Imaging Neural Excitability and Networks in Genetic Absence Epilepsy Models

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17.1 Introduction

Epilepsies and epileptic syndromes are complex disease states that are accompanied by abnormal hyper excitability and/or hyper synchronicity within the central nervous system (CNS). The classifications of the International League against Epilepsy distinguish generalized from focal epilepsies (<http://www.ilae.org/visitors/centre/Definition-2014.cfm>), although the classification for some of them has been debated. Absence epilepsies are among the most well-characterized seizure types in patients and in animal models, and represent a most fascinating disease group, classically considered as prototypical generalized epilepsies.^{1–6}

Genetic rat models of absence epilepsy have been studied since the 1980s and are believed to mimic more accurately the spontaneous seizures of human epilepsy than drug-induced animal models do.^{1,7–9} The GAERS (Genetic Absence Epilepsy Rats from Strasbourg) and WAG/Rij (Wistar Albino Glaxo, originating from the city of Rijswijk) strains are two commonly used and accepted genetic models for childhood absence epilepsy. Rats of both strains are endowed with spontaneously occurring spike-wave discharges (SWDs), with frequencies from 7 to 11 Hz, amplitudes of 200 to 1,000 μ V, lasting 1 to 45 seconds, concomitant to mild facial myoclonus.^{10,11}

Historically, the GAERS colony was derived from Wistar rats and selectively bred for the seizure phenotype so that 100% of progeny spontaneously develop epilepsy. A nonepileptic control (NEC) strain was also derived from the original colony by selectively breeding for the lack of seizure expression, providing a powerful control strain, since any differences between the two strains would have a high a priori chance of being etiologically associated with the epilepsy trait.

The WAG/Rij and GAERS rats share the same electroclinical phenotype in spite of some minor differences, similar in magnitude to those observed

within the various sublines of GAERS.^{12,13} SWDs in WAG/Rij become manifest in the cortical EEG at two to three months of age. At an age of 75 days, one out of six subjects shows SWDs. At six months, all animals are affected, and both male and female rats show about 16–20 discharges per hour, with an average duration of about 5 s, which amounts to several hundred discharges per day.^{14,15}

The WAG/Rij and GAERS strains also exhibit a range of behaviors indicative of affective disturbance, such as depressive-like behavior;^{16,17} in addition, GAERS show higher anxiety levels, compared to NEC rats.¹⁸ These complex phenotypes bear some resemblance with mood disturbances observed in clinical populations.^{19–22} Interestingly, early and chronic anti-epileptogenesis treatment could prevent the depressive-like behavior.²³ Other behavioral characteristics of WAG/Rij rats include a short latency to emerge from the home cage into familiar and novel environments, low open-field defecation, and high open-field ambulation. In the same animals, under stressful circumstances, ambulation is low. They also have a low apomorphine-induced gnawing score, a high running-wheel activity, a low amount of REM sleep and interrupted non-REM-REM sleep cycle, a circadian distribution of the SWDs, good two-way, active shock-avoidance acquisition, and poor spatial reference memory in a hole-board, normal working memory scores in two spatial memory tasks, normal prepulse inhibition, but clearly abnormal auditory sensory gating.^{9,10,17,24–26}

17.2 On the Origin of Spike-wave Discharges

Outcomes of early mapping, neurophysiologic, lesion, and pharmacologic studies in the genetic models confirmed the involvement of the thalamus^{27–32} in the occurrence of SWDs. Moreover, a pacemaker role of the reticular thalamic nucleus (RTN) and a different

role of the ventral basal complex and the RTN in the occurrence of SWDs was suggested. Ibotenic lesions of the lateral thalamus, including the rostral RTN, confirmed that an intact thalamus is a prerequisite for SWD occurrence. Thalamic lesions including the caudal pole of the RTN, however, enhanced SWDs, suggesting that the rostral and caudal poles of the RTN seemed to antagonize each other sustaining SWDs.³³

The role of the cortex became more obvious after a comprehensive study of network mechanisms responsible for the immediate onset, widespread generalization, and high synchrony of SWDs in the genetic models.^{34–37} Based on field potentials simultaneously recorded from multiple cortical and thalamic sites, as well as the analyses of cortico-cortical, intrathalamic, and cortico-thalamic interrelationships between these field potentials with various network analysis techniques, a focal area in the facial somatosensory cortex was identified, followed in time by an extremely fast and large-scale synchronization across cortex and thalamus. These results are incompatible with the classical assumption that the thalamus acts as the primary driving source for the discharges.⁵ Instead, network analyses indicate that a cortical focus plays a leading role in the origin of generalized SWDs characteristic of absence seizures in the genetic absence models. The existence of a focal zone as the initiation site has been confirmed and extended in the GAERS model through the identification of hyperexcitable cells in the deep layers of the facial area of the somatosensory cortex, and by inactivation studies with tetrodotoxine.^{38,39} Since SWDs can be evoked by cortical and thalamic low frequency electrical stimulation,^{34,40,41} it seems rather likely that absence epilepsy should be understood as originating in and engaging a cortico-thalamo-cortical (CTC) network.^{36,37,42} A trigger pulse applied either within or outside the network may initiate oscillations in the tightly and reciprocally interconnected CTC network, and SWDs may occur when the brain is in an appropriate state. Reviewing the literature over the last 50 years, it can be concluded that much of the historical controversies concerning the exact mechanisms and sites of origin of the SWDs can be ascribed to the usage of different experimental models^{5,43} and that it is risky to interpret oscillations in slices as genuine SWDs.

17.3 Cortical Excitability

Evidence for an excitable focal cortical region is suggested by an increased expression of Nav1.1 and Nav1.6

selectively at the focal region in the somatosensory cortex and only in 5- to 6-month-old WAG/Rij rats.⁴⁴ Other evidence comes from neurophysiologic studies, both in vitro and in vivo. In vitro studies showed an increased excitability in the somatosensory cortex in WAG/Rij rats: NMDA-sensitive late EPSP led to action potential discharges in 44% of regular bursting cells in deep neocortical layers, vs. 8% in control rats.⁴⁵ Whole cell patch-clamp recording from layer II–III cortical pyramidal neurons in WAG/Rij rats was complemented by immunohistochemical, Western blot, and PCR studies of HCN1–HCN4 subunits of the I_h channel. The fast component of I_h activation in neurons of WAG/Rij rats showed a 50% decrease in current density, and was four times slower than in neurons of non-epileptic control Wistar rats. The results of Western blot and PCR analysis corresponded to a decreased I_h current. A 34% decrease was found in HCN1 subunit protein levels in the cerebral cortex of WAG/Rij rats as compared to Wistar rats, but HCN1 mRNA expression was not different. The protein and mRNA levels of the other three I_h channel subunits (HCN2–HCN4) were not altered.⁴⁶ The rapid age-dependent decline in expression of HCN1 channels preceded the onset of SWDs, suggesting that the loss of HCN1 channel expression is inherited rather than acquired.⁴⁷ An in vivo electrophysiologic study in free-moving animals with electrical evoked potentials in the somatosensory and motor cortex in WAG/Rij and in nonepileptic, age-matched control rats confirmed the increased excitability in the somatosensory cortex since only the epileptic rats showed an increased response toward cortical electrical stimulation. Moreover, stimulation induced SWD-like afterdischarges, suggesting not only that the focal area was more excitable, but also that the whole CTC network became more prone to SWDs.⁴⁰ It has been proposed that this loss of neocortical HCN1 function but also many neurophysiologic and biochemical changes contribute to an increased cortical excitability.⁴⁸

Besides increased excitability, impairment of inhibitory processes was found in the frontal cortex of WAG/Rij rats in vivo when they were compared to three other rat strains in a paired pulse inhibition paradigm (sensory gating). The amplitude of the auditory evoked potentials toward the second stimulus was enhanced,^{17,25} suggesting functional inhibitory disturbances in the neocortex of WAG/Rij rats. Other evidence for diminished cortical inhibition was obtained from a pharmacological study in which tiagabine, a GABA reuptake blocker, was

locally applied in the somatosensory cortex. A dose-dependent decrease in SWD occurrence was found, suggesting that decreased cortical inhibition might also play a role in the pathogenesis of absence epilepsy.⁴⁵ It is not always clear, however, whether the differences between strains are found exclusively in the perioral region of the somatosensory cortex or if other cortical regions differ between the epileptic and control rats. The latter cannot be established without using a complete high-resolution cortical grid. The electrophysiologic studies lack a sufficient spatial resolution and therefore imaging techniques might be helpful to better delineate putative focal cortical zones, and to elucidate the involvement of and changes in wider brain regions than the classically assumed cortex and thalamus. In the next paragraph, first the structural imaging data will be reviewed, followed by some new MRI data collected in WAG/Rij rats. Next, fMRI studies will be reviewed, both regarding the interictal state in the absence models, as well as regarding the hemodynamic response in parallel with the occurrence of SWDs.

17.4 Imaging Absence Epilepsy

17.4.1 Structural MRI Studies

In a structural volumetric analysis (T2-w MRI, 4.7 T) of several brain structures in 14-week-old epileptic GAERS ($n = 11$) and NEC rats, all GAERS rats were reported to display SWDs.⁴⁹ It was found that the somatosensory cortex was thicker in GAERS than in NEC rats. Furthermore, the analysis of ROIs showed larger brain structures bilaterally, including the amygdalae, cortices, and cerebral ventricles in GAERS relative to NEC animals. The differences were not generalized, as no differences were observed in other regions, such as the striatum. On the other hand, GAERS rats showed also a hippocampal volume loss, undetectable with classical analyses, but observed with high-dimensional mapping, a postacquisition analysis technique that is sensitive to subtle changes in shape of the structure. The enlarged cortical width and volume could be explained by aberrant neuronal branching and arborization in this area, such as that described in the WAG/Rij model.⁵⁰

Diffusion tensor imaging (DTI), a method based on the detection of water diffusion in biological tissues, allows characterization of long-range white

matter networks and their modifications with neuropathology and treatment.⁵¹ WAG/Rij rats at two different developmental stages (1.7 and 8 months) were studied before and after the onset of epilepsy to identify DTI changes related to epileptogenesis, compared to age-matched nonepileptic (control) Wistar rats.⁵² The results were compared with 4.2-month GAERS and NEC rats to determine the specificity of these changes. Adult WAG/Rij exhibited a localized decrease in FA (fractional anisotropy, a measure of structural integrity of white matter) in the anterior part of the corpus callosum, compared to controls. The decreased FA in the anterior corpus callosum was not seen in young WAG/Rij rats before the onset of SWDs. Also GAERS exhibited a marked decrease in FA in the anterior corpus callosum vs. age-matched NEC. This decrease was more extensive than in WAG/Rij rats. Symptomatic WAG/Rij and GAERS also have an increased perpendicular diffusivity ($\lambda_{\perp}$) in the anterior corpus callosum, which could be the cause of the reduced FA observed in the epileptic animals. Tractography was used to identify the white matter pathways of the anterior corpus callosum; it showed that the fibers interconnect the facial region of the somatosensory cortex between the two hemispheres. All this suggests that SWDs lead to microstructural changes in white matter pathways interconnecting the regions where the SWDs have their site of origin. These *ex vivo* DTI results in both absence epilepsy models⁵² are an important step for understanding neurological difficulties in children suffering from absence epilepsy, suggesting that white matter abnormalities could contribute to chronic dysfunction in what has classically been considered exclusively a gray matter disorder.

Since treatment of WAG/Rij rats with ethosuximide (ESX) initiated before the developmental onset of SWDs and continued through adulthood was found to suppress seizures even 3 months after the medication was suspended,⁵³ the possibility that early and sustained blockade of SWDs by early and chronic administration of ESX could prevent white matter alterations was investigated by means of *ex vivo*, post-treatment DTI.⁵⁴ Cortical excitability in the form of electrical evoked potentials, but also CTC network activity by measuring SWDs and by electrical stimulation-induced 8 Hz afterdischarges, and depressive-like behavior were investigated. Four months of treatment with ESX suppressed SWDs during treatment, and 6 days and 2 months posttreatment. Also the

duration of afterdischarges was reduced 6 days post-treatment. Increased FA in corpus callosum and internal capsula on DTI was found, an increased amplitude of the evoked potential (P8, a positive component with a latency of 8 ms), and a decreased immobility in the forced swim test, the latter suggesting an antidepressant-like response. Shorter treatments with ESX had no large effects on any of the above parameters. Chronic ESX has widespread effects not only within but also outside the circuitry in which SWDs are initiated and generated, including preventing epileptogenesis, reducing depressive-like symptoms in WAG/Rij rats⁵⁴ and anxiogenic responses in GAERS.⁵⁵ It is thought that the treatment of patients before symptom onset might prevent many of the adverse consequences of chronic epilepsy.

17.4.2 Structural MRI: Original Data

We investigated structural changes in the major cerebral key players in SWD initiation and maintenance (lateral thalamus and somatosensory cortex) and in noninvolved control areas such as the hippocampus. We report here an original structural MRI study carried out to establish whether 4-month-old WAG/Rij rats (with a lower incidence of SWDs) and 9-month-old WAG/Rij rats (large amounts of SWDs) differ in T2-w values, indicating alterations in either water content or protein extravasation in the brain tissue. Given that the thalamus is not homogenous with respect to its role in SWD initiation and maintenance,^{33,37} we also investigated whether the lateral thalamus including the VPM might differ from the medial thalamus. Since hyperexcitable cells in GAERS have been identified in the subgranular layers of the somatosensory cortex, differences between the sub- and supragranular layers were investigated as well, thanks to the spatial resolution of our scanner.

The T2 values in the motor cortex, hippocampus, and medial section of the thalamus did not show differences between 4-month-old (4 m, $n = 6$, mildly symptomatic) and 9-month-old (9 m, $n = 5$, fully symptomatic) WAG/Rij rats, suggesting that becoming 5 months older does not induce large structural differences in presumably noninvolved brain areas. In contrast, age-dependent decrease (all $ps < .05$) were found exclusively for the three regions involved in the occurrence of SWDs: the supra-somatosensory cortex (supra SS: 4 m 87.2 ± 0.8 , 9 m 83.4 ± 0.9), the sub-somatosensory cortex (sub SS: 4 m 83.6 ± 0.7 , 9 m

81.3 ± 0.7), and the lateral thalamus (lat thal: 4 m 79.2 ± 0.3 , 9 m 76.4 ± 0.4). The data are presented in Figure 17.1 and Table 17.1.

Differences in T2 between supra SS and sub SS ($p < .05$) were found in the young WAG/Rij group only, suggesting that the difference disappeared when the number of seizures increase.

The reviewed data show an extended set of structural changes, mostly in the CTC network, although the limbic system also seemed affected in rats with hundreds of SWDs per day. The structural changes in the cortex were rather selective for the somatosensory cortex as they did not occur in the motor cortex and also selective for the lateral thalamus containing the VPM and VPL, as they did not occur in the limbic thalamus. An increase in FA was also observed in the anterior commissure and in the anterior part of the callosum. The latter connects the focal regions in the two hemispheres. All these fit well with the cortical focus theory. The volumetric changes in the amygdala could be related to the increased anxiety in GAERS. Treatment aimed at the prevention of expression of SWDs prevented the DTI changes in WAG/Rij rats, suggesting that at least some of the structural changes were caused by the frequently occurring SWDs.⁵⁴

17.5 Functional Imaging of the Dynamic Response Accompanying SWDs

Tenney et al.⁵⁶ used EEG-triggered fMRI (T2*-weighted echo planar imaging at 4.7 T) of SWDs in the WAG/Rij model to determine the activity of several thalamic nuclei and cortical areas that are supposed to be involved in the genesis and maintenance of SWD activity. Rats were first acclimated to the restrainer and next surgically implanted with MR-compatible epidural EEG electrodes, and Antisedan was administered so the combined EEG and MR study was done in fully conscious rats. BOLD activation associated with SWDs (34 episodes with a mean duration of 7 s) was clustered throughout the primary somatosensory, parietal association, and temporal cortices, along with several thalamic nuclei including the RTN, MD, VPM/VPL, and Po. No significant negative BOLD activation was seen for any seizures.

Also others were investigating whether the increases in oxygen delivery during SWDs are truly generalized throughout the entire brain, or whether the increases occurred preferentially in focal regions where neuronal firing is most intense. A second

question was whether neuronal activity was matched by changes in local cerebral blood flow.⁵⁷ WAG/Rij were anesthetized with a mix of neurolept-fentanyl anesthesia, which permits the occurrence of SWDs, closely mimicking those that occur spontaneously. A total of 256 SWDs obtained from 9 rats were investigated. The time course of the BOLD fMRI signal changed in the presence of SWDs. Clear increases in the BOLD fMRI signal were found in the facial part of the somatosensory cortex and in the thalamus. On the other hand, no significant changes related to SWD activity were observed in the primary visual cortex.

Table 17.1 Summary of T2 Values of Four Cortical and Three Subcortical Regions for the 4- and 9-Month-Old WAG/Rij Rats

Region	4 months	9 months
Motor cortex supragranular	88.35 ± 3.39	86.83 ± 3.06
Motor cortex subgranular	89.01 ± 4.51	84.01 ± 2.84
Somatosensory cortex supragranular	87.82 ± 2.90	84.14 ± 2.68
Somatosensory cortex subgranular	84.33 ± 3.32	81.22 ± 2.39
Hippocampus	90.09 ± 3.10	88.35 ± 4.42
Lateral thalamus	79.08 ± 2.18	76.31 ± 1.05
Medial thalamus	87.47 ± 3.99	84.85 ± 1.52

Data are expressed as mean ± standard deviation.

The most comprehensive fMRI study was done by Mishra et al.⁵⁸ A total of 1,856 SWD events were analyzed in 22 neurolept-fentanyl anesthetized WAG/Rij rats. BOLD activation, cerebral blood volume (CBV), as well as laser Doppler cerebral blood flow (CBF) were determined with a 9.4 T scanner. Local field potential (LFP) and multiunit activity (MUA) recordings were obtained in order to establish whether neuronal activity was matched by changes in local cerebral blood flow. The study confirmed and extended the previous results that BOLD signal changes during SWDs do not involve the whole brain uniformly. Rather, it showed both increases and decreases in specific cortical and subcortical brain regions, including intense bilateral increases in BOLD response in the facial zone of the somatosensory cortex, thalamus, anterior cingulate, posterior cingulate/retrosplenial cortex. Furthermore, BOLD increases in the superior colliculi were found in about 40% of the animals. The somatosensory cortex and thalamus showed increased fMRI, CBV, CBF, LFP, and MUA signals accompanying SWDs. However, the caudate-putamen showed fMRI, CBV, and CBF decreases despite increases in LFP and MUA signals. A dissociation between electrophysiological signals and fMRI activation also exists in the cortex, as SWDs can be found in frontal, central, and parietal cortex while the cortical BOLD increase is selective for the facial region of the parietal cortex.⁵⁷

Figure 17.1. Schematic representation of T2 values comparing mild symptomatic (4 months old) versus fully symptomatic (9 months) animals. Asterisks denote a difference for multiple areas in the same group of animals ($p < .05$). Arrows indicate differences between 4- and 9-month-old animals. Modified from Ott et al.²¹

Near-infrared spectroscopy (NIRS) is a recently developed technique for hemodynamic studies that is particularly suitable for dynamic recordings in humans and animals. It uses the particular absorption properties of living tissues in the near-infrared range to measure changes in the concentrations of oxy-, deoxy-, and total hemoglobin (HbO₂, HHb, and HbT, respectively) in tissues. In order to assess whether SWDs are accompanied by a cortical hemodynamic response, 444 SWD episodes from 6 GAERS rats in a quiet waking state were used as triggers for the analyses of the NIRS measurements.²⁶ It was found that the concentration of HbO₂ starts to decrease 13 s before SWD onset (20% of baseline), then increases (25% of baseline) from 1 s before the onset of SWDs, reaches a maximum 7 s after the onset, decreases (22% of baseline) from 1 s before the end of the EEG SWD, reaches a trough 9 s after the end, and finally returns to baseline. The concentration of HHb shows an inverse pattern of similar amplitude, while the concentration of HbT closely follows HbO₂.

Next, whether the cortical epileptic focus in the genetic models can be interpreted as a driving source was investigated by means of fMRI data collected in a 2.35 T magnet.⁵⁹ Standard techniques estimating functional connectivity measures are jeopardized by the variation of blood flow dynamics between regions. The authors were able to remove hemodynamic effects by using appropriate modeling techniques. Highly significant increases and decreases in CBV were found during SWDs at the group level in neurolept anesthetized GAERS in the facial area of the somatosensory cortex, the ventrolateral thalamus, but also in the central medial and mediodorsal thalamus, next to the reticular part of the substantia nigra, the cerebellum, and nuclei of the pons and medulla oblongata, while the visual cortex, the other parts of the somatosensory cortex, the visual and the secondary motor cortex were deactivated.⁵⁹ Functional connectivity was estimated from linear Granger Causality applied to CBV-weighted fMRI data and after deconvolution of hemodynamics the Granger causality showed that the facial area is indeed the neural driver, affecting thalamus and striatum.⁵⁹ Interestingly, an abnormally slow hemodynamic response function was also found in the focal region. EEG data collected from various subcortical regions in free moving GAERS indicated that the averaged cortical SWDs preceded the thalamic one and this validated the

concept that the focal region is indeed driving the thalamus and striatum.⁵⁹

It seems, therefore, that the increases in oxygen delivery during SWDs are not truly generalized throughout the entire brain. Rather, the data point to the facial area of the somatosensory cortex and the thalamus as the major actors in SWD occurrence. The increase in hemodynamic variables parallels the appearance of SWD, and in most regions an increase in CBV and CBF. The driving function of the facial region in the somatosensory cortex to the thalamus throughout the whole SWD was additionally proposed.

Brain networks were investigated in WAG/Rij rats by means of fMRI-based resting functional connectivity measures. Simultaneous EEG-fMRI data were acquired at 9.4 T in epileptic WAG/Rij rats compared to nonepileptic Wistar and WAG/Rij controls. Two focal regions were identified: one in the left and one in the right part of the facial zone of the somatosensory cortex. Both showed an fMRI increase during SWDs. These two regions were used for connectivity analysis to investigate whether chronic seizure activity is associated with changes in network resting functional connectivity. High degrees of interictal cortical-cortical correlations were found in all WAG/Rij rats, but not in NEC WAG/Rij rats. Strongest connectivity was seen between the bilateral somatosensory and adjacent cortices. This result, together with the DTI studies described above, suggest that the two focal regions in the left and right hemispheres are strongly functionally connected despite the decrease in FA. Both seemingly opposite changes occur, most likely, under the influence of hundreds of SWDs per day.⁵⁸

17.6 Interictal Imaging

We carried out DWI, rCBV, and rCBF imaging during interictal periods in 4- vs. 9-month-old WAG/Rij rats ($n = 5$ or 6). Age-related “epilepsy” effects were not always detected, however, consistent regional differences in the measured signals were found.

DWI values were lower (at 4 as well as at 9 months) in regions involved in the SWD occurrence (sub and supra SS and lateral thalamus) than the less involved regions (motor cortex, medial thalamus, hippocampus). Next, the subgranular cortical regions were always lower than the supragranular layers, and the difference was significant for both cortical regions

sub SS (0.00063 ± 0.00003) < supra SS (0.00065 ± 0.00004) and sub MOT (0.00068 ± 0.00005) < supra MOT (0.00075 ± 0.00005). Thus, WAG/Rij rats have higher supra than sub DWI values in the cortex as a whole and lower DWI values in the SS compared to the motor cortex. The data are presented in Figure 17.2.

rCBV data obtained from images acquired with a gradient-echo sequence before and after USPIO administration showed no significant effects of age on rCBV in any of the brain regions investigated. However, we found regional differences and an interaction between region and age. In particular, at 4 months of age, the motor cortex supragranular layer had higher ($p < .05$) rCBV values than the subgranular layer, at 9 months the subgranular layer had increased ($p < .05$) its rCBV value and it was now higher ($p < .05$) than the supragranular layer (Table 17.2). This demonstrated that the difference between supra- and subgranular layers and how they change over time are typical for the motor cortex, our control region, since they were not present in the somatosensory cortex.

Age and region effects were found for rCBF in all regions: 9-month-old rats had higher values (8.14 ± 0.53) than 4-month-old rats (5.34 ± 1.90 ; see Figure 17.3 and Table 17.3). Post hoc tests regarding the regional differences showed lower ($p < .01$) rCBF values in the somatosensory cortex than in the motor cortex and higher ($p < .05$ and $p < .01$) rCBF values in the sub- than in the supragranular cortical layers in the somatosensory and motor cortex, respectively. This all implies that the age-related increases in rCBF were not found in the SS cortex.

Table 17.2 Summary of rCBV Results for both Experimental Groups

Region	4 months	9 months
Motor cortex supragranular	7440 $\pm$ 1235	6122 $\pm$ 454
Motor cortex subgranular	5205 $\pm$ 678	7566 $\pm$ 315
Somatosensory cortex supragranular	5550 $\pm$ 2619	5050 $\pm$ 781
Somatosensory cortex subgranular	4611 $\pm$ 812	5238 $\pm$ 768
Hippocampus	7203 $\pm$ 1505	6269 $\pm$ 640
Lateral thalamus	5025 $\pm$ 1720	5121 $\pm$ 924
Medial thalamus	6310 $\pm$ 1472	4889 $\pm$ 318

Data are expressed as mean $\pm$ standard deviation.

We also found subcortical regional differences in rCBF. The highest values were in the hippocampus, lower ($ps < .05$) for the medial and lateral thalamus. Thus, rCBF data demonstrate cortical and subcortical regional differences and higher values for the older animals in both cortical and subcortical regions. However, this subcortical pattern does not clearly point toward selective changes rCBF in the networks involved in absence epilepsy.

17.7 Cortico-thalamic-cortical and Cortico-cortical Networks

A wealth of evidence strongly suggests that in the genetic rodent absence models the facial region of the somatosensory cortex contains a hyperexcitable zone. The fMRI data reviewed here, describing the hemodynamic changes accompanying SWDs, confirm a selective involvement of cortical and subcortical regions (lateral thalamus, but also other medial thalamic nuclei, the striatum, the cerebellum, and some brainstem nuclei) and are all in line with the cortical focus theory of absence epilepsy, which states that absence epilepsy in these genetic models is due to an increased cortical excitability in a focal region, accompanied by a reduced cortical inhibition. The foci in the left and right hemispheres form a strongly interconnected network. The regional selective increased BOLD, CBF, and CBV values accompanying the SWDs agree with the increased neuronal activity in most parts of brain. An interesting observation remains that SWDs are generalized over the cortex, while the hemodynamic responses are restricted, which suggests that the

Figure 17.2. Summary of DWI results (pooled groups). Data are expressed as mean $\pm$ SD. Asterisks denote a difference with the pooled noninvolved brain regions ($p < .05$). Black horizontal lines indicate differences between supra- and subgranular layer ($p < .05$).

presence of SWDs is not sufficient to increase oxygen demands. A driving function, as previously established,^{35,37,59} is a better candidate for an increased demand of oxygen in this area. The early changes (13 s pre SWD onset) as observed with NIRS deserve to be investigated in more detail. The increased hemodynamic response in the thalamus can be ascribed to the SWD accompanying increased tonic inhibition.⁶⁰

The functional interictal connectivity changes point toward an increase in white matter tracts, connecting the bilateral foci. Although DWI reductions

reflect pathological conditions in brain tissue that are only partially understood, and involve changes in the diffusion characteristics of intra- and extracellular water compartments and water exchange across permeable boundaries,⁶¹ they have been associated with acute cytotoxic edema.⁶¹ The observation that changes were exclusively present in the absence epilepsy brain circuitry may have some consequences for local changes in excitability or vice versa. And this merits further investigation.

The age-related changes in rCBV in control regions in 4- vs. 9-month-old WAG/Rij rats did not occur in the focal region, and the latter area was characterized by a lower rCBF than the cortical control region, suggesting that absence epilepsy is accompanied not only by an interictal but also by an ictal change in the hemodynamic response function.⁵⁹

The age-related increase in rCBF in all the cortical and subcortical regions and the higher rCBF values might be epilepsy related, but it can also be a general aging effect. It might point toward an increased glucose metabolism, resulting from increased inflammatory cell activation,⁶² commonly seen in aging.

The structural imaging data (T2 and DTI) are also in line with a special and perhaps unique role of the bilateral foci in the facial region of the somatosensory cortex, including the morphological changes expressed in the anterior corpus callosum, interconnecting the bilateral foci. It might be a reason, together with the functional changes that were reported, why the pathological activity seems

Table 17.3 Summary of rCBF Results for All Experimental Groups

Region	4 months	9 months
Motor cortex supragranular	5.62 ± 1.67	8.26 ± 0.31
Motor cortex subgranular	6.01 ± 1.15	8.67 ± 0.49
Somatosensory cortex supragranular	4.50 ± 2.54	7.73 ± 0.29
Somatosensory cortex subgranular	5.24 ± 2.16	7.92 ± 0.56
Hippocampus	6.11 ± 1.22	8.10 ± 0.30
Lateral thalamus	4.53 ± 2.70	7.26 ± 0.41
Medial thalamus	4.85 ± 2.20	7.38 ± 0.45

Data are expressed as mean ± standard deviation.

Bregma -2.80 mm; modified from Paxinos and Watson 1998

Figure 17.3. Schematic representation of rCBF results comparing 9-month-old with 4-month-old animals. Arrows indicate differences between age groups ($p < .05$).

generalized after being initiated in a single hemisphere. The structural data also point toward interictal changes in the lateral thalamus.

The decrease in T2 in the brain regions specifically involved in SWD occurrence might indicate a decline in extracellular water, and once more this might affect the excitability of neuronal tissue. As an alternative explanation, some authors⁶³ suggest that an increased concentration of deoxyhemoglobin causes a decrease in T2. This so-called negative BOLD effect has been shown to occur in MRI studies of hypoxia in the rat. Deoxyhemoglobin increases in situations in which the metabolic requirement for oxygen is not met by the delivery of oxygen.

The way in which structural and functional changes in local excitability and in the CTC and cortico-cortical networks relevant for absence epilepsy are related is by no means clear. What is clear, though, is that the loss of cortical HCN1 found in WAG/Rij rats preceded the onset of SWD, suggesting that the increased excitability is inherited, rather than required for SWD to occur.⁴⁷ Moreover, the same authors suggested that this loss of HCN1 provides a somatodendritic mechanism for increasing the synchronization of cortical output, and is therefore likely to play an important role in the generation of SWDs via the CTC and cortico-cortical networks.

An undisputed role is acknowledged for the corpus callosum in synchronizing the bilateral cortical and thalamic SWDs. Each hemisphere is able to initiate SWDs independently and SWDs become bilateral and symmetrical through the rapid interhemispheric communication via the monosynaptic callosal projections connecting homotopic regions of the somatosensory cortex. Interestingly, it has been demonstrated that the structural changes in the connectivity between the facial focal regions of the somatosensory cortex, as found by Hal Blumenfeld's group,⁴³ could be reduced by the prevention of SWDs, suggesting a causal relationship between the morphological callosal and cortical excitability changes. The reduction in excitability induced by antiepileptogenic treatment, as expressed by the enhancement of HCN1 channels, was accompanied by a reduction in the amplitude of the elicited local electrical evoked potential, and the reduction of SWDs and by stimulation-induced afterdischarges.^{53,54}

Changes in absence epileptic brains are not restricted to the mentioned networks, and functional changes in the limbic system are well documented

both in WAG/Rij and in GAERS rats: both strains showed a resistance to electrical amygdala kindling.⁶⁴ Imaging studies showed structural changes in the amygdala and hippocampus in GAERS. Whether the behavioral phenotype showing depressive-like and anxiety behavior is related to that needs to be investigated. It has been suggested that also these changes might be due to the daily occurrence of hundreds of SWDs.

Considering the wealth of other cortical and subcortical changes in, among others, GABAergic and glutamatergic neurotransmission functions, it is likely that other cortical functions are disturbed, such as sensory gating or the organization of sleep by the hypothalamus-brainstem network.

17.8 Conclusions

Work in genetic absence epilepsy models (WAG/Rij and GAERS) has revolutionized the theories on SWD generation in absence epilepsy and, more in general, on genetic generalized epilepsies. The analysis of EEG signals from in vivo studies has demonstrated the existence of cortical foci in the facial region of the somatosensory cortex, from which SWD activity drives other cortical and thalamic regions. Different types of high-resolution structural and functional imaging studies in these models have yielded a wealth of new data, demonstrating a selective involvement of brain structures in SWD occurrence, and highlighting functional and structural changes in neuronal networks within the CTC and cortico-cortical networks, but also in other subcortical brain regions. These data leave no room for the classical axiom that absence epilepsy is a generalized type of epilepsy. The terms "focal epilepsy" and "network epilepsy" are a better fit for the experimental neurophysiologic and imaging data. Arguably, they might be helpful in the understanding of cognitive, emotional, and sleep-related problems often encountered in persons with absence epilepsy.

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