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# Impairment of intracortical GABAergic inhibition in a rat model of absence epilepsy

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**Abstract**

The WAG/Rij rat strain is characterized in its EEG by the manifestation of spike-wave discharges which resemble in their spontaneous appearance and pharmacological sensitivity the absence epilepsy observed in humans. In order to test the hypothesis whether cellular intrinsic membrane and/or synaptic network properties in the neocortex are modified in this form of epilepsy, we analyzed with extra- and intracellular recording techniques the functional status of neocortical slices obtained from adult epileptic WAG/Rij rats and compared them with the data acquired from non-epileptic control Wistar rats. Intrinsic membrane properties, like resting membrane potential, neuronal input resistance and basic cellular firing characteristics, did not differ between these two strains. However, the analysis of extra- and intracellularly recorded synaptic responses revealed an intracortical hyperexcitability which was accompanied by a significant reduction in the efficiency of GABAergic inhibition. Our data indicate that the imbalance between intracortical excitatory and inhibitory mechanisms may at least contribute to the expression and augmentation of spike-wave discharges in epileptic WAG/Rij rats.

*Keywords:* WAG/Rij rat; Wistar rat; Cortical slice; Intra- and extracellular recordings; GABAergic inhibition; Hyperexcitability

## 1. Introduction

Currently there are two major theories on the emergence of electrophysiological signs of absence epilepsy. The first theory is based on experimental findings in cats that generalized spike-wave discharges can be induced by a single intramuscular injection of penicillin [11,17]. Sudden generalized bilaterally synchronous spike-wave discharges can be seen in the cortical EEG after blockade of the $GABA_A$ receptor with penicillin. According to Gloor et al. [11], systemic penicillin application gives rise to cortical neurons to respond to afferent thalamocortical volleys with spike-wave discharges, while in non-treated animals these volleys produce sleep spindles. According to this hypothesis, the development of spike-wave discharges primarily depends on a change in intracortical excitability [3]. However, since penicillin was applied systemically, the origin of the functional modification cannot definitely be restricted to the neocortex, but rather subcortical structures are also influenced. The second theory is based on experiments in cats and rats showing spontaneously occurring oscillations, such as sleep spin-

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dles and spike-wave discharges [4,35]. According to this hypothesis, rhythmic synchronous EEG patterns, such as sleep spindles and spike-wave patterns, originate from an intrathalamic circuitry. Anatomical data showed that the reciprocal thalamocortical fibers send and receive collaterals to the reticular thalamic nucleus (for review see [13]). Steriade et al. [35] propose a critical role of this reticular thalamic nucleus in the initiation and maintenance of thalamic oscillations. From lesioning experiments [2] and surgical disconnection of the reticular thalamic nucleus [36], it was concluded that the reticular thalamus acts as a pacemaker. Buzsáki [4] proposes that spike-wave discharges result from interactions between the relay thalamus and the reticular thalamic nucleus. In vivo experiments in the Genetic Absence Epilepsy Rat from Strasbourg (GAERS) by Avanzini et al. [2] indicate that $Ca^{2+}$ and/or $Ca^{2+}$-dependent $K^+$ conductances of reticular thalamic neurons profoundly contribute to the generation of cortical spike and wave discharges. However, a contribution of neocortical structures cannot be excluded and has to be analyzed in chronic models (for a recent discussion of this issue see [34]). Functional modifications at the neocortical level may result from (1) changes in the cells intrinsic conductances, (2) an increase in excitation, for example by augmentation of the thalamocortical drive or enhancement of intracortical *N*-methyl-D-aspartate (NMDA) components, or (3) a decrease in intracortical inhibitory mechanisms. In order to clarify the role of the neocortex in generating the spike-wave discharges, the functional properties of cortical structures were studied in adult WAG/Rij rats. Rats of this strain have a genetically determined type of epilepsy [28], with hundreds of spontaneously occurring spike-wave discharges per day. These discharges are accompanied by small, clinical manifestations, such as facial myoclonic movements [8,38], that frequently occur during periods of passive wakefulness and light slow wave sleep [10]. Spike-wave discharges in WAG/Rij rats can be suppressed by typical anti-absence drugs, such as trimethadione and ethosuximide, and by broad spectrum anti-epileptics, such as valproate, diazepam and loreclezole, while they are aggravated by carbamazepine, diphenylhydantoin and tiagabine [1,7,29]. In the present study a comparison was made between cortical excitability in the fronto-parietal cortex of adult WAG/Rij rats and non-epileptic Wistar controls. Parts of these results have been previously published in abstract form [21].

## 2. Methods

Subjects were 6 six-months-old WAG/Rij rats and 42 Wistar rats of about the same age. We selected this age group since a previous study has shown that the expression and frequency of spike-wave complexes in WAG/Rij rats is strongly age-dependent and that WAG/Rij animals younger than 3 months are almost seizure-free [9]. Extra- and intracellular in vitro recordings were obtained from the fronto-parietal cortex of the rats since these cortical parts show the largest amplitude of the spike-wave discharges and they receive extensive thalamic projections. The techniques for preparing and maintaining neocortical slices were similar to those described previously [19]. In brief, rats were deeply anaesthetized with pentobarbital sodium (50 mg/kg bodyweight i.p.), decapitated and a block of the brain including the frontoparietal cortex was rapidly removed. We consider it unlikely that the pentobarbital anaesthesia may have any long-term effects on the functional state of the neocortical slices because a previous study has shown that pentobarbital can be easily and repetitively washed out of the bathing solution under similar experimental conditions [16]. Coronal slices of 400-$\mu$m thickness were cut on a Dosaka vibratome, trimmed to smaller pieces and immediately transferred to an interface-type recording chamber or to an incubation-storage chamber. The bathing solution contained (in mM) 124 NaCl, 3 KCl, 1.25 $NaH_2PO_4$, 1.8 $MgSO_4$, 1.6 $CaCl_2$, 26 $NaHCO_3$, and 10 glucose, and had a pH of 7.4 when saturated with 95% $O_2$–5% $CO_2$. The temperature of the bathing solution in the recording chamber was 34–35°C and in the storage chamber 32–33°C. All preparations were performed between 9 and 10 a.m. to avoid functional changes due to a circadian rhythm in the expression of spike-wave discharges in WAG/Rij rats [39]. After an incubation period of $> 1$ h, extracellular field potential (FP) responses were recorded in layer II/III with 2- to 5-M$\Omega$ electrodes to electrical stimulation of the underlying white matter/layer VI. Electrical stimuli

of 200-$\mu$s duration and 2–40-V intensity were applied via a sharpened bipolar tungsten electrode at a frequency of $\leq 0.1$ Hz. The efficacy of intracortical inhibition in extracellular recordings was estimated by the use of a paired-pulse stimulation protocol (for details see [19,20]). Double stimuli of identical duration and intensity were applied at inter stimulus intervals of 15–30 ms and the peak-to-peak amplitudes of the first ($FP_1$) and second ($FP_2$) response were measured to calculate the amount of inhibition from the following formula:

$$\text{percentage inhibition} = [(FP_1 - FP_2)/FP_1] \times 100$$

Because the efficacy of inhibition estimated with this protocol is strongly dependent on the stimulus intensity (see Fig. 1 in [20]), the strength of the electrical stimuli was adjusted to twice the magnitude needed to evoke a maximal $FP_1$ response.

Intracellular recordings in layers II/III and layer V were performed with 60–120 M$\Omega$ microelectrodes filled with 2 M potassium acetate. Only cells with a stable resting membrane potential ($V_m$) more negative than $-65$ mV and with a neuronal input resistance ($R_N$) of more than 20 M$\Omega$ were included in the data analysis. Neurons were categorized according to their discharge pattern to injection of suprathreshold depolarizing current pulses into regular spiking, intrinsic bursting and fast spiking cells [25]. The synaptic network properties were analyzed with intracellular recording techniques by eliciting

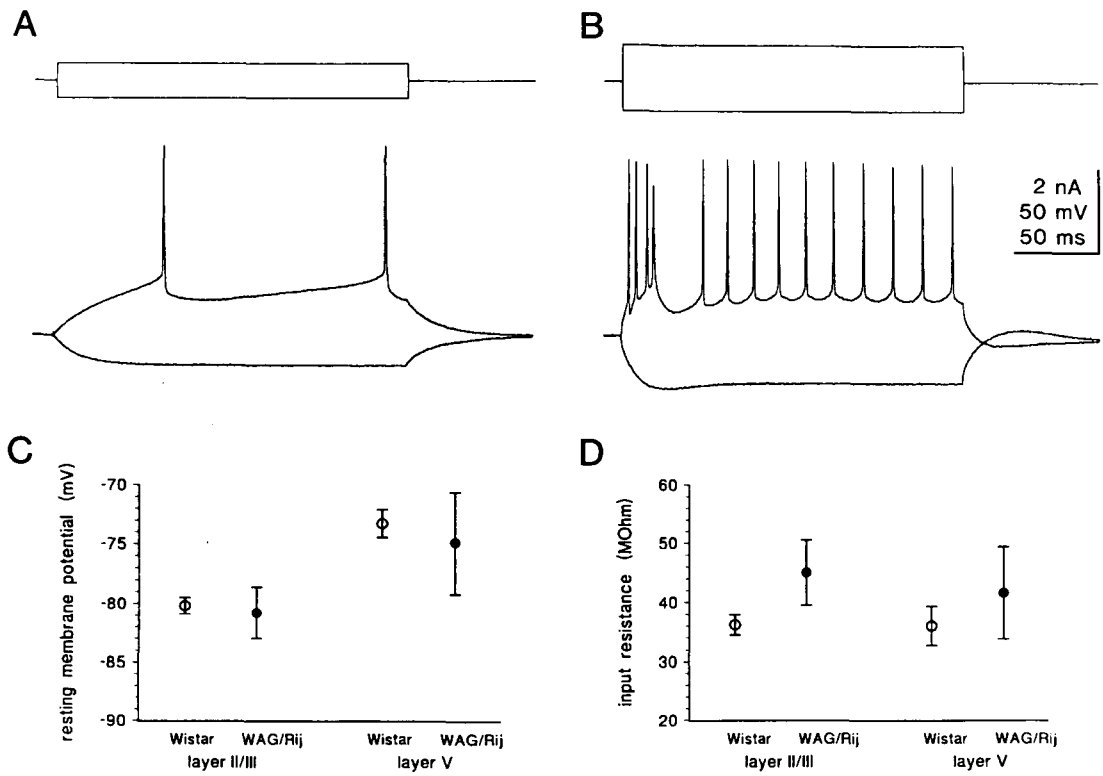


Fig. 1. Intracellular recordings and comparison of intrinsic membrane properties between Wistar and WAG/Rij rats. A: Characteristic response pattern of a regular spiking cell recorded in supragranular layers of a WAG/Rij rat at $V_m = -84$ mV. B: Typical intrinsic bursting neuron recorded in layer V of a WAG/Rij animal at $V_m = -66$ mV. Note pronounced burst discharge and repetitive single action potentials as response to injection of suprathreshold current pulse. C: Average resting membrane potentials of neurons recorded in layers II/III (left) and layer V (right) of Wistar (○) and WAG/Rij (●) rats. D: Membrane input resistance determined at $V_m$ in layers II/III (left) and layer V (right) neurons of Wistar (○) and WAG/Rij (●) animals. Bars on each symbol indicate standard error of the mean.

excitatory and inhibitory postsynaptic potentials (EPSPs and IPSPs, respectively). Subthreshold stimuli of various intensities were used to evoke an EPSP. To elicit an IPSP, stimulus intensities were adjusted to twice the threshold to evoke an action potential. The reversal potential of the IPSP ($E_{IPSP}$) was determined by linear regression from a plot of the IPSP peak response amplitude versus membrane potential. The IPSP peak conductance ($G_{IPSP}$) was calculated by linear regression analysis from the slope of the plot of the relation between the membrane potential deflection at the peak of the IPSP versus injected current. A contamination of voltage-activated conductances was minimized by performing this analysis in the linear range of the cell's current–voltage relationship. The pure IPSP peak conductance was then calculated by subtracting the cell's resting conductance from the estimated conductance at the IPSP peak amplitude [19,20]. The presence of long-latency epileptiform FP responses, multiphasic intracellularly recorded EPSPs or synaptically evoked burst discharges was investigated with electrical stimuli of different intensities. Stimulus-evoked FPs and single cell synaptic responses that

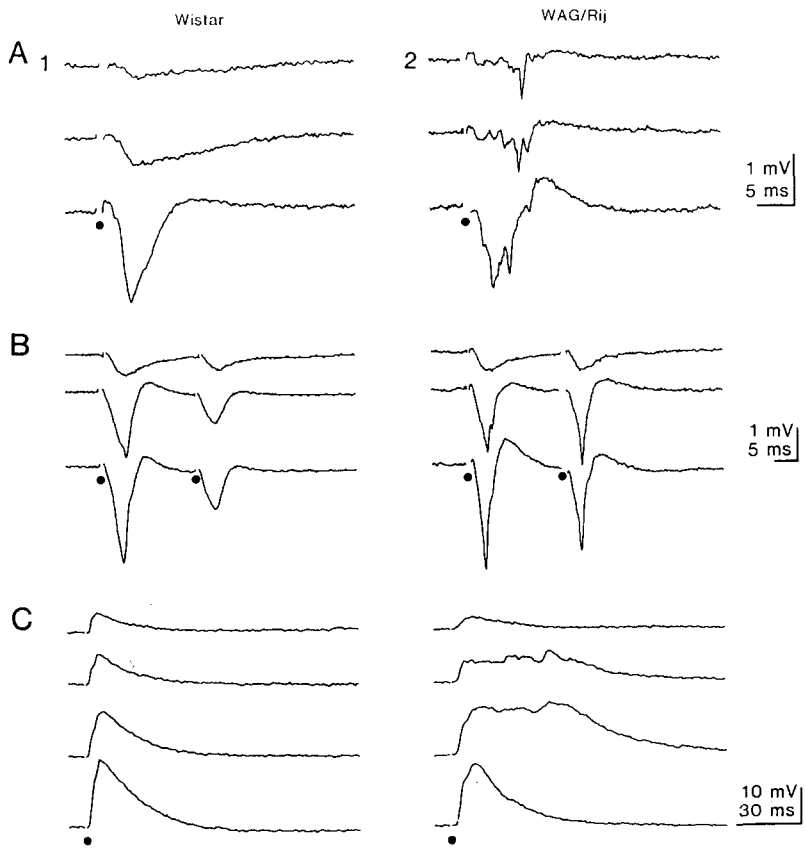


Fig. 2. Stimulus-evoked synaptic responses in neocortical slices obtained from Wistar (left column, 1) and WAG/Rij rats (right column, 2). A: Field potential responses recorded in layers II/III to three different stimulus intensities (●). Note expression of epileptiform activity in the recordings from the WAG/Rij animal. B: Field potential responses to a double stimulus with an inter-stimulus interval of 20 ms. Higher stimulus intensities evoke an increase in the initial response and, only in the Wistar rat, an increase in paired-pulse inhibition, as demonstrated by the suppression of the response to the second stimulus. Negativity in field potential recordings in A and B is shown downward. C: Intracellular recordings from a supragranular regular spiking cell in a slice obtained from a Wistar (left, $V_m = -80$ mV) and a WAG/Rij rat (right, $V_m = -91$ mV). Electrical stimuli of four different intensities evoke a gradually increasing monophasic EPSP in the Wistar rat and an epileptiform EPSP in the WAG/Rij animal.

revealed a multiphasic component were classified as epileptiform. For statistical analysis a Student's *t*-test was performed on the data. If not otherwise noted, values throughout this report are given as mean ± S.D.

## 3. Results

In vitro recordings were performed in coronal fronto-parietal cortical slices obtained from 42 adult Wistar and 6 adult WAG/Rij rats. Out of the 141 stable intracellular recordings in Wistar and 23 cells recorded in WAG/Rij rat cortical slices, 122 and 19 neurons were classified according to the criteria of McCormick et al. [25] as regular spiking cells, respectively (Fig. 1A). In addition, 12 and 2 typical fast spiking cells with non-accommodating high frequency (> 400 Hz) discharges could be recorded in Wistar and WAG/Rij slices, respectively. Intrinsic bursting behaviour could be observed in 5 and 2 neurons in Wistar and WAG/Rij cortical slices, respectively, and in both experimental groups the soma location of these cell types was restricted to layer V (Fig. 1B). Two of the 141 cells recorded in Wistar cortical slices could not be categorized because current–voltage analyses were not performed in these two neurons. No obvious differences in the functional properties of these three cell categories could be detected in comparison between the two experimental groups. In addition, other intrinsic membrane properties like resting membrane potential ($V_m$) and neuronal input resistance ($R_N$) were not significantly different between Wistar and WAG/Rij rats. In agreement with a previous report [24], neurons recorded in layer II/III revealed a more hyperpolarized $V_m$ as compared to the layer V cells (Fig. 1C). Layer II/III cells in Wistar and WAG/Rij neocortical slices had a $V_m$ of $-80.2 \pm 7.1$ mV ($n = 97$) and $-80.8 \pm 8.6$ mV ($n = 16$), respectively. The $V_m$ measured in layer V cells was $-73.3 \pm 5.7$ mV ($n = 44$) and $-74.9 \pm 10.5$ ($n = 7$) for Wistar and WAG/Rij rats, respectively. Neurons recorded in WAG/Rij rats tended to have a slightly higher $R_N$, but these differences were not significant at the $P < 0.05$ level (Fig. 1D). Supragranular neurons in Wistar and WAG/Rij cortical slices revealed a $R_N$ of $36.2 \pm 15$ MΩ ($n = 81$) and $45 \pm 20.5$ MΩ ($n = 15$), respectively. In layer V cells the $R_N$ values were estimated to $36 \pm 19.8$ MΩ ($n = 35$) and $41.6 \pm 19.1$ MΩ ($n = 7$), respectively.

In contrast to the intrinsic membrane properties, significant differences could be detected between Wistar and WAG/Rij rats in the synaptic properties of the neocortical network. Both extra- and intracellular recording techniques revealed a decrease in the efficacy of GABAergic inhibition and a hyperexcitability in neocortical slices obtained from WAG/Rij rats. Whereas all extracellular recorded field potential (FP) responses in Wistar cortical slices ($n = 23$) showed the normal short-lasting mono- or biphasic response pattern (Fig. 2A1), 15.6% of the FP responses ($n = 90$) recorded in supragranular layers of WAG/Rij neocortical slices clearly revealed a polyphasic and often long-lasting activity pattern (Fig. 2A2). Therefore these responses were classified as epileptiform. In addition to these strain-specific differences in the expression of neuronal hyperexcitability, WAG/Rij cortical slices were characterized by a significant reduction of paired-pulse inhibition (Fig. 2B). Whereas paired-pulse inhibition in supragranular layers of Wistar rats amounted to 68.3 ± 14.2% ($n = 23$, fig. 2B1), WAG/Rij animals revealed a strength of intracortical inhibition of only $59.8 \pm 19.4\%$ ($n = 90$, Fig. 2B2) ($P < 0.05$). Electrical stimuli of similar intensity (up to 40 V) were necessary in Wistar and WAG/Rij cortical slices to evoke a maximal FP response, but comparable stimulus intensities produced a smaller paired-pulse inhibition in the latter (compare Fig. 2B2 and Fig. 2B1).

In agreement with the extracellular recordings, intracellular synaptic responses in WAG/Rij cortical slices were also characterized by a hyperexcitability and a loss of GABAergic inhibition. Normal monophasic and short-lasting EPSPs could be observed in 94.3% of the cells recorded in Wistar ($n = 35$, Fig. 2C1) and in 76% of the neurons analyzed in WAG/Rij animals ($n = 21$). However, in five neurons recorded in WAG/Rij cortical slices electrical stimulation of the afferent pathway with relatively low intensity elicited a long-lasting EPSP that clearly consisted of multiple components (middle traces in Fig. 2C2). Similar multiphasic EPSPs could be observed in only 2 of the 35 cells recorded in Wistar rats.

The intracellular analysis of the efficacy of the

intracortical GABAergic system revealed a significant difference between the two experimental groups. A biphasic inhibitory postsynaptic potential (IPSP) consisting of a $GABA_A$ receptor-mediated fast (f-) IPSP and a $GABA_B$-mediated long-latency (l-) IPSP could be observed in every cell recorded in Wistar cortical slices ($n = 27$) and in 85.7% of the cells analyzed in WAG/Rij rats ($n = 15$) (Fig. 3A). The remaining neurons in WAG/Rij rats either showed a very small IPSP (Fig. 3B) or no postsynaptic hyperpolarizing component at all. Since supragranular neurons differ in their inhibitory synaptic input from layer V cells [6], the properties of IPSPs elicited in layer II/III only were analyzed in more detail and compared between the two experimental groups. The reversal potential of the $Cl^-$-dependent f-IPSP estimated $-74 \pm 5.7$ mV ($n = 27$) in Wistars and $-70.5 \pm 9.5$ mV ($n = 8$) in WAG/Rij rats (Fig. 3C, left). The $K^+$-dependent l-IPSP showed a more hyperpolarized reversal potential in both experimental groups and estimated $-90.3 \pm 6.1$ ($n = 27$) and $-88.8 \pm 6.7$ mV ($n = 8$) in supragranular neurons recorded in Wistar and WAG/Rij cortical slices, respectively (Fig. 3C, right). A prominent difference

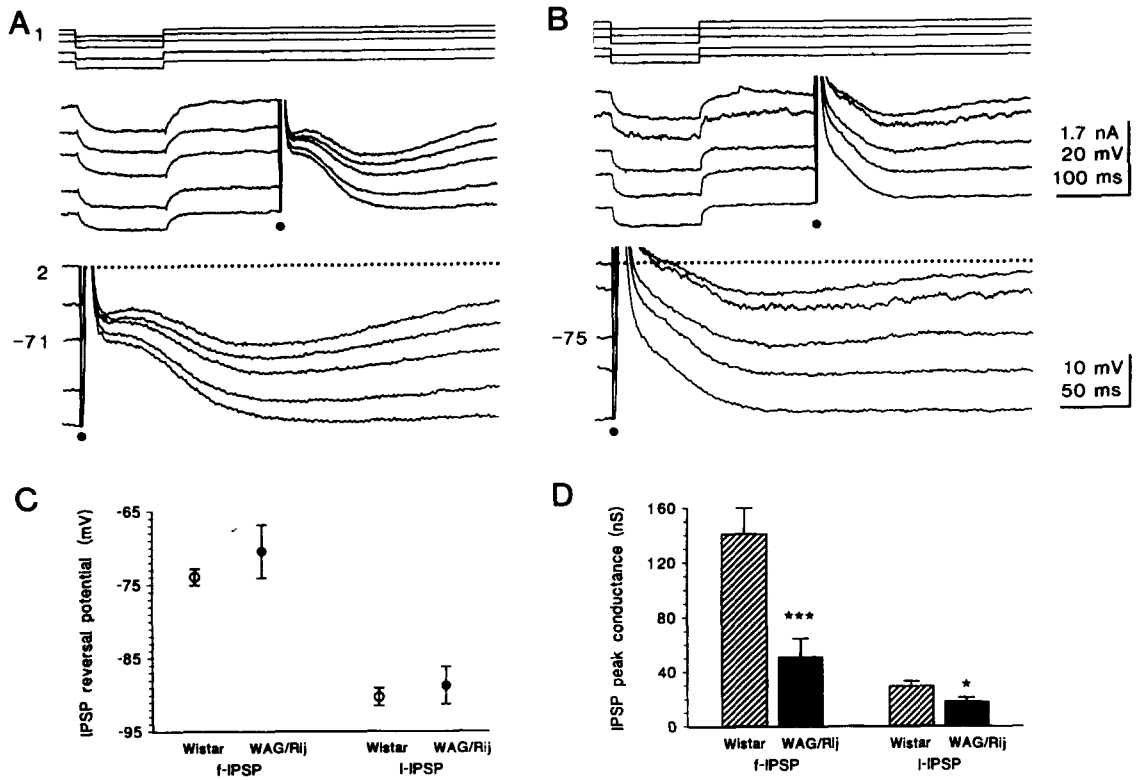


Fig. 3. Properties of stimulus-evoked IPSPs recorded in neocortical slices obtained from Wistar and WAG/Rij rats. A: Intracellular recordings from a layer II/III regular spiking cell ($V_m = -71$ mV) in a cortical slice obtained from a WAG/Rij rat. Current protocol (upper trace in A1) and corresponding voltage responses (lower trace in A1) at different de- and hyperpolarizing membrane potentials. Electrical stimulation (●) of the afferent pathway evokes a prominent biphasic IPSP that is shown in A2 at a higher gain. B: Recordings from a supragranular regular spiking cell in a WAG/Rij rat cortical slice ($V_m = -75$ mV). Note the lack of a hyperpolarizing f-IPSP and the small amplitude of the l-IPSP. Action potentials in A and B are truncated. Resting membrane potential of the cells is indicated in A2 and B2 to the left of the middle traces. Dotted line in A2 and B2 displays the most depolarized potential. C: Average reversal potential of the f-IPSP (left) and l-IPSP (right) in layers II/III neurons recorded in neocortical slices from Wistar (○) and WAG/Rij (●) rats. D: Average peak conductance of the f-IPSP (left) and the l-IPSP (right) calculated in supragranular neurons recorded in Wistar (○) and WAG/Rij (●) neocortical slices. Bars on each symbol and column symbolize standard error of the mean. Asterisks in D indicate significant differences between Wistar and WAG/Rij rats at the $P < 0.02$ (*) and $P < 0.001$ (***) level.

could be detected between Wistar and WAG/Rij rats in the efficacy of the f- and l-IPSP in layer II/III cells (Fig. 3D). The peak conductance of the f-IPSP was significantly ($P < 0.001$) smaller in WAG/Rij's ($50.8 \pm 36.5$ nS, $n = 8$) as compared to the control Wistar rats ($140.8 \pm 96.1$ nS, $n = 26$). A significant difference ($P < 0.02$) could be also detected for the relative strength of the l-IPSP between WAG/Rij rats ($17.9 \pm 7.5$ nS, $n = 8$) and Wistars ($29.2 \pm 19.1$ nS, $n = 26$).

## 4. Discussion

Our results show that the efficacy of intracortical GABAergic mechanisms is considerably reduced in fronto-parietal cortical slices of WAG/Rij rats when compared to non-epileptic Wistar controls. At first a note has to be made with respect to the non-epileptic character of Wistar rats. Also spike-wave discharges can be noticed in random bred Wistar rats, but the prevalence and incidence of these discharges is much lower compared to age-matched WAG/Rij rats [14]. In two studies it was shown that at an age of six months only 10 or 20% of random bred rats including Wistars show spike-wave discharges [15,31]. It is therefore safe to conclude that Wistar rats quantitatively differ in the amount of spontaneously occurring spike-wave discharges from WAG/Rij rats. All adult WAG/Rij rats abundantly show the bilateral generalized spike-wave discharges in the cortical EEG (for review see [8]).

Although significant differences could be detected between Wistar and WAG/Rij rats in the synaptic properties of the neocortical network, no differences could be discovered in the analyzed intrinsic properties of the cortical neurons of the two rat strains. The resting membrane potentials, the neuronal input resistances as well as the basic cellular firing properties, appeared not significantly different between Wistar and WAG/Rij rats. Nevertheless, both extra- and intracellular recording techniques revealed a significant decrease in the efficacy of GABAergic inhibition and a hyperexcitability in neocortical slices of WAG/Rij rats. This is best expressed both in the epileptiform extracellular field potential responses and in the EPSPs and IPSPs recorded intracellularly in slices of WAG/Rij rats. These data imply that the elements of the network are not changed, but that the tuning of the network is more sensitive to incoming volleys. The cortical circuitry is easier brought into a more extreme response, expressed in the EEG as spike-wave discharges. This implies that the present study confirms and extends the experimental data from Gloor and coworkers [11]. These authors showed that systemic penicillin application induced spike-wave discharges in cats and suggested that a pharmacologically induced change in cortical excitability was the underlying factor. They reasoned that spindle volleys coming from the thalamus were transformed into spike-wave discharges at the cortical level, when this level was made hyperexcitable by penicillin. We extend Gloor's theory to another species and to a genetic type of absence epilepsy. The latter is important since generalized epilepsies in man, including absence epilepsy, are also genetically determined [18,26].

Changes in the cortical excitability might be a permanent characteristic of the fronto-parietal cortex in this type of epileptic rat with a genetically determined high incidence of spike-wave discharges. Our results are also in good agreement with in vivo and in vitro studies on the GAERS rat strain by Pumain et al. [30]. By the use of different electrophysiological techniques, the authors characterize stronger NMDA receptor-mediated components in the sensori-motor cortex of rats with petit mal-like seizures. These long-lasting NMDA components are also more widely distributed throughout all cortical layers [30], suggesting a significant NMDA receptor-mediated intracortical hyperexcitability in GAERS rats. This hyperexcitability may result from molecular-biological (e.g. alterations in the subunit composition), anatomical (e.g. changes in the receptor density) and/or functional (e.g. reduction in $Mg^{2+}$ sensitivity) modifications of the NMDA receptor. However, they may be also subsequently caused by a decrease of intracortical GABAergic inhibition. Previous observations have shown, that already a minor decline in the efficacy of GABAergic inhibition induces epileptiform activity in rat neocortical slices [5,22,23]. Whether the observed reduction in intracortical GABAergic inhibition in WAG/Rij rats results from removal of excitatory input to inhibitory interneurons ('dormant basket cell hypothesis'), a decrease in GABA release, a loss of postsynaptic

GABA receptors, structural and/or functional modifications of GABAergic interneurons or changes in the expression pattern of GABA receptors needs to be clarified.

It is of interest that the present changes in cortical excitability are found in vitro, and that an intact thalamocortical circuitry is not a prerequisite for the increase in cortical excitability. The present data do not falsify the thalamic network hypothesis of Steriade et al. [35] and Buzsáki [4]. These authors emphasized the role of the thalamus in generating spike-wave complexes. Several mechanisms at the thalamic level probably contribute to the expression of this pathophysiological activity. An enhanced $GABA_B$ mediated inhibitory drive onto thalamic relay cells may augment low threshold calcium spikes and the pathogenesis of absence seizure-like rhythmicity in these neurons [12,32]. This increase in $GABA_B$ mediated input probably originates from an increased activity level of neurons situated in the thalamic reticular nucleus [40]. Recent observations by Tsakiridou et al. [37] in acutely isolated neurons of the thalamic nucleus reticularis obtained from GAERS rats demonstrated a selective increase in the amplitude of the low threshold calcium conductance by 55% when compared to non-epileptic controls. These genetically determined differences may result from an increase in T-type $Ca^{2+}$ channel number and/or single channel conductance and significantly contribute to the enhanced synchronization during absence epilepsy [37]. However, as also suggested by Tsakiridou et al. [37], additional mechanisms may be involved in the generation and manifestation of synchronous spike and wave discharges. These additional mechanisms are probably located in cortical structures, since in vivo studies have shown that the neocortex apparently plays a leading role in the transition to spike-wave discharges. The present results indicate that permanent cortical changes are associated with a higher incidence and prevalence of spike-wave discharges. One yet unresolved issue concerns the question whether these changes in neocortical excitability are the cause or the consequence of spike-wave complexes. The decrease in GABAergic inhibition and the expression of stimulus-evoked hyperexcitability may be the result of repetitive seizure activity [27,33], originating from the thalamic network. To elucidate this interesting question, a detailed developmental study on thalamic and neocortical neurons has to be performed in parallel.

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