From Generalized to Focal Absence Seizures

Jeneralize Nöbetlerden Fokal Absans Nöbetlerine

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The leading theory on the origin of absence seizures, the cortico-reticular theory, supposes that a subcortical pacemaker is responsible for normal sleep spindles and pathological spike-wave discharges (SWDs). It postulates that the thalamic relay cells in the basolateral complex of the thalamus, the reticular thalamic nucleus and the cortex, assembled in a thalamo-cortico-thalamic network, generate both types of EEG oscillations. Recently, Meeren et al. (2002) proposed a focal theory of absence epilepsy. This theory, based on experimental findings in the WAG/Rij rats supposes that the somatosensory cortex contains a focus that initiates a cascade of events that lead to the occurrence of bilateral and generalized SWDs. In this review, new data are presented which show that the cortex of WAG/Rij rats contains areas including the somatosensory cortex, which lack parvalbumin. Second, local deactivation of the focal zone with lidocaine reduces the incidence of SWDs. Finally, branching of dendrites of neurons is abnormal in this zone. All these new results provide further support for the focal theory of absence epilepsy. The question as to whether similar or comparable pathophysiological processes are present in humans needs to be elucidated. The new focal theory can be easily tested in humans with respect to the location of the origin of the EEG oscillations.

Key Words: Brain mapping; cerebral cortex/physiopathology; disease models, animal; electroencephalography; epilepsy, absence/physiopathology; rats.

Absans nöbetlerinin kökeni konusunda önde gelen teorilerden biri olan kortiko-retiküler teori, normal uyku iğlerinden ve patolojik diken-dalga deşarjlarından (DDD) subkortikal bir ritm merkezinin sorumlu olduğunu; talamusun bazolateral kompleksindeki talamik relay hücrelerinin, retiküler talamik nükleusun ve korteksin talamo-kortiko-talamik bir ağ oluşturduğunu ve EEG osilasyonlarının iki türünün de bu sistem tarafından üretildiğini varsaymaktadır. Yakın zamanda, Meeren ve ark. (2002) absans epilepsi konusunda fokal teoriyi ortaya atmışlardır. WAG/Rij sıçanlarından elde edilen deneysel bulgulara dayanan bu teori, somatosensoriyal kortekste, iki taraflı ve jeneralize DDD'lerin oluşumuna yol açan olaylar fırtınasını başlatan bir odak olduğunu ileri sürmektedir. Bu yazıda sunulan yeni veriler şu noktalara dikkat çekmektedir: *(i)* WAG/Rij sıçan korteksinde parvalbumin boyaması göstermeyen bölgeler (fokal bölge) bulunmuştur. *(ii)* Somatosensoriyal korteksteki fokal bölgenin lidokoinle lokal kortikal deaktivasyonu DDD'lerin azalmasına neden olmaktadır. *(iii)* Anılan fokal bölgede, dendritlerin anormal dallanmasının görüldüğü nöronlar bulunmuştur. Bunların hepsi, absans epilepside fokal teoriyi destekleyici verilerdir. Benzer patofizyolojik süreçlerin insanlarda da olup olmadığı açıklığa kavuşturulmalıdır. Bu yeni fokal teori, EEG osilasyonlarının yerleşiminin saptanması açısından insanlarda kolaylıkla sınanabilir.

Anahtar Sözcükler: Beyin haritalandırması; serebral korteks/fizyopatoloji; hastalık modeli, hayvan; elektroensefalografi; epilepsi, absans/fizyopatoloji; sıçan.

Absence seizures were first described by Poupart in 1705, and later, in 1770, Tissot introduced the term "petits accés". Finally they were called "absence seizures" in 1824 by Calmeil.[1] Absence seizures, also known as petit mal, fundamentally differ from another type of generalized epilepsy- grand mal seizures, in that symptomatically there is nei-

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ther an aura nor any convulsions in the former. Secondly, absence seizures have quite a different pharmacological profile than that of grand mal seizures, in that some of the well-known anti-convulsant drugs may provoke absence seizures.[2,3]

There are two essential hallmarks in absence epilepsy, the first being a specific clinical sign – abrupt and brief impairment of consciousness (absence). Ongoing activity is interrupted during the seizure, responsiveness is decreased and mental functioning is impaired. There are minimal myoclonic movements of the eyes as well as in the peri-oral area. The impairment in consciousness seems to be moderate since humans are sometimes able to recall information that was presented to them during the seizure and rats are able to discriminate between relevant and less relevant stimuli presented during the seizure.[4,5] The second is the characteristic paroxysmal electrical activity invading the whole brain, generalized 3-4 Hz spike-and-wave discharges (SWDs). The SWDs may be consistently brief (2 to 5 seconds) or long (15 to 30 seconds), with the intra-discharge frequency being in the range of 2.5-5 Hz.[6] They do not appear randomly in time, they tend to appear, in humans as well in rats, in clusters.[7] The term pyknolepsy, equivalent to absence epilepsy, refers to this characteristic. The SWDs appear in the frontal midline region of the cortex, with amplitude becoming gradually smaller in the lateral and posterior directions. They are of abrupt onset and termination; the attacks may be preceded and immediately followed by a normal EEG activity, especially when recorded in the waking (resting) state.

Typical absences usually begin in children at the age of 5 to 15 years old, more often in girls than in boys. In most cases, the reason for the seizures is unknown. They also occur in about 10%-15% of adults with epilepsies, often combined with other generalized seizures. They may remit with age or last lifelong. Typical absence seizures are often easy to diagnose and treat. Valproic acid and ethosuximide, alone or in combination, are the first-line therapy; absence seizures also respond well to the newer medications including lamotrigine and topiramate. However, and in contrast to the effect on all other types of seizures,[2,3] GABA-mimetics such as vigabatrin and tiagabine may exacerbate absence seizures and aggravate SWDs.[8,9]

Since the time when the SWDs were first described on the electroencephalogram (EEG) by Gibbs, Davis, and Lennox in 1935,[10] a lot of efforts have been made to establish the possible origin of these oscillations. Two theoretical lines are prevailing after prolonged extensive debates, namely, the "centrencephalic" concept describing a subcortical origin of the generalized SWDs[11] and the cortical theory. Many researchers have recently accepted a synthetic cortico-reticular theory derived from both theories.[12]

Towards the understanding of the nature of spike-and-wave discharges. A historical overview of some earlier theories

The global nature of the SWDs and their highly synchronous appearance in both hemispheres led the earliest investigators to the assumption that SWDs could rise from a central structure that sends intensive bilateral projections to the whole cortex and distributes paroxysmal activity over the entire cortex.[13]

As no evidence could be found for a cortical focus, Jasper and Kershman[13] proposed a subcortical pacemaker of SWDs, and a few years later, the "centrencephalic" concept was introduced concerning the origin of the generalized SWDs associated with absence seizures.[11] This 'centrencephalic integrating system,' thought to be located in the brain stem and the diencephalon, was held responsible for the bilateral EEG onset of the SWDs and the loss of consciousness. A number of other non-specific structures were implicated for the location of absence seizures including the mesencephalon,[14] the reticular formation,[15] or the caudatus putamen.[16] Walker and Marshall[17] showed that the SWDs could arise simultaneously in limbic structures and in one or many thalamic nuclei in humans. These experimental data were not sufficient to determine a certain anatomical localization for the "centrencephalon." The exploration of the thalamus itself and the interrelations between

the thalamus and the cortex gave rise to a hypothesis that the SWDs may originate from the non-specific part of the thalamus, the intralaminar nuclei, which are diffusely projected to many cortical regions. Moreover, electrical stimulation of the thalamus evoked a spindle-like EEG pattern over the large cortical area or bilaterally synchronous spike-and-wave oscillations on the cortical EEG, depending on the location of the stimulation electrodes. This pattern of SWDs was quite similar to that seen during absence seizures in humans. Ingvar[18] tried to reproduce experiments of Jasper et al.[19] in unanesthetized cats with electrical stimulation of more than 4,000 sites in the thalamus and midbrain, but he was not able to obtain bilateral spike-wave complex in the cortex. The role of the intralaminar thalamic nuclei in the generation of SWDs in humans remained rather uncertain; anatomical studies showed that, in mammals compared to humans, these structures are less developed and sometimes even absent.[14,20]

"Centrencephalic" concept underwent a gradual transformation into a "thalamic" theory. Simultaneous thalamic and cortical recordings of patients provided direct evidence for the SWDs to begin in the thalamus with a cortical delay of 1-2 seconds during absence attacks.[21] Currently, the "thalamic theory" is still valid and well accepted by some researchers; it is gaining further interest and support with the use of modern techniques such as positron emission tomography.[22]

One of the first experimental findings that cast doubt on the "centrencephalic" theory was that an injection of the convulsant pentylenetetrazole (PTZ) into the internal carotid artery, which supplies large parts of the cerebral cortex, in patients with petit mal seizures produced generalized spike-and-wave activity. Such responses were absent or rudimentary when PTZ was injected into the vertebral artery, which supplies most of the diencephalon and the brain stem.[23] Bancaud[24,25] who used depth recordings of epileptic patients noticed that spontaneous discharges occurring during a spontaneous petit mal or grand mal seizure may initially be localized to the cerebral cortex, in the neighborhood of an identified lesion, particularly in the frontal lobe. In addition, similar attacks could be reproduced by electrical stimulation of the same cortical epileptogenic zone.[26] These observations led Bancaud[27] to the suggestion that SWDs were always secondary to a focal discharge in the frontal cortex, with the latter being rapidly propagated through the whole cortex through various cortico-cortical pathways.

Also not compatible with the "centrencephalic" theory was the finding obtained from detailed analyses of time shifts between EEG channels during typical absence SWDs. It was shown that the bilateral synchrony was not perfect as previously believed. It appeared perfect only at normal "paper speed" of the electroencephalograph. With the use of an oscilloscope or digital signal processing techniques, interhemispheric latencies were found to differ up to 20 ms.[28] However, no consistency existed concerning the leading and the following hemisphere, and constant shifts from side to side were the rule. In addition, with the use of the so-called toposcopic technique, Petsche[29] found time shifts in the antero-posterior direction, suggesting that the SWDs were travelling waves over the cortex, with preferred points of origin at the vertex and frontal regions. Others, however, were not always able to confirm time differences in primary generalized seizures.[30]

The above-mentioned data have been the basis for the cortical theory.[31] It states that primary generalized epilepsy is the expression of cortical pathology. The thalamus, on the other hand, is certainly involved, but it only "plays second fiddle" in carrying out normal physiological thalamo-cortical interactions. Generalized spike wave bursts in primary generalized epilepsy are generated in the mesiofrontal cortex, whence they rapidly spread over other cortical areas. Another viewpoint was that it was frequently impossible to distinguish between partial and generalized seizures and that there should be a continuum between clear-cut partial seizures at one end of the spectrum and generalized seizures at the other, most seizures falling somewhere in between.[28,31] Lüders et al.[32] reasoned that, moving along the spectrum from partial to general-

ized seizures, one could deduce by extrapolation that generalized seizures were actually multifocal cortical seizures with an extreme tendency for secondary generalization. Mutual functioning of the thalamus and the cortex is undoubtedly important for the perpetuation of SWDs. However, the mechanism of the initiation of discharges is still uncertain. Several authors propose that SWDs are initiated at the frontal cortex in humans. It has been shown that generalized SWDs are triggered by a frontal epileptogenic cortical focus.[31,33] This idea is based on the finding that 3 Hz SWDs are initially located at the mesiofrontal cortex and rapidly propagate to the rest of the cerebral regions.[24]

A third theory has been put forward combining the thalamic and cortical processes in the initiation and perpetuation of absence seizures. Gloor[12] observed that the cortex played a role in the generation of generalized SWDs, proposing that a 'cortico-reticular' mechanism might be involved in the generation of these discharges. The experimental data for his theory were mainly derived from the feline penicillin model of generalized absence epilepsy.[34,35] With this model it has been shown that large doses of intramuscular penicillin, a convulsant with properties similar to those of $GABA_A$-receptor antagonists, induced bilaterally synchronous SWDs. Moreover, systemic penicillin transformed spontaneous sleep spindles into SWDs. Diffuse cortical application of penicillin was also capable of producing SWDs, whereas penicillin injection into the thalamus was not associated with SWDs, suggesting that the epileptiform discharges were the result of abnormal responses of the cortex, but not of the thalamus.[36] The crucial factor responsible for the SWDs here is a diffuse increase in the excitability of the cortex: cortical neurons respond to afferent thalamocortical volleys by generating SWDs instead of producing sleep spindles. In all, this theory assumes that the mechanism responsible for the genesis of SWDs is closely linked to the thalamo-cortical mechanism that generates sleep spindles: spindles elicited in the thalamus are transformed into SWDs when the hyperexcitable cortex receives them.

Recent studies on spike-and-wave discharges in the animal models of absence epilepsy

Over the last decades, evidence has accumulated for a pacemaking role of a particular part of the thalamus, the reticular thalamic nucleus (RTN), in the origin of the brain rhythmicity, mainly sleep spindles. Surgical isolation of the RTN abolished rhythmic bursting in thalamocortical relay (TCR) cells, but bursting was preserved in RTN neurons.[37] It has been demonstrated that TCR nuclei can only exhibit spontaneous spindle oscillations (7-14 Hz) when they receive projections from the RTN.[38] These and similar studies were mainly performed in cats in vivo[36] and in the ferret in vitro.[39] However, some physiological and anatomical features of rodent brains are crucially different from those of feline brains and might provide some peculiarities for rodent models of epilepsy: firstly, cat SWDs occur with frequencies ranging from 3 to 4.5 Hz, which is about the same range in humans, but in rat SWDs, the frequencies vary from 7 to 14 Hz.[12,40] Secondly, cat dorsal thalamus contains inhibitory interneurons and receive an additional inhibitory control from the RTN cells, in contrast to rats which nearly lack GABA-ergic interneurons in the TCR nuclei:[41] a localized population is only found in the lateral geniculate nucleus.[42] Moreover, lateral geniculate nucleus itself receives more powerful monosynaptic inhibitory afferents from the RTN than other parts do in the rat thalamus.[42,43] As a corollary, due to its anatomic specificity, the larger part of rat thalamus receives only external inhibitory inputs from the RTN.

In the last decade, genetic rodent models became the model of choice.[44-47]

Several lines of experimental evidence in ferrets,[39] GAERS (Genetic Absence Epilepsy Rats from Strasbourg)[48-50] and WAG/Rij rats[51] support the hypothesis that the RTN is a pacemaker for triggering SWDs. Expression of SWDs in GAERS has been shown to be suppressed after large electrolytic lesions of the lateral thalamus[52] and also after more restricted chemical lesions of the RTN.[53,54] These observations were also found to be the case in WAG/Rij rats: all SWDs were abolished after

ibotenic-induced lesions of large parts of the thalamus including the RTN.[55] However, rostral and caudal poles of the RTN appeared to be highly different in respect to the sustaining of SWDs. Lesions of the caudal part of the RTN provoked the opposite changes in the number of SWDs compared to analogous lesions of the rostral part together with the lateral thalamus.[55,56] In fact, if lesions were confined to the caudal and middle parts of the RTN, the number of SWDs was surprisingly increased. Probably, this part of the RTN could depress functioning of a hypothetical pacemaker of SWDs which is located in the rostral part of the nuclei, suggesting that disinhibition of this pacemaker might have taken place. The findings of all these studies are in favor of the cortico-reticular theory which assumes that there is a thalamic pacemaker in the rostral pole of the RTN.

It is currently taken for granted that SWDs are initiated in the RTN; however, this does not imply that the RTN is a primary focus of SWDs. Detailed studies in GAERS failed to find any essential structural alterations in neurons of the RTN as compared to non-epileptic rats, and no neuronal loss occurred in the RTN of epileptic animals.[57] However, some observations on the intrinsic neuronal properties of the RTN seem to be somewhat inconsistent with this common assumption.[58,59] Also the relative contributions of the cortex and the thalamus and the exact mechanisms are still a matter of debate. A putative disadvantage of the penicillin model is that the role of the cortex might have been overemphasized, as the SWDs are in the first place in the pharmacologically-induced increase in cortical excitability.[55] Moreover, for studying the mechanisms of the idiopathic types of epilepsy such as childhood absence epilepsy, current research endeavors show more predilection for genetic models than for chemically-induced models. Nevertheless, the concepts developed by Gloor et al.,[34,36] and recently reviewed by Kostopoulos[35] are still considered of vital importance.

The role of the cortex in generalized absence epilepsy revisited

In their scheme for the generation of SWDs, authors of the cortical theory did not assume a single focus or a focal area,[24,25,31] neither did the authors of a cortico-reticular theory.[12,34] Regarding mechanisms of SWDs, both theories considered the cortex in general and did not pay attention to the specific local neurophysiological properties that could make one cortical region more favorable for the epileptogenesis than another.

Studies in GAERS and WAG/Rij rats for the role of the cortex demonstrated that cortical deactivation and spreading depression evoked by a unilateral injection of KCl resulted in immediate abolishment of SWDs not only in the injected cortex but also in the ipsilateral thalamus.[52,55] The same effect was found in cats with penicillin-induced SWDs during cortical spreading depression.[34,60] These findings were in favor of the cortico-reticular theory, suggesting that the cortex was a key element for the generation of SWDs.

This theory assumes that, in the epileptic subjects, the cortex becomes more easily excitable than does in non-epileptic animals, therefore thalamic sleep spindles that reach the cortex through afferent volleys could be transferred into SWDs. Tolmacheva et al.[61] investigated the thresholds for various types of after-discharges and seizure types at the sensorimotor cortex. Generally, they noted no differences between WAG/Rij and ACI rats in the seizure thresholds and after-discharges: only the threshold for limbic seizures showed a decrease in WAG/Rij rats compared to the latter. Therefore, it does not seem likely that the cortex of WAG/Rij rats is more hyperexcitable than that of non-epileptic control rats, at least in terms of thresholds of various types of epileptoformic after-discharges.

Several basic electrophysiological properties of the cortical tissue in GAERS do not principally differ from those of non-epileptic rats. This mainly concerns passive cortical properties such as the complex waveform of the field potentials and the percentage of pyramidal neuron populations (intrinsically bursting and regular spiking cells).[58] However, essential functional differences were found in the neocortex of GAERS that could make it hyperexcitable compared to the cortex of non-epileptic rats, including an enhanced cortical response to

N-methyl- D-aspartate activation in the frontal area,[62] and changes in glutamatergic[63] and GABA-ergic[58] transmission. In WAG/Rij rats, cortical inhibitory GABA-ergic mechanisms are impaired, as shown in the frontal cortex with extra- and intracellular recordings of synaptic responses.[64] A high level of impairment in the inhibitory processes was found in the frontal cortex of WAG/Rij rats compared to three other rats' strains in a paired-pulse inhibition paradigm. Briefly, if two consecutive auditory stimuli are interspersed with a short time delay (500 msec), the second stimulus is attenuated by an inhibitory effect of the first. An inhibitory gating deficit was found in WAG/Rij rats, suggesting functional disturbances in the neocortex.[65]

Neurochemical and neuroanatomical investigation of the neocortex in WAG/Rij rats

As discussed in the previous section, one of the largest impairments in the neocortex of WAG/Rij rats, compared to other rat strains, involves the inhibitory mechanisms. Further investigations of the cortical GABA-ergic system were performed in WAG/Rij rats using parvalbumin (PV) staining, a calcium-binding protein which is substantially co-localized with GABA. Since 90 per cent of PV-immunopositive neurons are GABA-egic, PV-immunostaining can quantify some (not all) of the GABA-ergic cells and may shed light on the spatial distribution of the inhibitory interneurons.[66,67]

Parvalbumin is abundantly present throughout the neocortex,[68] especially in the RTN.[69] Another advantage of this technique is that it allows to judge neuronal functions. As PV influences the membrane potential by buffering calcium ions entering the cell upon depolarization,[70] changes in neuronal activity can be estimated by changes in neuronal PV content, hence, in PV-immunoreactivity. Considering the important role of both voltage-activated calcium channels and calcium-activated potassium conductances in the genesis of SWDs in GAERS[59] and in WAG/Rij rats,[71] it was hypothesized that absence epilepsy in the WAG/Rij rat was related to a disturbed PV distribution within the major key structures of SWDs, namely, the RTN and the cortex. Such a disturbance may account for the increased excitability, the generation and/or maintenance of SWDs. This hypothesis was tested by the immunocytochemical assessment of the presence of PV in the RTN and in several cortical areas, comparing absence epileptic WAG/Rij rats with age-matched, non-epileptic ACI controls.

It was found that some brain regions such as the RTN and the pyramidal cell layers of the hippocampus were much more strongly stained than others such as the molecular hippocampal layers and the thalamic nuclei (Fig. 1.2b),[72] which is in agreement with data from Houser et al.[69] It was surprising to note that both ACI and WAG/Rij rats had structures or even regions that hardly showed or did not show at all PV-immunoreactive cells. This observation implies that these regions are not actually devoid of neurons and that lack of immunoreactivity is due to the inability of neurons to stain with the anti-PV serum. Apparently, such cells do not contain enough PV to become immunopositive. The unstained regions were localized exclusively in the cerebral cortex, though the location and the size of these areas strongly differs among animals. WAG/Rij rats showed more unstained regions than did ACI rats (21 vs 14 regions); however, this difference was not statistically significant and there was no specific cortical area in each WAG/Rij rat that consistently remained unstained. Quantification of PV-positive cells showed clear differences in the parietal (Par1) and in the forelimb area (FL) of the somatosensory cortex, where ACI rats showed about two times as many PV-positive cells as WAG/Rij rats (Fig. 1.3a,b; Fig. 2). The most prominent difference was seen in the olfactory tubercle (Tu), where the former demonstrated a substantial number of PV-positive neurons whereas the latter almost completely lacked such cells (Fig. 2). Par1 and FL are parts of the somatosensory cortex and Par1 contains peri-oral projections. The lack of PV in these regions may destabilize intraneuronal Ca^{2+} homeostatic processes such as excitability, intracellular signalling, and neurotransmitter release. In neurons devoid of PV, a too high Ca^{2+} concentration might adversely affect such processes. Considering the co-localization of PV with glu-

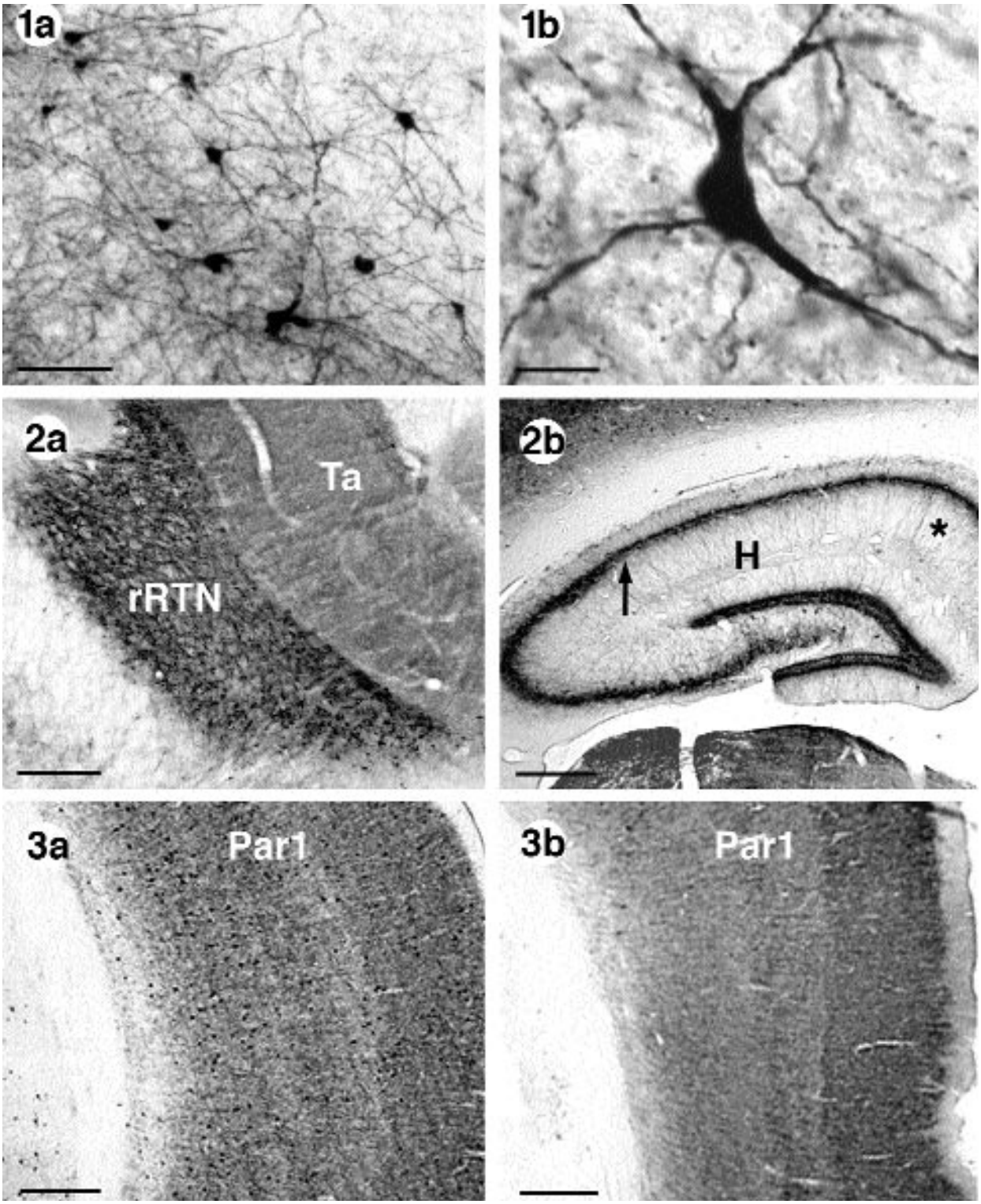

Fig. 1

PV-immunoreactive staining in neuronal tissue of ACI and WAG/Rij rats. **1.** Neurons in the piriform cortex of ACI rat. **(a)** Bar: 50 µm. **(b)** Detail. Bar: 10 µm. **2.** Brain slices of the WAG/Rij rat. **(a)** Rostral reticular thalamic nucleus (rRTN) and adjacent thalamic nuclei (Ta). **(b)** Hippocampus (H). Arrow indicates the pyramidal cell layers of the hippocampus; asterisk indicates the molecular hippocampal layers. PV-immunoreactive cells are present as black dots in the RTN, while they are absent in the adjacent other parts of the thalamus. Bars: 500 µm. **3.** Brain slices of parietal cortical area 1 (Par 1) in ACI rat. **(a)** PV-positive neurons are numerous but they are scarce in WAG/Rij rat. **(b).** Bar: 400 µm. (Adapted from van de Bovenkamp-Janssen et al.)[72]

tamic acid decarboxylase (GAD) and GABA, it is assumed that WAG/Rij and ACI rat cortices contain areas with few or even no GABA-ergic cells. Probably, PV-immunoreactive (GABA-ergic) neurons in the somatosensory cortex and olfactory tubercle of WAG/Rij rats contain less or even no PV, resulting from local cortical alterations of inhibition. This is in agreement with the findings of Luhmann et al., who demonstrated impairment in GABA-ergic inhibition in the frontal cortex of WAG/Rij rats compared to Wistar rats.[64]

Temporal and spatial properties of SWDs: demonstration of a cortical focus

Meeren et al.[55,73] investigated the spatio-temporal properties of SWDs in order to elucidate some of the network mechanisms that are responsible for the immediate widespread generalization and high synchronization of discharges, characterizing the generalized nature of absence seizures. Simultaneous field potentials were recorded from multiple cortical and thalamic sites in freely moving WAG/Rij rats. By definition, epileptic seizure activity manifests highly synchronized coherent discharges of the large neuronal population that is reflected in local field potentials. Meeren et al.[73] were able to record concurrent electrical activity in the cortical and subcortical areas, to measure some spatio-temporal characteristics, and to judge the propagation of oscillations in the large-scale network. The cortico-cortical, intrathalamic, and cortico-thalamic interrelationships between these field potentials were quantified using a non-linear association analysis.[74] In this way, a direct measure of the strength of association, and thus, of the degree of correlation between the events recorded at the different sites were obtained, which in turn provided an indication of the degree of functional coupling between the two underlying neuronal populations. In addition, the method provided an estimate of the time delay between signals.

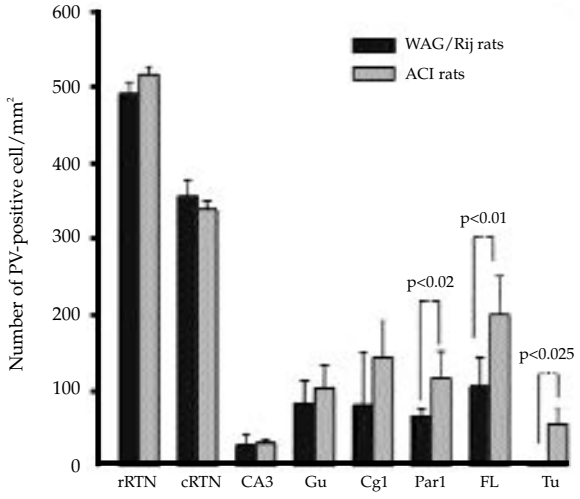

Fig. 2

Number of PV-positive neurons per mm^2 in various brain areas of WAG/Rij (n=5) and ACI (n=5) rats. (Adapted from van de Bovenkamp-Janssen et al.)[72]
CA3: CA3 region of the hippocampus; Cg1: Cingulate cortical area 1; cRTN: Caudal reticular thalamic nucleus; FL: Forelimb area of the somatosensory cortex; Gu: Gustatory cortex; Par1: Parietal cortex; Tu: Tuberculum; rRTN: Rostral pole of the reticular thalamic nucleus.

The non-linear association analysis revealed a consistent cortical 'focus' within the peri-oral region of the somatosensory cortex both throughout the seizure and across seizures.

Figure 3 illustrates a generalized SWD in WAG/Rij rat and the results of the non-linear analysis, the existence of a cortical focus originating from one of the 16 electrodes in the somatosensory cortex. Spike-and-wave discharges recorded at other cortical sites consistently lagged behind this focal site, with time delays that increased with electrode distance, resulting in a mean propagation velocity of about 1.5 meter per second. Intra-thalamic relationships were more complex and could not account for the observed cortical propagation pattern. Functionally interconnected cortical and thalamic sites appeared to influence each other, while the direction of this bi-directional coupling varied throughout one seizure. However, during the first 500 milliseconds, the cortical focus was consistently found to be in advance of its thalamic counterpart. Thereafter, the cortex and the thalamus were found to alternately precede or succeed in an unpredictable way. These results were incompatible with the common assumption that the thalamus acts as the primary driving source for the discharges. Instead, they indicated that a cortical focus played a leading role in the generation of generalized spike-wave discharges characteristic for absence seizures in the rat.

The large-scale synchronization characterizing bilaterally synchronized SWDs appears to

be mediated by the fast propagation of seizure activity from this focal site through cortico-cortical networks. Once the oscillation has been set into motion, however, the cortex and the thalamus form a unified oscillatory network in which both structures drive each other. The role of the thalamus probably lies in providing a resonant circuitry to amplify and sustain the discharges.

In the light of these findings, it is proposed that the following mechanisms are responsible for the initiation, generalization, and maintenance of absence seizures in the rat. The generation of bilaterally generalized spike-wave discharges is only possible in the presence of an anatomically and functionally intact cortico-thalamic network, which, furthermore, itself should be in a suitable state. This suitable state is characterized by a light to moderate hyperpolarization of the intrinsically bursting cortical pyramidal cells, of the thalamocortical relay and the reticular thalamic cells, which makes them highly prone to produce high-frequency bursts of action potentials, as is the case during transitions from waking to sleeping, during drowsiness and light non-REM sleep.[75]

The initial event to take place is the generation of an epileptiform spike at the site of the cortical focus. Figure 4 illustrates the pooled results of eight WAG/Rij rats investigated, in each of which the focal zone was found to occupy a small (2 x 5 mm) part in the projective area of the snout and vibrissae.

Functionally, the somatosensory cortex of rodents is rather unique, with peculiar properties to be taken into account if rats and other rodents are used for study of mechanisms of absence epilepsy. Some regions of the somatosensory cortex in rodents are predetermined to generate and sustain its own intrinsic rhythms and might bear a part in the genesis of SWDs. A closer look at the inner features of somatosensory neurons might bring some "neurophysiological" light on why the somatosensory cortex specifically contains the "leading" site of SWDs.

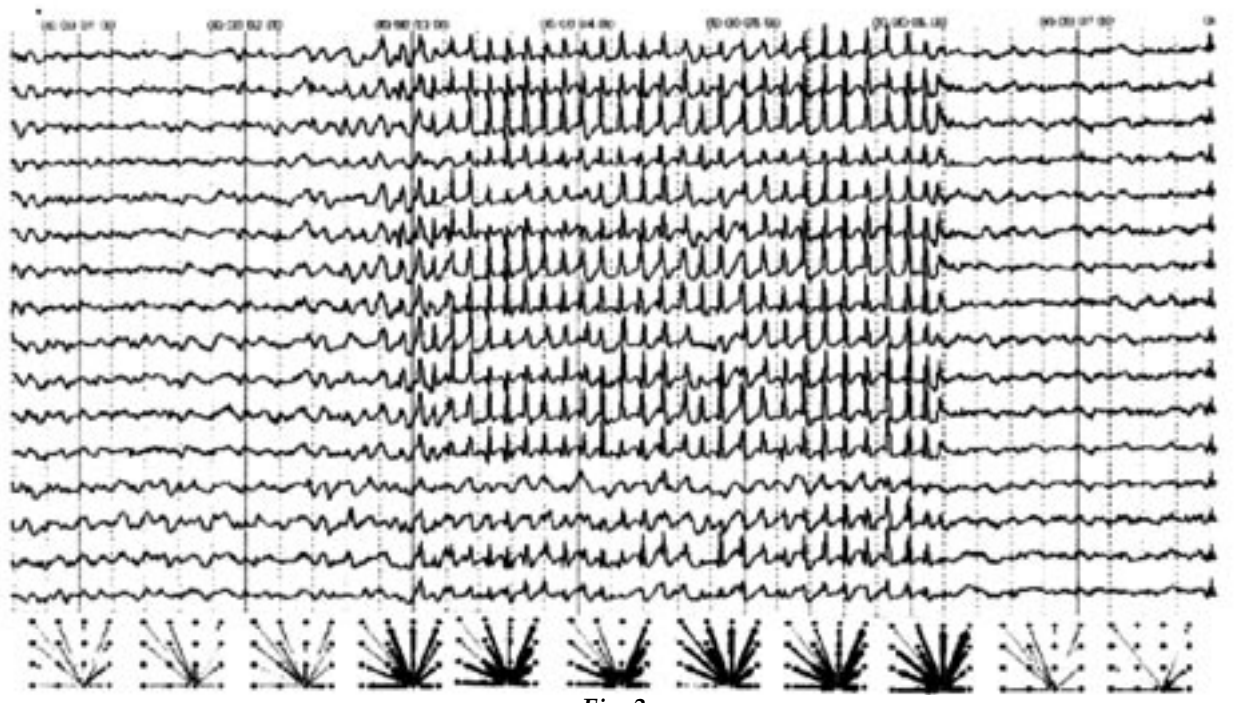

Fig. 3

A generalised spike-wave discharge recorded from the lateral convexity of the neocortex of a WAG/Rij rat. Below the discharge, the results of the non-linear association analysis are presented as performed on EEG epochs of 500 msec. The start point of arrows reflects the beginning of activity at the somatosensory cortex in all eleven time frames. The thickness of the arrows represents the strength of the association, while the arrowheads point into the direction of the lagging site. The results of the analysis consistently suggest a cortical focus. Spike-wave discharges recorded at other cortical sites lag the focal site with time delays that increase with electrode distance. (Adapted from Meeren et al.).[73]

Firstly, some cortical neurons are endowed with intrinsic abilities for the generation and synchronization of sustained oscillations. In fact, neocortical pyramidal neurons of the deep cortical layers, independently from the thalamus, could fire in "intrinsically bursting" mode with rhythmic recurrent spike bursts of 5-10 Hz, thus generating rhythmic oscillations in the frequency of SWDs.[76-78] Secondly, cortical neurons are capable to synchronize their bursting activity in specific oscillatory networks that contain pyramidal cells and inhibitory interneurons.[79] Thirdly, the cortical neurons (mostly pyramids) could effectively control thalamic activity by means of descending projections from the deepest cortical layers,[80] which are several times more intensive than ascending ones originating from the thalamus.[81]

In the thalamocortical network in which SWDs are generated, connections between the cortex and the thalamus are assumed to form closed loops where cortical neurons are excited by thalamic neurons and projected back (rule of reciprocity). Cortical neurons also project to other thalamic nuclei that have no direct terminals to certain cortical areas. Therefore, corticothalamic connections comply with a more general rule, the rule of parity.[80] With this type of connections excitation can spread throughout and even beyond the thalamocortical system so that paroxysmal activity can be distributed outside the prime neocortical site.[38]

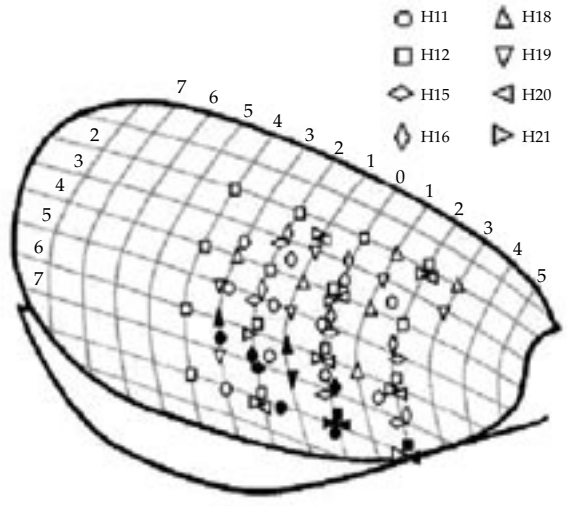

Fig. 4

Pooled data from eight rats, all with leading sites (filled symbols) in the somatosensory cortex. Open symbols represent lagging sites. The numbers from 5 (anterior) to 7 (posterior) represent the coordinates of the cortical area in mm, while the numbers 2 to 7 represent the lateral coordinates in mm. (Adapted from Meeren et al.).[73]

Based on *in vitro* investigations into the cortical neuronal activity, Silva et al.[82] showed the capability of cortical neurons to produce synchronous self-sustained 5-12 Hz oscillations. These authors were the first to suggest that networks of neocortical neurons might be the exclusive pacemakers for some EEG rhythms. Later, this view received support from Nicolelis et al.[83] who described the 7-12 Hz "somatosensory rhythm" in freely moving rats, originating from the neocortical projection area of vibrissae. Rodents frequently use rhythmic movements of their facial whiskers to obtain sensory information about location and texture of external objects, where humans rely on their fingers. Tactile perception with vibrissae is called "whisking", consisting of rhythmical protraction and retraction of whiskers with a frequency of 5-15 Hz. Most likely, the initiation and control of the movements of these whiskers are due to top-down propagation of the cortical "somatosensory" rhythm.[84-86] Although the frequency of this rhythm is similar to sleep spindles (7-12 Hz), it resembles more the "mu"-rhythm described in humans and "sensorimotor" rhythm in cats.

In contrast with sleep spindles, the "somatosensory rhythm" (1) rarely occurs during light sleep, and (2) is characterized by much longer (more than 1-2 s) and less stereotyped episodes.[83] Whisking is preceded by distinctive widespread 7-12 Hz synchronous oscillations in the neocortical projective area that descend to the subcortical structures of the trigeminal system in the ventroposterior medial nucleus of the thalamus and brainstem.[83] Having a cortical origin, the "somatosensory rhythm" is spread over the same corticothalamic circuitry that is involved in the genesis of SWDs. It is proposed that not only in rats with genetic predisposition to absence epilepsy, namely, WAG/Rij rats and GAERS, but also in many older Wistar, WKY and Sprague-Dawley rats, normal somatosensory oscillations are transformed into SWDs. It has been shown by the investigators of the somatosensory rhythm that genetic models of absence seizures can be pro-

duced by selective inbreeding of rat strains that show clear 7-12 Hz oscillations. However, extensive analysis has disparaged this view by showing that 7-12 Hz oscillations alone cannot lead to seizure activity. Instead, genetic manipulations such as selective inbreeding or selection are required to make epileptic activity emerge.[87] This genetic predisposition seems to be more readily present in albino rats than in others, taking into account the higher incidence of SWDs in albino than in hooded, brown and agouti rats.[88] This might result from the presence of the albino gene, resulting in a deteriorated visual system, favoring development of other sensory systems such as whisker areas, which in some cases become more excitable; hence, the source of pathological rhythms.

In addition to that, there is experimental evidence that the 7-12 Hz cortical rhythm may control the thalamic bursting activity, in that inactivation of the somatosensory cortex with the GABAa agonist muscimol abolished both whisker twitching and bursting activity in the ventroposterior medial nucleus of the thalamus in awake rats.[89] The observation that SWDs are often accompanied by abnormal whisker twitching may at least partly account for the same cortical mechanism involved in both whisking and SWDs.[44,90]

We propose that the somatosensory cortex of the rodent models of absence epilepsy produces, due to specific morpho-physiological characteristics, a spontaneous 7-12 Hz rhythm, which in turn activates a cortico-thalamo-cortical loop and, therefore, facilitates epileptogenesis in rats with genetic predisposition to absence seizures.

We attempted to deactivate the assumed focal area in WAG/Rij rats by local application of 2% lidocaine.[91] As known, lidocaine temporarily blocks sodium channels and reversibly inhibits neuronal activity.[92,93] It was hypothesized that blocking neuronal activity in the specific somatosensory area would lead to a unilateral elimination of the cortical triggering of SWDs, resulting in a temporal decrease in their number. Experiments were made in freely moving animals following microinjections (1 µl) of either 2% lidocaine or saline. Just after each injection, EEG activity was monopolarly recorded from four cortical sites including adjacent and remote areas. Lidocaine injection was associated with a local decrease in the spectral amplitude of EEG in the surrounding areas and a decrease in the number of SWDs. However, the difference in the number of SWDs between saline- and lidocaine-treated animals gradually diminished within two

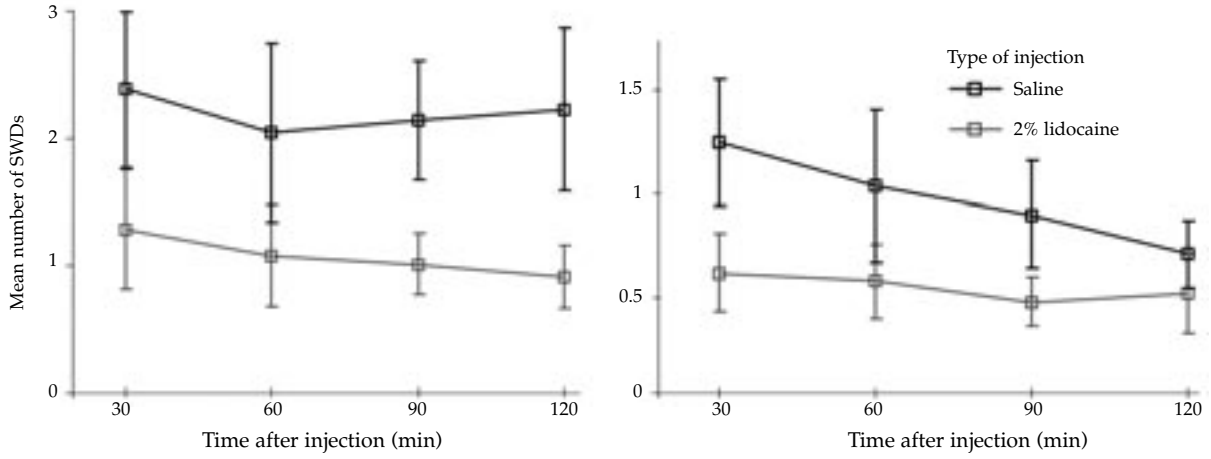

Fig. 5

Time course of SWDs during two hours after injections of saline and 2% lidocaine. Amount of SWDs were calculated in four successive 30-min intervals (mean per 5 min±s.e.m). The left plot presents numerical data disregarding state of vigilance. The right plot represents data corrected for the state of vigilance.. Application of 2% lidocaine resulted in a continuous decrease in the number of SWDs in comparison to saline. ANOVA analysis revealed that this effect was significant for uncorrected ($F=8.02$, df 1,12, $p<0.02$) and for the vigilance-corrected data ($F=4.75$, df 1,12, $p<0.05$). Orthogonal trend analyses showed that the two groups differed in the linear trend ($Flin=4.23$, df 1,12, $p<0.05$), demonstrating that after taking the behavioral influences (the right graph) into account, the initial difference between lidocaine and saline is getting smaller over time. (From Sitnikova and van Luijtelaar).[91]

hours after injection. These results, as presented in Figure 5, demonstrate that functional deactivation of the driving cortical source provokes a clear effect on the expression of SWDs, implicating a possible role of this specific cortical site in the initiation of SWDs.

A second experimental approach to study the inner properties of the focal area in the somatosensory cortex, which might underlie the initiation of SWDs, is the morphometric analysis of neuronal geometry. In this work, it was investigated *(i)* whether the structure of

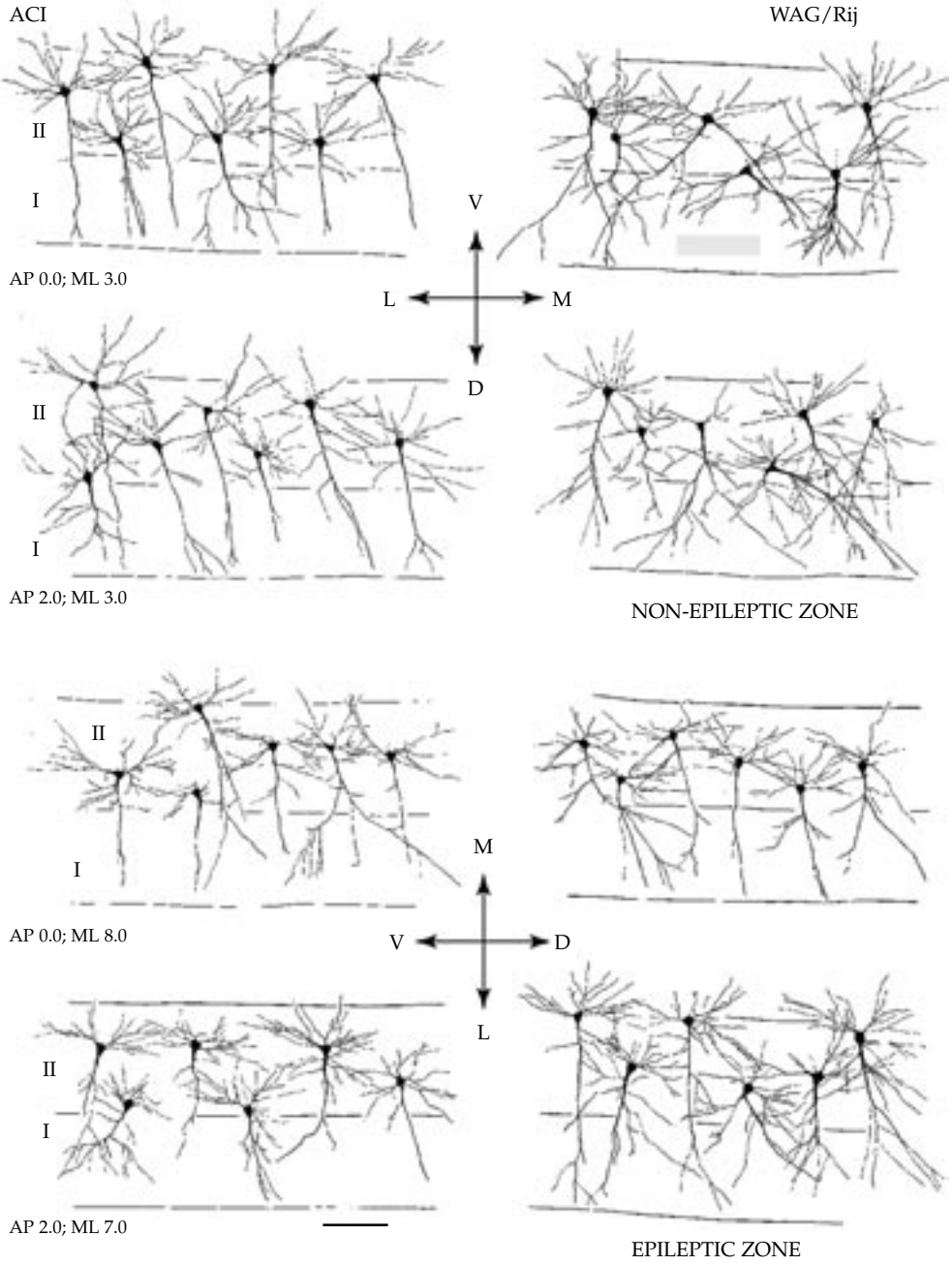

Fig. 6

Composite extended drawings of the Golgi-impregnated neurons of two cortical areas, frontal (presumably less-epileptic) and somatosensory (epileptic) areas, in ACI (non-epileptic) and WAG/Rij rats . Coordinates of each zone are given below pictures according to the atlas of rat brain (Paxinos and Watson, 1998).[94/96] Dendritic spines are not drawn, cortical layers are shown in roman numerals. Scale = 100 µm. (Adapted from Karpova et al., 2004).[97]

cortical cells in the focal cortical zone in WAG/Rij rats, a prerequisite for SWDs, would differ from other cortical periphery, and *(ii)* whether the morphology of neurons in the cortex of WAG/Rij rats is different from that of non-epileptic ACI rats. There is a general belief that typical absence epilepsy is purely a "functional" disease since no structural lesion of any kind has been identified as its probable substrate.[31,94] Morphological studies are rare; however, microdysgenesis was demonstrated in childhood absence epilepsy with an increased number of dystopic neurons in the neocortex and subcortical white matter of the frontal lobe.[95] Structural alterations have never been reported in cortical tissue of absence epileptic rats, neither in general nor specifically in the somatosensory area. Being in the framework of the focal theory of absence seizures, this analysis particularly focused on the investigation of the neuronal structure in that specific part of the somatosensory cortex containing the focal epileptic area. The cellular composition and geometry of dendritic trees were established with the Golgi-staining technique and quantitative morphometric analysis (Karpova et al. 2004).[97] Striking disturbances were noted in the distribution of pyramidal cells in the superficial layer (II) of the somatosensory and motor cortex of WAG/Rij rats compared to non-epileptic ACI rats. Apical dendrites of the superficial pyramidal cells were often split in two branches, declined, and exhibited a non-perpendicular course (Fig. 6). Morphometric measurements of the dendrites revealed that the superficial pyramidal cells in WAG/Rij rats were significantly different from those in ACI rats. More specifically, the total length of the dendrites, the mean length of the dendritic segment, and the size of the dendritic arbor were increased. In WAG/Rij rats, the pyramidal cells specifically within the focal zone showed clear and significant increases with respect to the length and branching of the dendrites. These dendritic morphologic features assume a different intracortical connectivity pattern in the cortex of WAG/Rij rats; elongation of the dendrites with higher branching, as specifically noted in the somatosensory neurons of epileptic rats, might reflect an enlargement of an intracortical network which facilitates spreading of SWDs from the site of initiation. Interestingly, differences that were found in pyramidal cells of the cortical layer II, which is the main source of the intracortical interconnections, could play a role in the generalization and propagation of SWDs.

The results of the above-mentioned studies made in the somatosensory cortex of WAG/Rij rats (PV-immunostaining, microinjection of lidocaine, and analysis of the dendritic structure with Golgi-staining) support our theory that a specific cortical focus controls and drives corticothalamic widespread networks during spontaneous SWDs in genetically predisposed rats and that global neurochemical changes in functioning of the GABA-ergic system and local morphometric changes in pyramidal cells in the focal zone might contribute to the transformation of spontaneous oscillations into those of pathological.

Whether the same mechanisms are involved in the pathophysiology of human primary generalized epilepsy needs to be established. Nevertheless, some recent evidence suggests that generalized absence epilepsy may arise in the cortex. An independent frontal cortical source at the beginning of a SWD was described with the aid of Independent Component Analysis.[98] A cortical source was demonstrated using dipole source analysis of EEG in patients with absence seizures.[99] It was concluded by Leutmezer et al.[100] that findings of new imaging studies were in good accordance with EEG findings which suggested that generalized epileptiform discharges were generated in the frontal cortex or at least appeared in the frontal cortex earlier than they did in other parts of the brain.[101]

It is of our opinion that SWDs accompanying absence seizures do not appear due to a general increase in cortical excitability, as proposed by the cortico-reticular theory. Instead, a focal cortical area is likely to be the origin for SWDs. The paroxysmal activity which is probably originated from a normal somatosensory rhythm might arise from the somatosensory cortex due to a decreased GABA-ergic inhibition and reduced Ca^{2+} buffering capacity. Through the descending cortico-thalamic pathways, the RTN and the

relay nuclei are involved in generating SWDs. More intense intracortical interconnections may play a role in the generalization and propagation of SWDs.

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