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Cortical and limbic excitability in rats with absence epilepsy

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Abstract

The classical cortico-reticular theory on absence epilepsy suggests that a hyperexcitable cortex is a precondition for the occurrence of absence seizures. In the present experiment seizure thresholds and characteristics of cortical and limbic epileptic afterdischarges (AD) were determined in a comparative cortical stimulation study in young and old adult genetically epileptic WAG/Rij, congenic ACI and Wistar rats. Fifteen-second series of 8 Hz stimulation of the sensory-motor cortex were applied in 80- and 180-day-old rats with implanted electrodes.

Strain differences were found for the threshold for movements directly induced by stimulation, low frequency spike-and-wave AD, maximal clonic intensity of seizures accompanying direct stimulation, and frequency characteristics of low frequency AD. None of these results agreed with a higher cortical excitability exclusively in WAG/Rij rats. However, WAG/Rij rats had the longest duration of the low frequency AD, and the lowest threshold for the transition to the limbic type of AD. The decrease of this threshold correlated with the increase of the incidence and total duration of spontaneous SWDs in WAG/Rij rats.

It is concluded that the elevated excitability of the limbic system or pathways mediating the spread of the epileptic activity into this system can be attributed to the development of genetic epileptic phenotype in WAG/Rij rats.

Keywords: Excitability; Cortex; Sensory-motor cortex; Rats WAG/Rij; Absence epilepsy; Afterdischarges

1. Introduction

The pathophysiology of idiopathic generalized absence epilepsy is not fully understood. Gloor's concept of cortico-reticular epilepsy, nowadays widely accepted, postulates that an abnormally excitable cortex

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interacts with thalamus and brain stem reticular formation (Gloor and Fariello, 1988; Kostopoulos, 2000). Gloor and co-workers showed that systemic administration of high doses of penicillin induced spike-wave discharges (SWDs) 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 SWDs at the level of the cortex, when this was made hyperexcitable by penicillin (Gloor and Fariello, 1988). However, it is not clear whether the cortex is hyperexcitable permanently (i.e. also interictally) or only during the appearance of SWDs.

The inbred WAG/Rij albino rat strain is considered to be an appropriate genetic animal model for absence epilepsy (van Luijtelaar and Coenen, 1986; Coenen et al., 1992; Crunelli and Leresche, 2002; Coenen and van Luijtelaar, 2003). This type of epilepsy, a generalized non-convulsive form, is associated with spontaneously occurring bursts of bilateral synchronous SWDs, which can be recorded in the electroencephalogram (EEG). SWDs in WAG/Rij rats consist of trains of spike and waves with duration of 1–30 s and a frequency between 7 and 10 Hz. At 2–3 months of age, SWDs start to become present in the EEG and the number of SWDs increases with age. In 6-month-old rats 16–18 SWDs emerge per hour, adding up to about 400 SWDs per day. Mild behavioral concomitants can be seen during the presence of a SWD, such as episodes of vibrissae twitching; otherwise the animals are immobile (van Luijtelaar and Coenen, 1986). Increased cortical excitability in WAG/Rij rats is conceivable since Meeren et al. (2002) found that cortex of WAG/Rij rats contains zones in which the epileptoformic activity may be triggered before being fully synchronized in or by the cortico-thalamic network.

Local rhythmic electrical stimulation of the sensorimotor cortex in freely moving rats was used to investigate whether genetic epileptic rats are endowed with a more excitable cortex interictally. This stimulation paradigm allows the study of four events with different mechanisms:

1. Movements elicited by individual stimuli, due to a direct activation of the motor system,
2. Afterdischarges (AD) characterized by low frequency (around 3 Hz) spike-and-wave EEG rhythm, generated by a thalamocortical system,
3. Clonic seizures of head and forelimbs muscles accompanying AD, indicating a spread of epileptic activity into the motor system, and
4. Transition into AD similar to those elicited by stimulation of limbic structures (Dyer et al., 1979) accompanied by behavioral arrest or automatisms—e.g. elements of orienting reaction in a well known cage and wet dog shakes. These phenomena presumably are representative of the epileptic activity spreading into limbic structures. A detailed description of this stimulation is at our disposal because this test was routinely used to study the ontogeny of cortical epileptic AD (Mareš et al., 2002) and the effects of anticonvulsant (Kubová et al., 1996, 1999; Haugvicová et al., 2002) as well as convulsant drugs (Koryntová et al., 2002; Živanović et al., 2003).

In order to investigate whether increased interictal cortical excitability may underlie the pathogenesis of absence epilepsy in WAG/Rij rats, we used this stimulation paradigm and determined threshold values and characteristics of AD. Two groups of WAG/Rij rats were used: 2- to 3-month-old, which has only few SWDs and 6-month-old with many SWDs. As well, age matched control rats from an inbred strain with no or a minimal number of SWDs (ACI, Inoue et al., 1990; De Bruin et al., 2001) and from an outbred control strain (Wistar) were used. This design allowed us to compare the effects of age and genotype (epileptic versus non-epileptic and inbred versus outbred) and their interaction in order to figure out whether differences between groups can be attributed to the development of genetic absence seizures.

2. Materials and methods

2.1. Animals

Experiments were performed in 2–3 (80 days) and 6-month-old male WAG/Rij ($n = 12$, $n = 11$), ACI ($n = 12$, $n = 11$) and Wistar rats ($n = 8$, $n = 8$). The animals were housed under standard conditions (temperature, $22 \pm 1\,^{\circ}C$; 12/12 light–dark cycle with light onset at 6:00 a.m.). WAG/Rij rats were purchased from Charles River Co., ACI from Harlan Winkelmann GmbH, Borchen, and Wistar rats from the breeding colony of the Institute of Physiology Academy of Sciences of

the Czech Republic. All experiments were approved by the Animal Care and Use Committee of the Institute of Physiology and declared to be in agreement with Czech Animal Protection Law (fully compatible with European Community Council directives 86/609/EEC).

2.2. Electrode implantation

Rats were surgically prepared under pentobarbital anesthesia (Nembutal® Abbott, 40 mg/kg i.p.). Silver ball electrodes were placed epidurally; two stimulation electrodes over right sensorimotor cortex (AP = −1 and +1; $L = 2.5$ mm in relation to bregma), recording electrodes over left sensorimotor cortex (AP = 0; $L = 2.5$ mm), left parietal cortex (AP = −3; $L = 3$ mm) and over occipital, visual cortical areas of both hemispheres (AP = 6; $L = 4$ mm). A reference electrode was inserted into the nasal bone, ground electrode into the occipital bone. All electrodes were connected to a plug and the whole assembly was fixed to the skull with fast curing dental acrylic cement. Experiments started after a one-week recovery period.

2.3. ECoG recording and cortical stimulation

Animals were put into Plexiglas cages and connected to the input of a paperless ECoG (sampling rate of 200 Hz) to perform video-ECoG monitoring in a semi soundproof room with regulated air temperature. Base-line recordings (duration 2 h) of spontaneous ECoG started after 2-h adaptation to experimental conditions, and allowed us to establish the number and duration of spontaneously occurring SWDs.

Next the animals were transferred to another room with the stimulation facilities. For recording of AD, the ECoG activity was digitized at a rate of 500 Hz. A constant current stimulator was used. Current intensity had to be confirmed before the stimulation started and was registered by the computer. It was possible to mark directly on the recording so that motor as well as behavioral phenomena were registered. Series of 15-s stimulation (biphasic rectangular pulses of 1-ms duration and 8-Hz frequency) were applied. Stimulation series were repeated after at least a 10-min interval, the intensity of stimuli was always increased for the next series in the following steps: 0.2; 0.4; 0.6; 0.8; 1.0; 1.2; 1.4; 1.6; 1.8; 2.0; 2.2; 2.4; 2.6; 2.8; 3.0; 3.5; 4.0; 4.5; 5.0; 6.0; 8.0; 10.0; 12.0; 14.0; 15.0 mA up to the transition of the AD into the limbic type. At the moment this second type of AD was present, stimulation intensities were not further increased. Electrocorticographic activity was always recorded 20 s before the start of stimulation and at least 2 min after the end of stimulation. The same protocol was used in earlier studies on the ontogenetic development (Mareš et al., 2002) and on the influence of GABA receptor antagonists (Živanović et al., 2003) on the AD thresholds in Wistar rats.

Concerning the events induced by electrical stimulation, four different phenomena were evaluated: movements of head or contralateral paws (usually forepaw) directly elicited by stimulation, epileptic AD characterized by low frequency spike-and-wave rhythm (Fig. 1), clonic seizures of head and forepaw muscles accompanying spike-and-wave AD and epileptic AD consisting of huge delta waves and fast low amplitude spikes (Fig. 1) that were accompanied by behavioral automatisms. Means and variability measures of threshold current intensities for these four phenomena were calculated in all six groups. In addition, the duration of spike-wave AD as well as severity of movements directly elicited by stimulation and clonic seizures accompanying epileptic AD were measured in the stimulation series with the threshold and two times threshold intensities for spike-and-wave AD elicitation. The severity of seizures was expressed by means of the slightly modified Racine's five-point scale (Racine, 1972) as described by Kubová et al. (1996). Frequency of low frequency spike-and-wave AD was measured in the first and the last 3-s sections of all spike-and-wave AD longer than 6 s. Animals were used only once since pilot experiments demonstrated marked changes of thresholds at the second exposure (Haugvicová et al., 2002).

SWDs were identified in WAG/Rij rats in the 2-h period of ECoG recording according to well-known criteria (van Luijtelaar and Coenen, 1986) and expressed as number of SWDs per hour. Data from both age groups were put together to have animals with low and high number of SWDs for calculation of possible correlations.

2.4. Statistics

Two-way ANOVA with age (2 levels) and strain (3 levels) as between groups factors with subsequent pairwise comparisons by LSD tests were used for statistical evaluation of the thresholds, a three-way ANOVA for

the frequency of the low-frequency AD (begin and end of the AD was used as within groups factor, strain and age as between groups factors). Spearman rank correlations between individual threshold current intensities for elicitation of spike-and-wave AD and hourly number and mean duration of spontaneously occurring SWDs in WAG/Rij rats were conducted. The incidence of fast activity during AD was statistically evaluated by means of Fisher exact test. The level of statistical significance was set at 5% (two-tailed tests) for all variables.

3. Results

All rats included in the present study exhibited the first three phenomena that were evaluated: movements during stimulation, low frequency spike-and-wave AD (Fig. 1) and clonic seizures accompanying this type of AD. Transition into the second, limbic type of AD (Fig. 1) was recorded under our experimental conditions (15 mA as the highest intensity of stimulation current) in 12–13 rats in each strain (i.e. in 80–100% of animals) without any significant strain or age difference in the number of animals showing this transition.

3.1. Thresholds for movements elicited by stimulation

The ANOVA showed significant strain effects for the thresholds for elicitation of stimulus-bound movements, reflecting direct activation of the motor cortex ($F_{2,56} = 28.1$, $p < 0.001$). The results are presented in Fig. 2. There were neither age effects nor an interaction between age and strain. Post hoc tests showed that Wistar rats demonstrated a lower excitability of the motor cortex in comparison with WAG/Rij as well as ACI rats ($p < 0.01$).

3.2. Threshold of low frequency spike-and-wave AD

A significant strain effect was found for the threshold intensity necessary for elicitation of low frequency spike-and-wave AD ($F_{2,56} = 33.1$, $p < 0.001$), see also Fig. 2. This threshold was in all subjects equal to that for elicitation of clonic movements of head and forelimbs muscles accompanying this type of AD. Both ACI and WAG/Rij rats had significantly lower thresholds

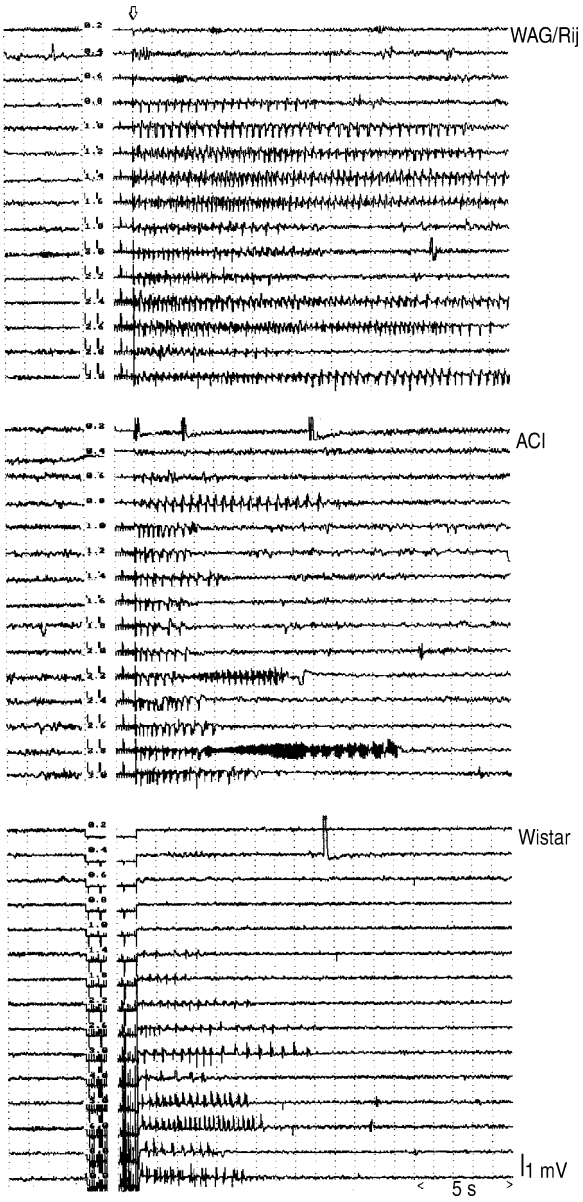

Fig. 1. Recordings of EEG effects of cortical stimulations with increasing intensities from 3-month-old rats. From top to bottom: WAG/Rij rat; ACI rat; Wistar rat. Individual sections from top to bottom: current intensities from 0.2 to 3.0 mA in WAG/Rij and ACI rats, from 0.2 to 10.0 mA in Wistar rats. Left part of each curve: last 4 s before the stimulation, right part: the last second of stimulation and 19 s poststimulation. Time mark 5 s, amplitude calibration 1 mV. Note the difference in thresholds and fast activity in ACI rat (afterdischarges elicited by 2.2 and 2.8 mA).

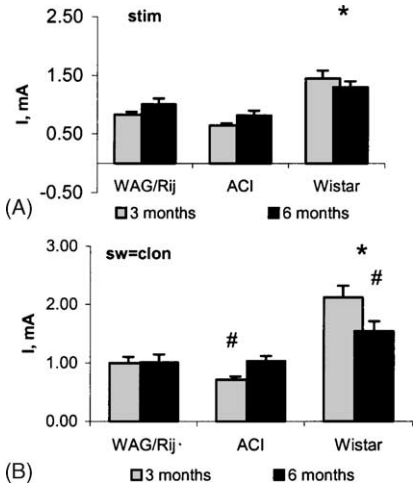

Fig. 2. Thresholds current intensities (mean ± S.E.M.) necessary for (A) elicitation stimulus-bound movements (stim) and (B) low frequency spike-and-wave afterdischarges (sw)/clonic seizures (clon) in WAG/Rij, ACI and Wistar rats at 3 (gray columns) and 6 months (black columns). Ordinate: current intensity in mA. $^{*}p < 0.001$ significant difference in comparison with both other strains, $^{\#}p < 0.05$ significant age difference.

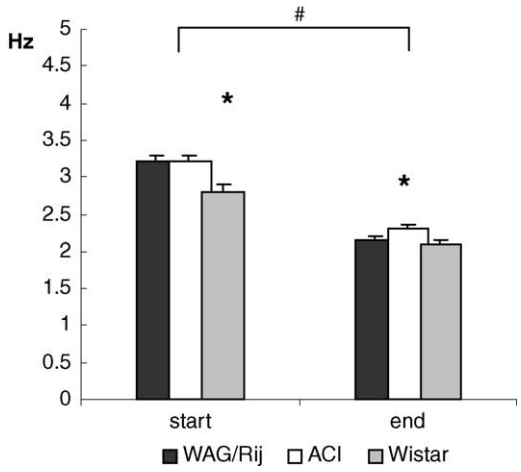

Fig. 3. The frequency of spikes (Hz) (mean ± S.E.M.) in the first three (start) and the last three seconds (end) of low frequency spike-and-wave afterdischarges in Hz (ordinate) in WAG/Rij, ACI and Wistar rats. $^{*}p < 0.05$ significant difference between Wistar and two inbred strains (start); $^{*}p < 0.05$ significant difference between ACI vs. Wistar and WAG/Rij strains. $^{\#}p < .0001$ statistically difference between start and end of the afterdischarge.

for elicitation of these phenomena in comparison with Wistar rats ($p < 0.01$). There was no age effect but a significant interaction between age and strain was found ($F_{2,56} = 6.4$, $p < 0.01$), pointing to difference in age-related shifts among the tested strains. Both thresholds (for low-frequency spike-and-wave AD) and elicitation of clonic movements of heads and shoulders tended to increase with age in both inbred strains, whereas they declined significantly ($p < 0.01$) with age in Wistar rats. This demonstrates that the differences between strains were attenuated with age.

3.3. Frequency of low frequency spike-and-wave AD

Frequency of the low frequency spike-and-wave AD (data are presented in Fig. 3) was always higher at the beginning of the AD than at the end ($F_{1,54} = 217.7$, $p < 0.0001$). There was no age effect, however, the strain difference was significant ($F_{2,54} = 7.44$, $p < 0.001$). Post hoc analyses for this strain effect demonstrated that the frequency (overall) was lower for Wistar than for ACI and WAG/Rij rats. The interaction between strain and begin–end of the AD just failed to reach significance ($0.05 > p > 0.10$), however, it prompted us to analyse the parameters in more detail. Separate analyses were performed for the frequency of this type of AD at the beginning, the end and for the difference score. For the beginning only a strain effect ($F_{2,54} = 5.54$, $p < 0.01$) was found; post hoc tests showed that the frequency is higher for ACI and WAG/Rij rats compared to Wistar rats. For the frequency at the end of the AD again a strain effect ($F_{2,54} = 4.84$, $p < 0.05$) was found; post hoc's for the strain effect showed that now the frequency is higher for ACI compared to Wistar and WAG/Rij rats. The ANOVA on the difference pre–post again revealed only a strain effect ($F_{2,57} = 3.15$, $p = 0.05$), post hoc tests show that the change in frequency is larger for WAG/Rij than for Wistar rats, but not for ACI. In addition, ACI rats often exhibited a change in the ECoG pattern of the AD. Section of fast spikes (Fig. 1) suddenly appeared in the course of AD in 70% of 3-month-old and in 35.7% of 6-month-old animals. Frequency of these spikes varied in the range from 9 to 12 Hz. Similar phenomenon was registered in only one out of 10 WAG/Rij rats 3-month-old and never in the remaining three (6 months WAG/Rij, 3 and 6 months Wistar) groups of animals; the distribution of these probabilities is significant.

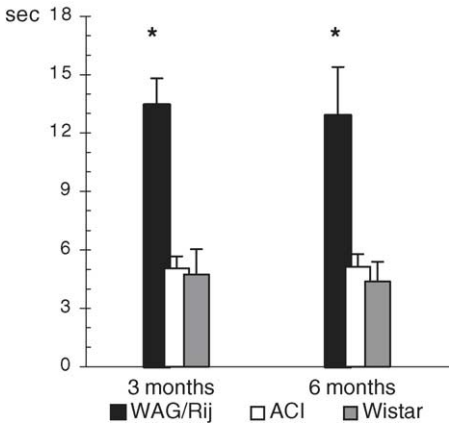

Fig. 4. Mean duration of low frequency spike-and-wave afterdischarges in seconds (mean ± S.E.M.), calculated from individual data for the threshold and twofold threshold current intensities in 3- and 6-month-old WAG/Rij, ACI and Wistar rats. *Significant difference in comparison with both other strains, $p < 0.05$.

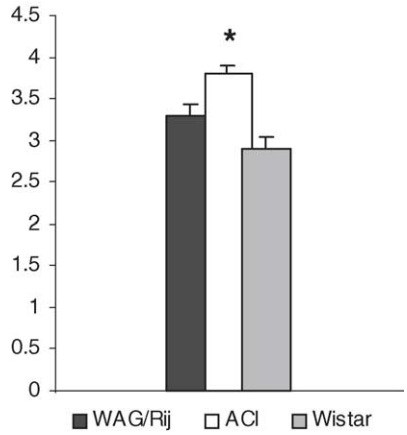

Fig. 5. Maximal severity of seizures (mean ± S.E.M.), accompanying stimulation and low frequency spike-and-wave afterdischarges in WAG/Rij, ACI and Wistar rats at 3 and 6 months, pooled data. Y-axis seizure severity. Racine's five point scale modified by Kubová et al. (1996) was used. *$p < 0.05$ significant difference in comparison with both other strains.

3.4. Duration of low frequency spike-and-wave AD

A strain effect ($F_{2,56} = 28.43$, $p < 0.000$) was found for duration of low frequency spike-and-wave AD; the data are presented in Fig. 4. WAG/Rij rats demonstrated a significantly longer duration of spike-and-wave AD compared to both ACI and Wistar rats ($p < 0.01$ in both cases). There were no other effects.

3.5. Maximal intensity of clonic seizures

Maximal intensity of clonic seizures accompanying AD (Fig. 5) exhibited a strain effect ($F_{2,47} = 16.01$, $p < 0.001$). ACI rats demonstrated significantly ($p < 0.001$) more severe clonic seizures than WAG/Rij as well as Wistar rats. Sections of fast spikes in ACI rats were accompanied by fast clonic movements of forelimbs synchronous with individual spikes.

3.6. Threshold for transition into limbic type of AD

Both an age effect ($F_{2,56} = 4.75$, $p < 0.05$) and a strain effect ($F_{2,56} = 13.4$, $p < 0.001$) were found for the fourth phenomenon—the threshold for the transition to the limbic type of AD, indicating spread of epileptic activity into the limbic system (see Fig. 6). The interaction between age and strain was not significant. WAG/Rij rats showed a significantly ($p < 0.01$) lower threshold for this phenomenon compared to each of the other strains. No difference was found between ACI and Wistar rats. Older rats had significantly ($p < 0.05$) more excitable limbic system than younger rats. The fact that, no interaction between age and strain was found, suggests that the differences between strains remain stable.

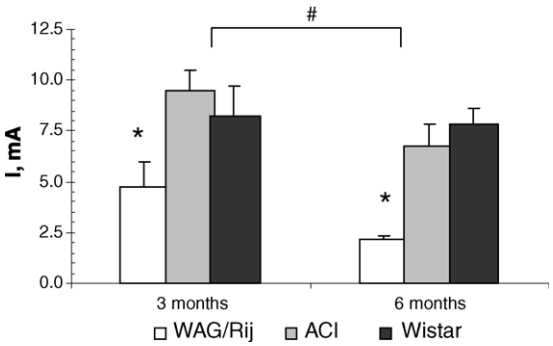

Fig. 6. Thresholds current intensities (mean ± S.E.M.) necessary for elicitation limbic type of afterdischarges in WAG/Rij (black columns), ACI (white columns) and Wistar (gray columns) rats 3- (A) and 6 (B) month-old. Ordinate: current intensity in mA. *$p < 0.001$ significant difference in comparison with both other strains, #$p < 0.05$ significant difference between 3 and 6 months rats.

3.7. Relation to spontaneously occurring spike-wave discharges

The number of spike-and-wave episodes counted in WAG/Rij rats during the base-line recording varied between 9 and 63, the total duration between 27 and 264 s. The correlation between individual threshold current intensities for transition into limbic seizures and hourly number and mean duration of spontaneously occurring SWDs in WAG/Rij rats showed a significant inverse correlation between the threshold and both mean and total duration of SWDs ($R = -0.57$, $R = -0.53$, $p < 0.05$).

4. Discussion

The present study was designed to investigate whether genetically epileptic rats are endowed with an increased cortical excitability interictally. The outcomes of cortical stimulation study demonstrated no difference in excitability of sensorimotor cortex between epileptic WAG/Rij and congenic ACI rats. All the first three phenomena, reflecting (i) direct activation of motor cortex (stimulus-bound movements), (ii) spread of epileptic activity into the motor system (clonic movements following after stimulation period) and (iii) involvement of cortico-thalamic system (low frequency AD) had the same thresholds in WAG/Rij rats as in ACI rats. However, comparison between Wistar rats and the two inbred strains showed a difference in excitability. Both inbred strains had from 1.5- to 2-fold lower thresholds for these three phenomena than age-matched Wistar rats. Consistent with this, Wistar rats demonstrated a lower intensity of seizures and stimulus-bound movements compared to ACI and WAG/Rij rats. Similar results were found in an in vitro study, in which neocortical slices of WAG/Rij rats in comparison to Wistar rats exhibited significant reduction in the efficiency of GABAergic inhibition concomitant with hyperexcitability (Luhmann et al., 1995). Another report showed that WAG/Rij rats have a lower seizure threshold than Wistar rats in a model of convulsive epileptic seizures (pentylenetetrazol) (Kliouevaet al., 2001). However, in contrast to what has been found before, our study showed that both inbred rats are characterized by increased interictal cortical excitability of the sensorimotor cortex as well as motor system compared to outbred Wistar rats. Since ACI rarely show SWDs, our data imply that precondition of higher interictal cortical excitability is necessary but not sufficient for the development of absence epileptic phenotype.

Our experiment tested the cortical excitability only during the interictal period and did not test cortical excitability during or immediately before episodes of SWDs. However, it might be that only a temporal ictal (or periictal) change in cortical excitability is necessary for the appearance of SWDs in WAG/Rij rats. Moreover, there might be only local changes in excitability (Meeren et al., 2002; Van de Bovenkamp-Janssen et al., 2004), which could not be found in the stimulation paradigm used in the present experiment. These possibilities remain to be further explored.

Oscillation frequency within the AD is a parameter that describes thalamo-cortical interaction. First of all, we found that the frequency of the low-frequency oscillations was always higher at the beginning compared to the end of the AD. Interestingly, the decrease in frequency over the course of spontaneous SWDs was also found in WAG/Rij rats and in children with absence epilepsy and might be a reflection of a mechanism which is involved in arresting of an AD or train of SWDs. At the beginning, the frequency of the AD was higher in WAG/Rij and ACI rats than in Wistar rats. The decrease in frequency was not identical in the three strains: it was larger in WAG/Rij than in ACI and Wistar rats. Therefore, ACI rats have at the end of ADs higher frequency than both Wistar and WAG/Rij rats. In addition, old WAG/Rij rats did not differ from young WAG/Rij rats. These outcomes do not give strong evidence to assume that frequency characteristics of this type of AD are associated with the presence of SWDs in WAG/Rij rats. On the other hand, of interest, ACI rats were found to be peculiar: they were characterized by a higher frequency at the end of the AD and not in the beginning. Moreover, ACI rats frequently demonstrated a sudden shift from low 3 to 4 Hz much higher (till 9–12 Hz) frequency of oscillations within AD. In comparison, a similar shift was recorded in only a single WAG/Rij rat. We observed this fast activity in Wistar rats in other experiments (unpublished observations). These facts suggest that different reactivity rather than threshold characteristics of nervous cells may account for increased excitability in agouti (ACI) versus albino

(Wistar and WAG/Rij) rats. Since majority of Wistar rats also develop spontaneous SWDs during aging (van Luijtelaar et al., 1995), we suggest that this difference in reactivity of cortico-thalamic cells in response to the same stimuli may also underlie the development of SWDs.

A parameter that revealed a difference between epileptic WAG/Rij and other two strains was the duration of low frequency spike-and-wave AD. Both young and old WAG/Rij rats had considerably longer duration of low frequency spike-and-wave AD in comparison to age-matched ACI and Wistar rats. This result clearly indicates impaired inhibition of generalized thalamocortical oscillations in WAG/Rij rats. Interestingly, the lack of either age effect and age-strain interaction for this parameter suggests that this seizure arresting mechanism is already altered before the presence of SWD in WAG/Rij rats. To date, little is known about seizures arresting mechanisms. Adenosine is one of the neurotransmitters that affect duration of different epileptic phenomena. In our laboratory, it was shown that caffeine, an antagonist of adenosine receptors, prolongs epileptic AD more efficiently than agents compromising GABA-ergic inhibition (Koryntova et al., 2002). On the other hand, the adenosinergic system is altered in genetic epileptic rats from Strasbourg (GAERS). Reticular (nRT) and anterior ventral (AV) thalamic nuclei as well as basal ganglia of GAERS have a lower density of A1 receptors (15% decrease) compared to control animals (Ekonomou et al., 1998). An adenosinergic inhibition is considered to exert an anti-oscillatory effect on thalamic nuclei by suppressing (via A1 receptors) excitatory as well as inhibitory neurotransmitter release (Kostopoulos, 2000). However, an injection of adenosine induces a dose-dependent increase in the appearance of SWDs in WAG/Rij rats (Ilbay et al., 2001). Taken together, these results may indicate diverse, region specific role of adenosine in the control of SWDs and in the duration of AD.

The last finding concerned the fourth phenomenon that indicated the excitability of the limbic system. Unexpectedly, WAG/Rij rats exhibited the lowest threshold for the spread of epileptic activity into the limbic structures in comparison with the two other strains; moreover, in all strains this threshold decreased with age. The low threshold of this transition in WAG/Rij rats may be due to either an augmented excitability of structures mediating spread of epileptic activity into the limbic system (probably thalamic nuclei with limbic projections) or an increased excitability of limbic structures in WAG/Rij rats. Little evidence is available concerning the role of limbic system in absence epilepsy. The inverse correlation between individual threshold current intensities of this phenomena and individual total and mean duration of spontaneously occurring SWDs found in WAG/Rij rats suggests that the excitability of the limbic system is apparently related to the occurrence of spontaneous SWDs in WAG/Rij rats.

Currently, it is difficult to account for the role of the limbic system in genetic absence epilepsy. However, there are some data in GAERS that also point to changes in the limbic system. In comparison to non-epileptic control rats, at postnatal day 21 (before the occurrence of SWDs), GAERS have higher brain metabolic activation in limbic regions, but not in the thalamo-cortical loop (Nehlig et al., 1998). There is no longer any difference between brain regions in adult GAERS expressing SWDs. It was suggested that metabolic changes are present throughout the brain and translated into SWDs in the cortico-thalamic loop. Lason et al. (1992) found also elevated levels of a-neoendorphin in the hippocampus of 6-month-old WAG/Rij rats in comparison with younger rats of the same strain and age matched ACI rats. GAERS showed a high resistance to propagation of amygladoid-kindled seizures (Eskazan et al., 2002). Finally, the rostral pole of the nucleus reticularis thalami (nRT), which is known to play a role in the generation of SWD, does belong to the limbic system. The nRT is a key structure in the generation of sleep spindles and spike-wave discharges (Avanzini et al., 1992). Moreover, lesions of the lateral thalamus including the rostral pole of the nRT abolished SWD in GAERS and WAG/Rij rats (Avanzini et al., 1992; van Luijtelaar and Weltink, 2001; Meeren, 2002). The rostral pole is connected with various motor and limbic centres, the middle and caudal parts of the nRT are connected with the thalamocortical relay cells (Lubke, 1993). Most of the projections to and from the nRT are ipsilateral. However, commissural connections from the rostral pole of the nRT to selected nuclei of the contralateral thalamus have been reported in the rat (Chen et al., 1992; Raos and Bentivoglio, 1993; Battaglia et al., 1994). Through these bilateral connections the nRT may influence the activity of wide territories of

the cerebral cortex of both hemispheres and these areas might be involved in the synchronization of spontaneously occurring SWD as well as the limbic seizures. A final argument for the role of the thalamic relay nuclei and the nRT in limbic seizures was demonstrated by injections of carbachol in the ventral basal complex of the thalamus and in the nRT. These injections induce behavioural and electrocortical limbic seizures in rats (Mraovitch and Calando, 1995). The strain difference suggests that the lowered seizure threshold for limbic seizures precipitates the presence of SWD in the ECoG. Therefore, the mechanisms involved in the limbic seizure threshold might be causative for the development of SWD from 2 to 3 months onwards. In addition, the age-dependent decrease of the threshold for limbic seizures may reflect general characteristic of the aging process of the limbic part of the brain. The role of the limbic system in the development of absence seizures deserves to be further investigated.

In addition, it should be taken into account that repeated electrically induced seizures lead to a stepwise increase in the corticosterone response (Young et al., 1990). Neurons of the limbic system containing large quantities of mineralocorticoid receptors are one of the first potential targets affected by corticosterone (Joels and de Kloet, 1992; Reddy and Rogawski, 2002). Strain differences in sensitivity to stressful events are well known (e.g. Stohr et al., 2000). Therefore, considering the long duration (4–5 h) of the present experiments, the effects of endogenous corticosterone levels on excitability of limbic system may interfere with effects of cortical stimulation and contribute to the strain differences found in the present experiment.

In conclusion, 6-month-old WAG/Rij rats have lower thresholds than Wistar but not ACI rats for most tests. WAG/Rij rats have a lower threshold for the seizure spread into the limbic system and prolonged duration of low frequency of spike-and-wave AD. This outcome is in disagreement with Gloor's theory that absence seizures are due to a hyperexcitable cortex. The threshold of limbic type of AD is susceptible to aging processes and parallels an age-dependent increase in the occurrence and duration of SWDs. In addition, the correlation between the number and duration of SWDs with the limbic seizure threshold in WAG/Rij rats further emphasizes a role of the limbic system in generalized absence epilepsy.

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