visual cortex may be a common change across the life-span and in the disease state.

Decreased ReHo

Results from the one sample *t*-test in our study demonstrated the altered spatial and intensity pattern of ReHo in DMN in mTLE patients. Differing from the typical ReHo pattern in DMN in the healthy controls, absence of increased ReHo in MPFC, and predominantly decreased ReHo in PCC and precuneus were the main characteristics in the mTLE patients. Moreover, group comparisons with two sample *t*-test confirmed decreased ReHo in the DMN and cerebellum.

Deactivation or suspension of the DMN activity in epilepsy has been reported by previous studies (Archer et al.,

2003; Gotman et al., 2005). In contrast, Mankinen et al. (2011) reported significantly increased ReHo in the PCC in pediatric patients with nonlesion TLE. However, Archer et al. (2003) reported deactivation in PCC during generalized spike and slow-wave discharges. It is widely accepted that exogenous or endogenous stimulation of the brain will interrupt the resting state and cause the deactivation and suspension of the DMN. Based on the above theory, the interictal activity could be considered as internal stimulation, which may decrease or interrupt the DMN. The contrary result between our study and Mankinens study may be due to differences in patients' profiles, with adult patients with mTLE-HS in our study and pediatric patients with nonlesion temporal lobe epilepsy in Mankinens study; however, further studies are required to confirm the current findings.

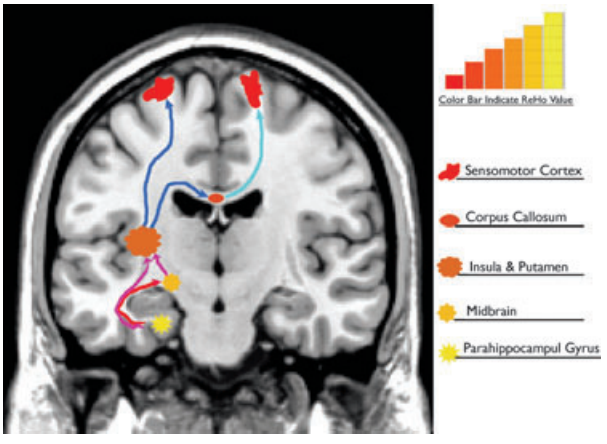
It has been repeatedly shown that the DMN is one of the most important networks of resting-state network, which maintains the baseline brain activities related to self-awareness, episodic memory, and interactive modulation between internal mental activities and external tasks (Fox et al., 2005; Buckner et al., 2008). MPFC has been associated with cognitive operations and emotional processes. PCC and precuneus are recognized as the most salient nodes in DMN (Jiao et al., 2011). Reduced synchronization (as measured by ReHo) in DMN suggests reduced network connection in this region. So, we speculated that decreased ReHo in the DMN of mTLE patients may be due to the long-term injurious effects of epileptic activity, which may eventually cause DMN functional impairments.

Bilateral posterior cerebellum showed decreased ReHo in mTLE group compared to the healthy control group, prominently in the contralateral side of the temporal lesion (right side). Mankinen et al. (2011) also found decreased ReHo in cerebellum, but in cerebellar culmen not in the posterior lobe. In contrast, Zhong et al. (2011) revealed increased ReHo in bilateral cerebellum in generalized tonic–clonic seizure during interictal seizures. This significant difference may be due to the different types of seizures. Van Paesschen et al. (2003) also found hypoperfusion in contralateral posterior cerebellum lobe using SPECT, which is consistent with our finding. This phenomenon was called "crossed cerebellar diaschisis," and was considered as an indication of disconnection of the glutamatergic corticopontocerebellar tracts (Nelissen et al., 2006). Because one of the main functions of cerebellum is motor coordination, it is possible that reduced ReHo could be a sign of decreased motor coordination.

In summary, these findings provide additional evidence to support epileptogenic network theories based on a relatively novel method, the Regional Homogeneity method applied to resting state fMRI data. The regions showing alterations of ReHo in patients with mTLE could be considered as part of an organized network. Based on the ReHo values observed in this study, we propose that abnormal increased ReHo (reflecting increased spontaneous synchronization amongst regions) in the hippocampus, midbrain, insula, and frontoparietal subcortical structure comprise a network, which might be responsible for the seizure genesis and propagation (Fig. 4). The regions showing abnormal decreased ReHo, specifically the visual cortex, cerebellum, and DMN, may be a part of a network of regions that are functionally impaired, leading to impairments in vision, motor coordination, and cognition, respectively, which is frequently reported in these patients.

Limitations

There are several limitations of this study. First, the sample size is modest, which can reduce sensitivity and accuracy of our results, so further work is needed to confirm our findings. Second, in order to increase the sample size and make a homogeneous group, we flipped the ReHo maps for four right-sided mTLE patients. Blumenfeld used the same method to study cortical and subcortical networks in patients with secondarily generalized tonic–clonic seizure (Blumenfeld et al., 2009). Although the possibility is minimal, flipping the ReHo maps may lead to some uncertainty in the results. Third, we did not have neuropsychological evaluations in these patients for correlation analysis of behavior with brain activity. Finally, the lack of simultaneous fMRI-EEG in this study limits our ability to confirm our ReHo result of increased ReHo regions in descending order representing a pathway for epileptiform discharge propagation.



COLOR

Figure 4.
Hypothesized epileptogenic network of seizure propagation for mTLE-HS based on ReHo value in this study. The decreasing order of significant ReHo values noted from parahippocampal gyrus to midbrain to insula/putamen to corpus callosum to frontal white matter (ipsilateral and contralateral) to sensorimotor cortex as shown in Table I suggests a directionality in terms of seizure origination and propagation through this network.
Epilepsia © ILAE

Conclusions

We used ReHo, one of the indices of the RS-fMRI, which measures the synchrony of local brain region, to study the mTLE-HS patients by comparing them to healthy controls. Altered (both increased and decreased) regional homogeneity in the resting state networks of mTLE-HS patients were found. Decreased ReHo in several areas were found, which may correspond to wide functional impairments found in these patients. We propose that the increased ReHo regions that were found may compose a network that is responsible for seizure genesis and propagation. This method may have the potential ability of detecting epileptogenic zone or network in seizure patients.

Acknowledgment

The project described was supported by the Clinical and Translational Science Award (CTSA) program, previously through the National Center for Research Resources (NCRR) grant 1UL1RR025011, and now by the National Center for Advancing Translational Sciences (NCATS), grant 9U54TR000021. The project described was supported by Award Number R25GM083252 from the National Institute of General Medical Sciences. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute of General Medical Sciences or the National Institutes of Health. We acknowledge Benjamin J. Swan for his help in exporting and converting the functional image data of some subjects.

Disclosure

We declare that we have no conflict of interest. We confirm that we have read the Journals position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

Reference

Archer JS, Abbott DF, Waites AB, Jackson GD. (2003) fMRI "deactivation" of the posterior cingulate during generalized spike and wave. *Neuroimage* 20:1915–1922.

Beckmann CF, DeLuca M, Devlin JT, Smith SM. (2005) Investigations into resting-state connectivity using independent component analysis. *Philos Trans R Soc Lond B Biol Sci* 360:1001–1013.

Berg AT. (2008) The natural history of mesial temporal lobe epilepsy. *Curr Opin Neurol* 21:173–178.

Berg AT, Berkovic SF, Brodie MJ, Buchhalter J, Cross JH, van Emde Boas W, Engel J, French J, Glauser TA, Mathern GW, Moshe SL, Nordli D, Plouin P, Scheffer IE. (2010) Revised terminology and concepts for organization of seizures and epilepsies: report of the ILAE Commission on Classification and Terminology, 2005–2009. *Epilepsia* 51:676–685.

Bettus G, Wendling F, Guye M, Valton L, Regis J, Chauvel P, Bartolomei F. (2008) Enhanced EEG functional connectivity in mesial temporal lobe epilepsy. *Epilepsy Res* 81:58–68.

Biswal B, Yetkin FZ, Haughton VM, Hyde JS. (1995) Functional connectivity in the motor cortex of resting human brain using echo-planar MRI. *Magn Reson Med* 34:537–541.

Blumenfeld H, McNally KA, Vanderhill SD, Paige AL, Chung R, Davis K, Norden AD, Stokking R, Studholme C, Novotny EJ, Jr., Zubal IG, Spencer SS. (2004) Positive and negative network correlations in temporal lobe epilepsy. *Cereb Cortex* 14:892–902.

Blumenfeld H, Varghese GI, Purcaro MJ, Motelow JE, Enev M, McNally KA, Levin AR, Hirsch LJ, Tikofsky R, Zubal IG, Paige AL, Spencer SS. (2009) Cortical and subcortical networks in human secondarily generalized tonic–clonic seizures. *Brain* 132:999–1012.

Bouilleret V, Dupont S, Spelle L, Baulac M, Samson Y, Semah F. (2002) Insular cortex involvement in mesiotemporal lobe epilepsy: a positron emission tomography study. *Ann Neurol* 51:202–208.

Buckner RL, Andrews-Hanna JR, Schacter DL. (2008) The brains default network: anatomy, function, and relevance to disease. *Ann N Y Acad Sci* 1124:1–38.

Cao Q, Zang Y, Sun L, Sui M, Long X, Zou Q, Wang Y. (2006) Abnormal neural activity in children with attention deficit hyperactivity disorder: a resting-state functional magnetic resonance imaging study. *NeuroReport* 17:1033–1036.

Chao-Gan Y, Yu-Feng Z. (2010) DPARSF: a MATLAB Toolbox for "Pipeline" data analysis of resting-state fMRI. *Front Syst Neurosci* 4:13.

Cukiert A, Burattini JA, Mariani PP, Cukiert CM, Argentoni-Baldochi M, Baise-Zung C, Forster CR, Mello VA. (2009) Outcome after extended callosal section in patients with primary idiopathic generalized epilepsy. *Epilepsia* 50:1377–1380.

David O, Guillemain I, Saillet S, Reyt S, Deransart C, Segebarth C, Depaulis A. (2008) Identifying neural drivers with functional MRI: an electrophysiological validation. *PLoS Biol* 6:2683–2697.

Detre JA. (2006) Clinical applicability of functional MRI. *J Magn Reson Imaging* 23:808–815.

Di Bonaventura C, Vaudano AE, Carni M, Pantano P, Nucciarelli V, Garreffa G, Maraviglia B, Prencipe M, Bozzao L, Manfredi M, Giallonardo AT. (2006) EEG/fMRI study of ictal and interictal epileptic activity: methodological issues and future perspectives in clinical practice. *Epilepsia* 47(Suppl. 5):52–58.

Fox MD, Raichle ME. (2007) Spontaneous fluctuations in brain activity observed with functional magnetic resonance imaging. *Nat Rev Neurosci* 8:700–711.

Fox MD, Snyder AZ, Vincent JL, Corbetta M, Van Essen DC, Raichle ME. (2005) The human brain is intrinsically organized into dynamic, anticorrelated functional networks. *Proc Natl Acad Sci USA* 102: 9673–9678.

Gotman J, Grova C, Bagshaw A, Kobayashi E, Aghakhani Y, Dubeau F. (2005) Generalized epileptic discharges show thalamocortical activation and suspension of the default state of the brain. *Proc Natl Acad Sci USA* 102:15236–15240.

He Y, Wang L, Zang Y, Tian L, Zhang X, Li K, Jiang T. (2007) Regional coherence changes in the early stages of Alzheimers disease: a combined structural and resting-state functional MRI study. *Neuroimage* 35:488–500.

Jiao Q, Lu G, Zhang Z, Zhong Y, Wang Z, Guo Y, Li K, Ding M, Liu Y. (2011) Granger causal influence predicts BOLD activity levels in the default mode network. *Hum Brain Mapp* 32:154–161.

Khatamian YB, Fahoum F, Gotman J. (2011) Limits of 2D-TCA in detecting BOLD responses to epileptic activity. *Epilepsy Res* ??????;??????–??????. IS

Liang S, Li A, Zhao M, Jiang H, Meng X, Sun Y. (2010) Anterior temporal lobectomy combined with anterior corpus callosotomy in patients with temporal lobe epilepsy and mental retardation. *Seizure* 19:330–334.

Mankinen K, Long XY, Paakki JJ, Harila M, Rytky S, Tervonen O, Nikkinen J, Starck T, Remes J, Rantala H, Zang YF, Kiviniemi V. (2011) Alterations in regional homogeneity of baseline brain activity in pediatric temporal lobe epilepsy. *Brain Res* 1373:221–229.

Menon V, Uddin LQ. (2010) Saliency, switching, attention and control: a network model of insula function. *Brain Struct Funct* 214:655–667.

Morgan VL, Gore JC, Abou-Khalil B. (2007) Cluster analysis detection of functional MRI activity in temporal lobe epilepsy. *Epilepsy Res* 76: 22–33.

Morgan VL, Gore JC, Abou-Khalil B. (2010) Functional epileptic network in left mesial temporal lobe epilepsy detected using resting fMRI. *Epilepsy Res* 88:168–178.

Nelissen N, Van Paesschen W, Baete K, Van Laere K, Palmini A, Van Billoen H, Dupont P. (2006) Correlations of interictal FDG-PET metabolism and ictal SPECT perfusion changes in human temporal lobe epilepsy with hippocampal sclerosis. *Neuroimage* 32:684–695.

Norden AD, Blumenfeld H. (2002) The role of subcortical structures in human epilepsy. *Epilepsy Behav* 3:219–231.

Ortega GJ, Menendez de la Prida L, Sola RG, Pastor J. (2008) Synchronization clusters of interictal activity in the lateral temporal cortex of epileptic patients: intraoperative electrocorticographic analysis. *Epilepsia* 49:269–280.

Paakki JJ, Rahko J, Long X, Moilanen I, Tervonen O, Nikkinen J, Starck T, Remes J, Hurtig T, Haapsamo H, Jussila K, Kuusikko-Gauffin S, Mattila ML, Zang Y, Kiviniemi V. (2010) Alterations in regional homogeneity of resting-state brain activity in autism spectrum disorders. *Brain Res* 1321:169–179.

Ponten SC, Bartolomei F, Stam CJ. (2007) Small-world networks and epilepsy: graph theoretical analysis of intracerebrally recorded mesial temporal lobe seizures. *Clin Neurophysiol* 118:918–927.

Schevon CA, Cappell J, Emerson R, Isler J, Grieve P, Goodman R, McKhann G Jr., Weiner H, Doyle W, Kuzniecky R, Devinsky O, Gilliam F. (2007) Cortical abnormalities in epilepsy revealed by local EEG synchrony. *Neuroimage* 35:140–148.

Smith SM, Fox PT, Miller KL, Glahn DC, Fox PM, Mackay CE, Filippini N, Watkins KE, Toro R, Laird AR, Beckmann CF. (2009) Correspondence of the brains functional architecture during activation and rest. *Proc Natl Acad Sci USA* 106:13040–13045.

Song XW, Dong ZY, Long XY, Li SF, Zuo XN, Zhu CZ, He Y, Yan CG, Zang YF. (2011) REST: a toolkit for resting-state functional magnetic resonance imaging data processing. *PLoS ONE* 6:e25031.

Spencer SS. (2002) Neural networks in human epilepsy: evidence of and implications for treatment. *Epilepsia* 43:219–227.

Tyvaert L, Chassagnon S, Sadikot A, LeVan P, Dubeau F, Gotman J. (2009) Thalamic nuclei activity in idiopathic generalized epilepsy: an EEG-fMRI study. *Neurology* 73:2018–2022.

Van Paesschen W, Dupont P, Van Driel G, Van Billoen H, Maes A. (2003) SPECT perfusion changes during complex partial seizures in patients with hippocampal sclerosis. *Brain* 126:1103–1111.

Wang Z, Lu G, Zhang Z, Zhong Y, Jiao Q, Tan Q, Tian L, Chen G, Liao W, Li K, Liu Y. (2011) Altered resting state networks in epileptic patients with generalized tonic–clonic seizures. *Brain Res* 1374:134–141.

Wu T, Zang Y, Wang L, Long X, Li K, Chan P. (2007) Normal aging decreases regional homogeneity of the motor areas in the resting state. *Neurosci Lett* 423:189–193.

Wu T, Long X, Zang Y, Wang L, Hallett M, Li K, Chan P. (2009) Regional homogeneity changes in patients with Parkinsons disease. *Hum Brain Mapp* 30:1502–1510.

Yan L, Zhuo Y, Wang B, Wang DJ. (2011) Loss of coherence of low frequency fluctuations of BOLD FMRI in visual cortex of healthy aged subjects. *Open Neuroimag J* 5:105–111.

Zang Y, Jiang T, Lu Y, He Y, Tian L. (2004) Regional homogeneity approach to fMRI data analysis. *Neuroimage* 22:394–400.

Zhong Y, Lu G, Zhang Z, Jiao Q, Li K, Liu Y. (2011) Altered regional synchronization in epileptic patients with generalized tonic–clonic seizures. *Epilepsy Res* 97:83–91.

Author Query Form

Journal: EPI
Article: 12066

Dear Author,
During the copy-editing of your paper, the following queries arose. Please respond to these by marking up your proofs with the necessary changes/additions. Please write your answers on the query sheet if there is insufficient space on the page proofs. Please write clearly and follow the conventions shown on the attached corrections sheet. If returning the proof by fax do not write too close to the paper's edge. Please remember that illegible mark-ups may delay publication.
Many thanks for your assistance.

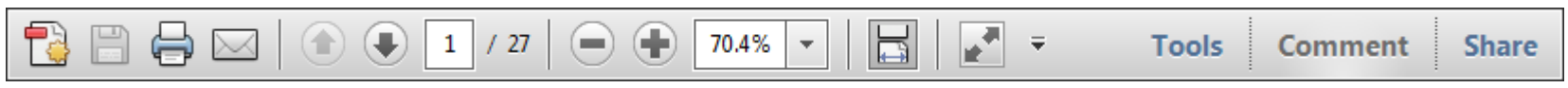
Query reference	Query	Remarks
1	**AUTHOR: change in title for readability okay?**	
2	**AUTHOR: A running head short title was not supplied; please check if this one is suitable and, if not, please supply a short title of up to 40 characters that can be used instead.**	
3	**AUTHOR: Please check heading levels.**	
4	**AUTHOR: mean age ± SD correct as added here?**	
5	**AUTHOR: written okay as added to informed consent?**	
6	**AUTHOR: Please provide missing manufacturer information here.**	
7	**AUTHOR: please spell out TR and TE**	
8	**AUTHOR: please spell out DPARSF, SPM8, REST, and any other abbreviations that appear in this article that are not already spelled out at first mention. Thank you.**	
9	**AUTHOR: Please check all website address and confirm that they are correct. (Please note that it is the responsibility of the author(s) to ensure that all URLs given in this article are correct and useable.)**	
10	**AUTHOR: Please give manufacturer information for 'REST software': company name, town, state (if USA), and country.**	
11	**AUTHOR: please spell out FEW in figure**	
12	**AUTHOR: sub-pos?-precentr**	
13	**AUTHOR: patients' groups correct as set? Or?**	
14	**AUTHOR: please clarify Rrs-fMRI**	
15	**AUTHOR: Please provide the volume number, page range for reference Khatamian et al. (2011).**	

WILEY-BLACKWELL

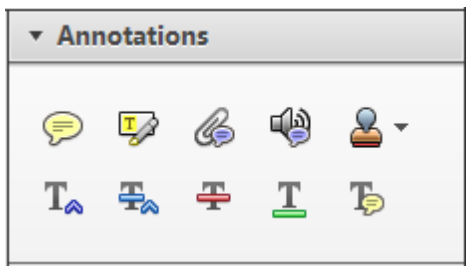
USING e-ANNOTATION TOOLS FOR ELECTRONIC PROOF CORRECTION

Required software to e-Annotate PDFs: Adobe Acrobat Professional or Adobe Reader (version 7.0 or above). (Note that this document uses screenshots from Adobe Reader X)
The latest version of Acrobat Reader can be downloaded for free at: http://get.adobe.com/uk/reader/

Once you have Acrobat Reader open on your computer, click on the Comment tab at the right of the toolbar:



This will open up a panel down the right side of the document. The majority of tools you will use for annotating your proof will be in the Annotations section, pictured opposite. We've picked out some of these tools below:

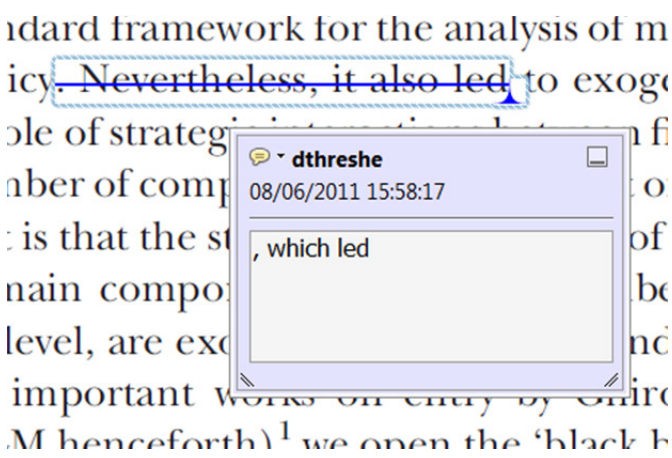


1. Replace (Ins) Tool – for replacing text.

Strikes a line through text and opens up a text box where replacement text can be entered.

How to use it

- Highlight a word or sentence.
- Click on the Replace (Ins) icon in the Annotations section.
- Type the replacement text into the blue box that appears.

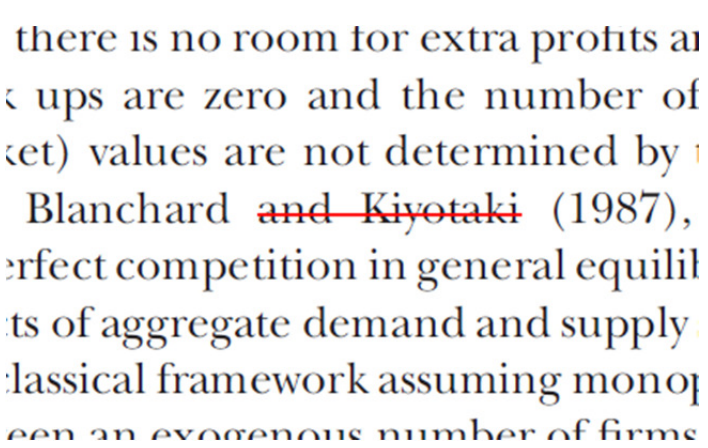


2. Strikethrough (Del) Tool – for deleting text.

Strikes a red line through text that is to be deleted.

How to use it

- Highlight a word or sentence.
- Click on the Strikethrough (Del) icon in the Annotations section.

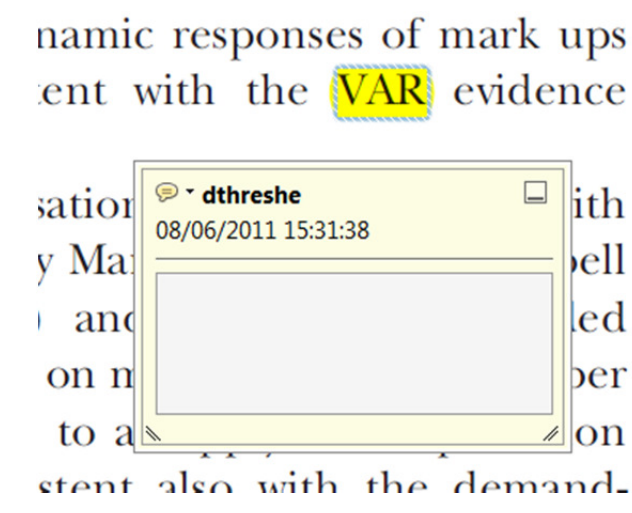


3. Add note to text Tool – for highlighting a section to be changed to bold or italic.

Highlights text in yellow and opens up a text box where comments can be entered.

How to use it

- Highlight the relevant section of text.
- Click on the Add note to text icon in the Annotations section.
- Type instruction on what should be changed regarding the text into the yellow box that appears.

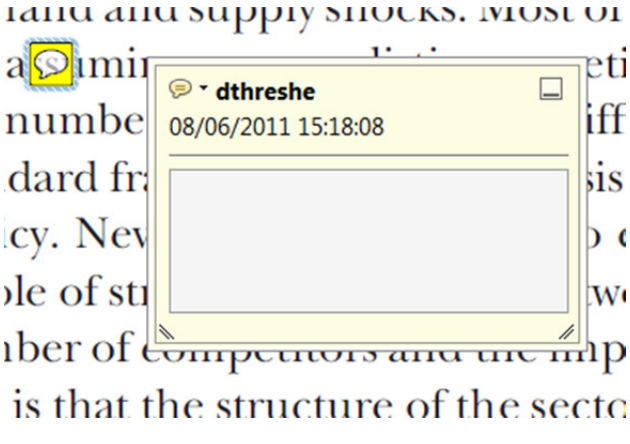


4. Add sticky note Tool – for making notes at specific points in the text.

Marks a point in the proof where a comment needs to be highlighted.

How to use it

- Click on the Add sticky note icon in the Annotations section.
- Click at the point in the proof where the comment should be inserted.
- Type the comment into the yellow box that appears.



WILEY-BLACKWELL

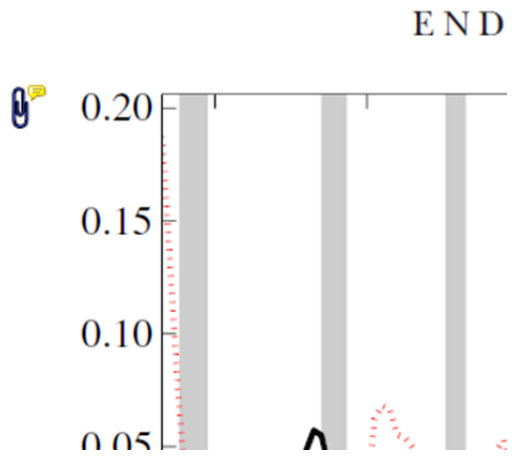
USING e-ANNOTATION TOOLS FOR ELECTRONIC PROOF CORRECTION

5. Attach File Tool – for inserting large amounts of text or replacement figures.

Inserts an icon linking to the attached file in the appropriate pace in the text.

How to use it

- Click on the Attach File icon in the Annotations section.
- Click on the proof to where you'd like the attached file to be linked.
- Select the file to be attached from your computer or network.
- Select the colour and type of icon that will appear in the proof. Click OK.

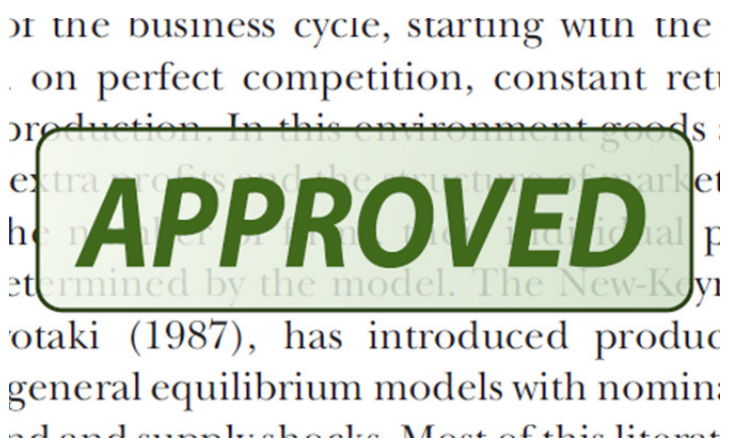


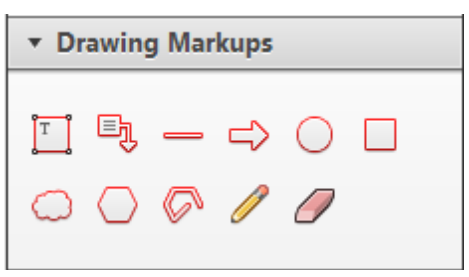
6. Add stamp Tool – for approving a proof if no corrections are required.

Inserts a selected stamp onto an appropriate place in the proof.

How to use it

- Click on the Add stamp icon in the Annotations section.
- Select the stamp you want to use. (The Approved stamp is usually available directly in the menu that appears).
- Click on the proof where you'd like the stamp to appear. (Where a proof is to be approved as it is, this would normally be on the first page).



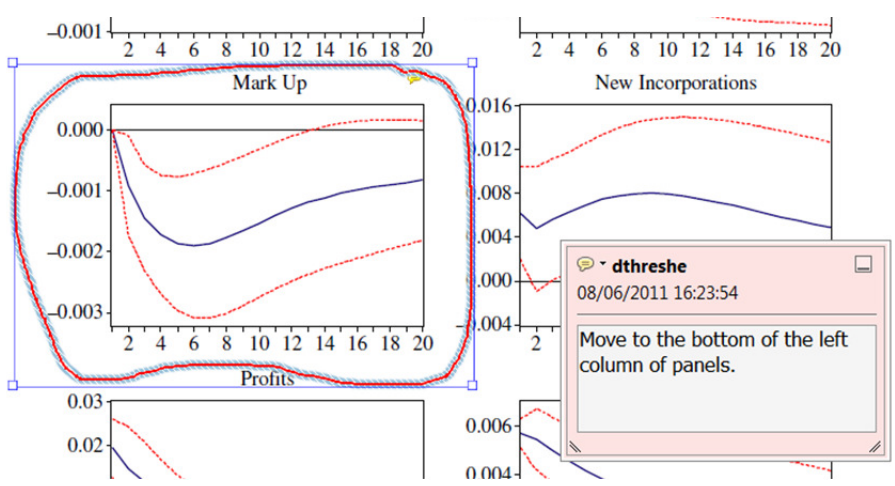


7. Drawing Markups Tools – for drawing shapes, lines and freeform annotations on proofs and commenting on these marks.

Allows shapes, lines and freeform annotations to be drawn on proofs and for comment to be made on these marks..

How to use it

- Click on one of the shapes in the Drawing Markups section.
- Click on the proof at the relevant point and draw the selected shape with the cursor.
- To add a comment to the drawn shape, move the cursor over the shape until an arrowhead appears.
- Double click on the shape and type any text in the red box that appears.



For further information on how to annotate proofs, click on the Help menu to reveal a list of further options: