

Clinical Study

Functional connectivity changes in patients with absence epilepsy studied using resting-state functional MRI

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ABSTRACT

Functional connectivity is altered in several mental disorders. We used resting-state functional MRI to examine the alterations in functional connectivity that occur in patients with absence epilepsy. We found an altered functional connectivity within and between functional modules in patients with absence epilepsy. Some brain regions had a greater number of altered connections. The functional connectivity within and between modules in absence epilepsy patients showed an increase in the number of positive connections and a decrease in the number of the negative connections. In particular, the superior frontal gyrus demonstrated both an increased number of connections with other nodes of the frontal default mode network and a decreased number of connections with the limbic system. These findings provide a new perspective and shed light on how the balance of connections within and between modules may contribute to the development of absence epilepsy.

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1. Introduction

Absence epilepsy (AE) is characterized by brief episodes of impaired consciousness, which last 2–10 s and consist of 2.5–5.0 Hz spike-and-wave complex discharges (SWD). These discharges may be generated by the oscillations between the thalamus and slightly hyperexcitable cortex and depend on long-range cortico-thalamic and cortico-cortical network interactions.¹

In addition, diffusion tensor imaging studies have revealed that there are fiber changes associated with AE in an animal model,² and these have been confirmed in human patients by the observation of increased mean diffusivity (MD) values bilaterally in the thalamus, putamen and left caudate nucleus, and increased fractional anisotropy (FA) values in the bilateral caudate nuclei. These results suggest an important role for functional connections between cortical areas and subcortical regions in AE. Additional indirect evidence to support this assumption comes from a case report of a 4.5-year-old girl with periventricular nodular heterotopia that induced typical absence seizures, probably because the heterotopia influenced the formation and excitability of the striato-thalamo-cortical network.³

Resting-state functional MRI (fMRI) can be used to measure the blood oxygen level-dependent (BOLD) signals of brain regions and is presumed to reveal spontaneous, intrinsic and functional connec-

tivity. The use of resting-state fMRI provides a good opportunity to directly explore the cortico-thalamic network in AE by examining its functional connectivity. A previous study that utilized this approach investigated the relationship between AE and the default mode network (DMN) by exploring their functional connectivity.⁴ These authors defined DMN nodes by using the posterior cingulate cortex (PCC) as a seed to determine the resting-state functional connectivity and found a decrease in functional connectivity in several links of the DMN in patients with AE when compared with control subjects. However, a limitation of this study was that only nodes in the DMN were evaluated. Therefore, any possible alterations of connections within and between different brain modules, such as attention networks or the somatosensory system, were not examined.

Instead of defining relevant functional nodes by a seeded region of interest (ROI), an approach commonly used in small-world network studies is to parcellate the brain into multiple regions or nodes by a previously defined automatic template.⁵ These nodes form distinct functional modules that can be revealed by resting-state functional connectivity and fit well with other task-involved fMRI results.⁶ In the present study, we use the predefined brain mask to study functional connectivity in patients with AE instead of defining the ROI as the seed. We found that patients with AE demonstrated an alteration in several functional links and there was also a significant divergence of functional connectivity within and between brain modules. In patients with AE, nodes in the limbic system, including the amygdala and the putamen, showed decreased functional connections to the DMN; in contrast, connections to the somatosensory system were increased. We also

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found that frontal nodes of the DMN showed increased connections within the DMN module and decreased connections to the limbic system of patients with AE.

2. Methods

2.1. Subjects

We recruited a total of 12 patients (20.0 ± 10.5 years old, between 8 and 40 years of age; five males and seven females) with AE from Xuanwu Hospital. All patients underwent a clinical MRI to examine their brain structure and long-term video electroencephalogram (EEG) monitoring. No patient exhibited any radiological abnormalities. A diagnosis of AE was established according to the classification published by the International League Against Epilepsy in 2001.⁷ These patients experienced absence seizures from 1–2 times per year to 5–10 times per day (Table 1). No patient reported an absence seizure during the MRI scans. A separate group of 14 normal subjects (24.6 ± 1.5 years old, between 22 and 28 years of age; eight males and six females) were selected as controls. All control subjects were healthy and had no history of seizures. All patients and healthy subjects gave informed consent before the experiment. The experimental procedures were approved by the Xuanwu Hospital Institutional Review Board.

2.2. Data acquisition

The fMRI scans were conducted with a Siemens Tim 3Tesla system (Siemens Healthcare, Erlangen, Germany). BOLD responses were acquired using echo-planar imaging (EPI) with the following parameters: field of view (FOV) = 192 mm, repetition time (TR) = 2000 ms, echo time (TE) = 30 ms, flip angle = 90 degrees and voxel size = $3 \times 3 \times 3.5$ mm³. The functional scans consisted of 33 axial slices that covered the whole brain, and each scan had 240 functional volumes. A rapid anatomical scan that had the same center and slice orientation as the functional scans was obtained using the magnetization-prepared rapid acquisition gradient echo (MP-RAGE) sequence with the following parameters: TR = 810 ms, TE = 2.39 ms, flip angle = 15 degrees, voxel size = $1.1 \times 0.8 \times 2$ mm³ and 144 axial slices. An anatomical scan with an iso-resolution was obtained using the same MP-RAGE sequences with different parameters: TR = 2600 ms, TE = 3.02 ms, flip angle = 8 degrees, voxel size = $1 \times 1 \times 1$ mm³ and 176 sagittal slices.

2.3. Functional connectivity

The preprocessing of the fMRI data involved a MATLAB toolbox called Data Processing Assistant for Resting-State fMRI.⁸ All 240

time points in the functional scan were included in the analysis. The data were first corrected for slice timing and head motion before being normalized into Montreal Neurological Institute space using the default EPI templates in the toolbox. The data were then smoothed with the default full width at half-maximum parameter set as 4 mm.

After detrending and filtering with a default value of 0.01–0.08 Hz, the data were parcellated into 90 cortical and subcortical regions using an already established anatomical automatic-labeling template⁵ provided with the toolbox. Nuisance covariates, including six head-motion parameters, whole brain, white matter, and cerebrospinal fluid signals, were regressed out before a calculation was made to determine the functional connectivity matrix of the 90 regions.

A 90×90 functional connectivity matrix was generated for each subject (control or patient). An independent *t*-test was performed for each entry of the functional connectivity matrix to determine the difference between the AE and control groups. A threshold of $P < 0.01$ (uncorrected) was established to generate the difference matrix, in which an entry was set to 1 if the AE group had a significantly larger functional connectivity than the control group or -1 if the functional connectivity of the AE group was significantly smaller than the control group.

The difference matrix was randomly shuffled 1000 times to apply a multi-comparison correction. The node with the maximum number of connections was selected from the shuffled matrix, and the number of maximum connections that had been established by that node was determined. The 1000 permutations generated 1000 maximum connections (Fig. 1A). The sorted results (Fig. 1B) revealed that for the difference matrix, there is an extremely small ($P < 0.05$) chance that the number of maximum connections a node may have due to random factors is ≥ 10 . Thus, a threshold of 10 was applied for correcting the multi-comparisons on the difference matrix.

2.4. Module definition and visualization

The 90 brain regions were separated into five modules: I, somatosensory, motor and auditory; II, visual processing; III, attention processing; IV, DMN; V, limbic/subcortical system. Different node colors were used for each module (Supplementary Fig. 1). The coordinates of the center of mass for each brain region were used to lay out the 90 nodes in three-dimensional (3D) space in MATLAB using a previously described method.⁴ The edges between the nodes were constructed on the basis of the differences of the correlation matrix between AE and control subjects; red edges indicate that the AE group has a larger functional connectivity than

Table 1
Clinical information collected from the patients with absence epilepsy (AE)

Patient	Age (years)	Gender	Epilepsy duration (years)	Epilepsy subtype	Medication	Reported absence seizure frequency
1	12	F	5	AE	None	1–2/day
2	37	F	20	AE + GTCS	Carbamazepine	2–3/month
3	13	M	0 ^a	AE	None	1–2/month
4	10	M	4	AE + GTCS	Valproate	10/day
5	8	F	0 ^b	AE	None	4/year
6	25	F	10	AE + GTCS	Carbamazepine	1–2/month
7	14	M	2	AE + GTCS	Carbamazepine	1–2/month
8	40	F	30	AE + GTCS	None	2/year
9	20	M	6	AE + GTCS	Carbamazepine	1–2/day
10	21	F	13	AE	None	3–5/day
11	27	F	3	AE	None	2–3/week
12	13	M	0 ^a	AE + myoclonus	None	10/day

M = male, F = female, GTCS = generalized tonic-clonic seizures.

^a 3 months.

^b 7 months.

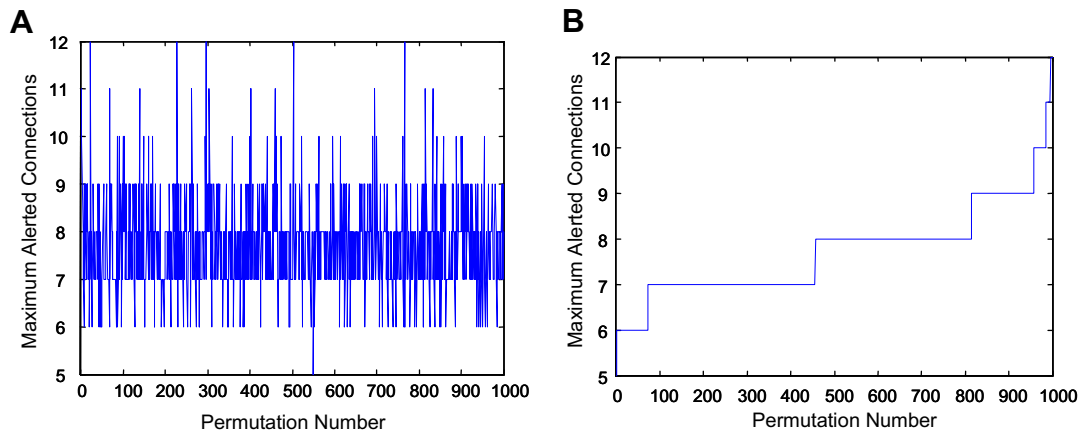


Fig. 1. The difference matrix was randomly shuffled 1000 times to apply a multi-comparison correction. (A) The 1000 permutations generated 1000 maximum connections. (B) The sorted results revealed that for the difference matrix, there is a very small ($P < 0.05$) chance that the number of maximum connections a node may have due to random factors is ≥ 10 .

normal and blue edges represent a decreased functional connectivity in the AE group.

2.5. Identification of important nodes

To examine the importance of the nodes, the number of altered connections of each region/node was examined as a node-connection distribution. The resulting histogram showed that most nodes have only one or two altered connections and that only a few nodes have multiple altered connections. The mean and standard deviation (SD) were calculated for this node-connection distribution and a threshold of mean + 2.326 SD was used to select the nodes with the greatest number of altered connections. To correct for the multi-comparison problem for this test, a 1000-round permutation procedure similar to the one used for the difference matrix was applied. However, instead of looking for the maximum number of connections a node may have from the matrix, the distribution and histogram of the node connections were generated, and the value of mean + 2.326 SD for each permutation was determined (Fig. 2A). This method allowed for a multi-comparison correction at the threshold of $P < 0.001$ to identify the most important nodes (Fig. 2B).

For each selected node (with the greatest number of altered connections), an average value of the altered functional connections within the module and between modules was calculated; this

average was calculated from the original correlation matrix (r value, instead of the binarized difference matrix) for each patient and each control subject. A mixed, repeated-measures general linear model (GLM) was used for further statistical analysis in which relation to modules (within versus between modules) was a within-subject factor and subject group (AE versus control) was a between-subject factor.

2.6. Differential connectivity

One of the frontal nodes in the DMN module, the superior frontal gyrus, showed more positive connections with other frontal nodes of the DMN (within module) in patients than in control subjects; this same area also demonstrated more negative connections to the limbic system (between modules). A differential connectivity was defined to index this divergence within and between modules as follows (Eq. 1):

$$\text{Differential connectivity} = |\text{connections to other frontal DMN nodes}| + |\text{connections to limbic system}| \quad (1)$$

Differential connectivity was also calculated for other pairs of nodes that had similar divergence between modules.

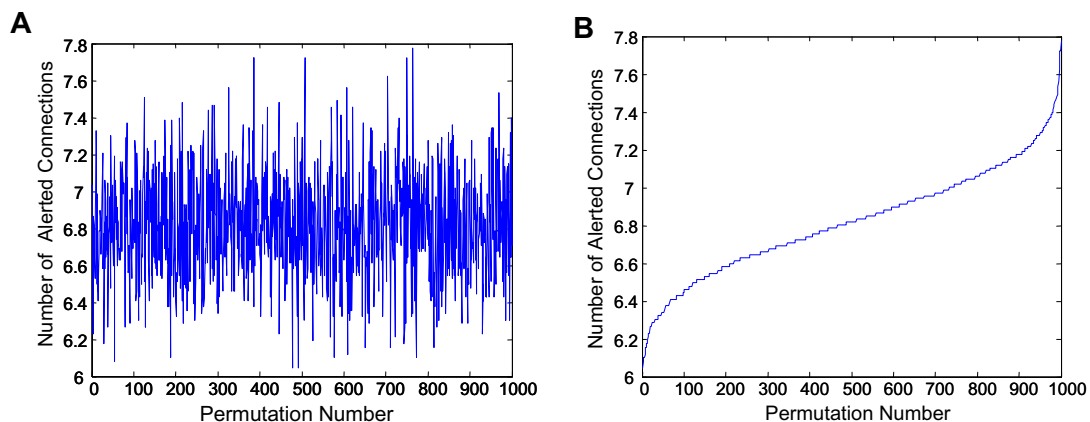


Fig. 2. Identification of important nodes. (A) The distribution and histogram of the node connections were generated, and the value of mean + 2.326 SD for each permutation was determined. (B) Multi-comparison correction at the threshold of $P < 0.001$ to identify the most important nodes.

3. Results

3.1. Altered functional connectivity between the AE group and the control group

A significantly altered functional connectivity matrix was constructed by comparing the difference between the AE group and the control group for each entry in the functional connectivity matrix with an independent *t*-test at a threshold of $P < 0.01$ (Supplementary Fig. 1). The entries in red indicate that the AE group showed a significantly larger functional connectivity than the control group in those links, while the entries in blue indicate that the AE group showed a significantly smaller functional connectivity than the control group.

To achieve a more comprehensive understanding of the results, the matrix was visualized in a three-dimensional (3D) space from both side and top views (Supplementary Fig. 2), and 90 brain region nodes were divided into five differently colored modules. An examination of both the uncorrected and the corrected results (Supplementary Fig. 2) reveals clearly that the AE group has a decreased functional connectivity between nodes in the DMN (pink nodes), specifically in nodes in frontal areas and the nodes in the limbic system (blue nodes). It is also significant that some nodes in the limbic system (the putamen and amygdala) showed increased functional connectivity to sensorimotor systems (red nodes) in the AE group. When the value of the differences in each link with a threshold $P < 0.0005$ (uncorrected) is used to emphasize the severely altered connections to the nodes, it becomes clear that the frontal DMN nodes have increased connections to frontal nodes and decreased connections to limbic nodes (Supplementary Fig. 3).

Positive correlations were observed between the superior frontal gyrus (left orbital) and the superior frontal gyrus (left medial orbital) ($R = 0.581, P = 0.048$), the superior frontal gyrus (left orbital) and the superior frontal gyrus (right medial orbital) ($R = 0.742, P = 0.006$), the precuneus (right) and the middle temporal gyrus (right) ($R = 0.710, P = 0.0096$), and the middle temporal gyrus (right) and the temporal pole of the left middle temporal gyrus ($R = 0.65, P = 0.022$); a negative correlation between the insula (right) versus the precuneus (right) ($R = -0.576, P = 0.0498$) was also observed.

3.2. Important brain regions with the highest altered functional connectivity

The number of significantly altered connections for each of the 90 brain regions is illustrated in Fig. 3A. While the number of significantly altered connections for each node ranges from 0 to 17, only a small subset of regions had more than four altered connections. A histogram of the distribution (Fig. 3B) confirmed that the number of nodes with each number of altered connections decreased until a value of six altered connections was reached. This distribution has a mean of 2.911 and a standard deviation of 3.364; therefore, a threshold of 11 altered connections was chosen to find the most important brain regions. After applying this threshold, the three most important brain regions with at least 11 altered functional connections were identified as the superior frontal gyrus (orbital part), the right amygdala and the left putamen (Supplementary Fig. 4A). It is interesting to note that the 3D graphs (Supplementary Fig. 4B, C) constructed from these three nodes and their altered connections have almost the same key features as the original altered functional connectivity matrix shown in Supplementary Figure 2.

One of the frontal DMN nodes, the orbital part of the superior frontal gyrus, showed a clear pattern of altered connections. The links to other default network nodes were stronger while links to

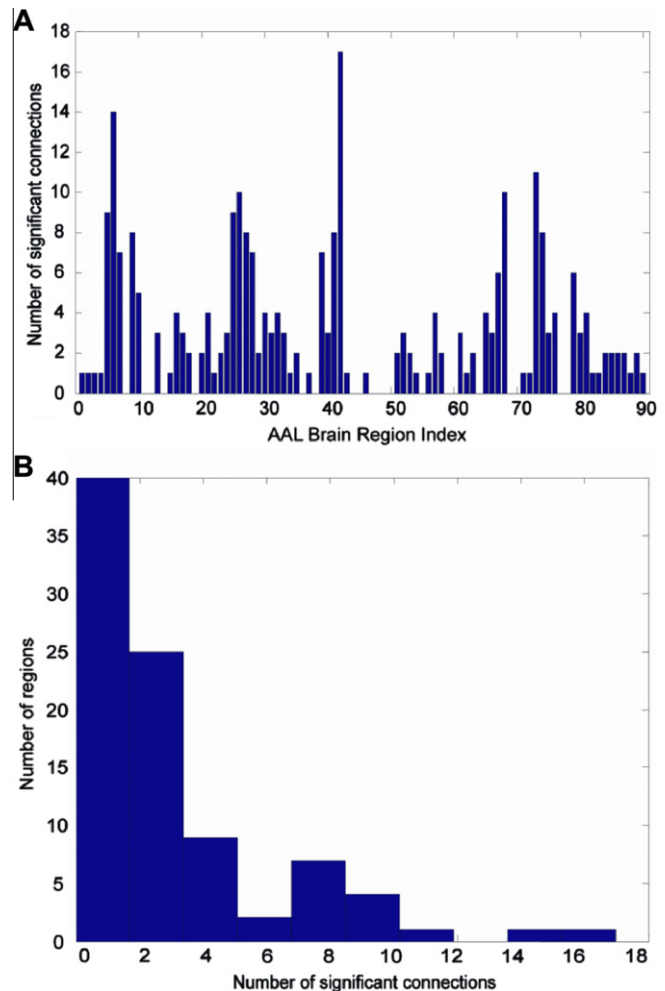


Fig. 3. Statistical characteristics of significantly altered connections for each node/region. (A) The number of significantly altered connections was determined. It is clear that among the 90 nodes, most had fewer than four significantly altered connections, while some nodes have more than 10 significantly altered connections. (B) A histogram shows the distribution of the number of significantly altered connections among the 90 nodes. Importantly, only three nodes have more than 11 significantly altered connections, and there is a bump in the distribution above six connections. AAL = Automated Anatomical Labeling.

the limbic system were weaker in the AE group when compared with the control group (Supplementary Fig. 5A, B). The average value of these connections showed that while there are positive functional connections between this node and other frontal DMN nodes in the control group, patients with AE have even more positive connections than normal; in contrast, while functional connections to the limbic system are negative or near zero in controls, patients with AE have more negative connections (Supplementary Fig. 5C). A significant interaction ($F(1,24) = 105.44, P < 0.0001$) confirmed that the changes in the connections to the DMN nodes and the limbic system occur in the opposite direction.

The two limbic system nodes (the right amygdala and the left putamen) shared the same increase in links to the sensorimotor system and the same decrease in links to DMN nodes in patients with AE (Supplementary Fig. 5D, E, G, H). An average value of these connections showed that connections to sensorimotor nodes become more positive and connections to DMN nodes become more negative in the AE group when compared with the control group (Supplementary Fig. 5F, I). Significant interactions ($F(1,24) = 35.45, P < 0.0001$ and $F(1,24) = 22.95, P < 0.0001$, respectively) confirmed these observed differences.

4. Discussion

In this study, we compared the inter-regional functional connectivity between patients with AE and controls using resting-state fMRI. We identified functional nodes/regions with severe alterations in their connection with other nodes and analyzed the divergence within and between modules for these nodes.

4.1. Altered inter-regional functional connectivity

Inter-regional functional connectivity showed that the patients with AE had stronger connections between frontal DMN nodes and other frontal nodes as well as between posterior DMN nodes and frontal/parietal nodes. These data conflict with a previous study that found decreased functional connectivity in the DMN for patients with AE.⁴ However, the current results should not be explained in the same context as the previous study. While the DMN in the previous study was defined by the functional connectivity that was seeded at the PCC and determined by combining all of the fMRI data from both patients with AE and control subjects, the brain regions/nodes in the current study were automatically defined by an anatomical template. The modules of these nodes were defined by a previous small-world network study.⁶ We believe that this approach could provide more sensitive and detailed information about the alteration in functional networks. In addition, when we considered only the links from both sides of the PCC, we found a similar decrease in connections to the right temporal cortex as was observed previously,⁴ although the links to the left frontal cortex are increased in patients with AE. The inconsistency between the current results and the previous study may indicate that different dynamics exist within the frontal and posterior DMN regions if they are parcellated into subregions. However, the brain regions/nodes revealed by the altered connections, including thalamic regions and the frontal and parietal cortices, are consistent with previous EEG/fMRI results obtained from patients with AE.

We also found an increased functional connectivity to the somatosensory system that originated primarily from the limbic system. Because somatosensory regions were not obvious regions of interest when the DMN was defined by seeding at the PCC, the current results show for the first time how the interaction between the DMN and other functional modules is altered in patients with AE. The increased connections to the somatosensory system fit well with the observation that the excitability of somatosensory behavior changes in patients with AE.⁹ If we consider the increased within-frontal DMN connections together with these increased connections from subcortical structures to the somatosensory system, these results may provide solid support for the idea that a slightly hyperexcitable cortex is one of the main factors in AE.¹⁰ The changes in SWD that were revealed by an EEG/fMRI study reflect the brain regions that are directly involved in seizure discharges,¹¹ while the current results are based on resting-state fMRI and therefore focus on alterations that occur in the absence of seizures. These functional or anatomical changes that occur during periods where patients do not exhibit seizures may contribute to our understanding of the underlying mechanism of AE and its dynamics during seizure development.

Another significant feature of the altered network is the decreased connections from the limbic system. The present study is the first to observe the functional relationship between subcortical structures and cortical areas in AE. The results are somewhat unexpected because in most EEG/fMRI studies, the SWD-triggered activation of thalamic areas is increased while the activation in cortical areas is decreased.¹² The different activation pattern observed in subcortical and cortical areas suggests that each plays a different

role in the dynamics of absence seizures. The current results show that in addition to their differing involvement in SWD dynamics, the thalamic and cortical areas have different functional dynamics even under resting-state conditions. This finding adds information to the long-debated field of SWD generation in thalamo-cortical networks¹³ by suggesting that the decreased functional connections between subcortical and cortical areas should be considered in any attempt to understand the dynamics of seizures during AE.

4.2. Divergence of within/between module connections in patients with AE

We identified three brain nodes/regions that showed a significantly higher number of altered connections after a multi-comparison correction was applied. The connections from these nodes showed almost the same patterns as the original network, suggesting that these three nodes may behave as hubs in the small-world network. More importantly, these nodes showed an increased divergence of within/between-module connections, which were defined as differential functional connectivity. This result is the first in human patients to demonstrate that the balance between functional connectivity and within/between brain modules plays a role in the development of AE. Anatomical changes that are related to AE may contribute to this divergence. This idea is supported by previous findings. Some studies have shown that the volume and concentration of gray matter increases in some cortical regions and thalamus but decreases in others,¹⁴ while other studies have revealed changes in the diffusion parameters of subcortical nuclei and a lower thalamic N-acetylaspartate to creatine (NAA/Cr) ratio in patients with AE.¹⁵ Several animal model studies may help to reconcile these results. In one study using WAG/Rij and GAERS rats, a diffusion tensor image scan revealed a decreased FA value in the anterior corpus callosum. This change was related to SWD onset, and the decrease in FA was induced by an increased perpendicular diffusivity.² Although this study did not directly demonstrate any changed fiber connections between subcortical and cortical brain areas, it suggested that fiber changes related to absence seizures do occur and that these changes are positively correlated with epilepsy discharges. After cortical stimulation, the lowest threshold was observed for the transition to the limbic type after discharges in WAG/Rij rats,¹⁶ indicating that the connection between the cortex and the limbic system has been altered to be more sensitive in these animals.

5. Conflicts of Interest/Disclosures

The authors declare that they have no financial or other conflicts of interest in relation to this research and its publication.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.jocn.2012.02.044>.

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