Epilepsia, **47**(1):33–40, 2006
Blackwell Publishing, Inc.

Amygdala Kindling in the WAG/Rij Rat Model of Absence Epilepsy

*Rezzan Gülhan Aker, *Hasan Raci Yananli, *Ayten Azizova Gurbanova, *Aydan Ergün Özkaynakçi, †Nurbay Ateş, ‡Gilles van Luijtelaar, and *Filiz Yilmaz Onat

*Department of Pharmacology and Clinical Pharmacology, School of Medicine, Marmara University, Istanbul, and †Department of Physiology, School of Medicine, Kocaeli University, Kocaeli, Turkey; and ‡NICI-Biological Psychology, Radboud University Nijmegen, Nijmegen, The Netherlands

Summary: *Purpose:* The kindling model in rats with genetic absence epilepsy is suitable for studying mechanisms involved in the propagation and generalization of seizure activity in the convulsive and nonconvulsive components of epilepsy. In the present study, we compared the amygdala kindling rate and afterdischarge characteristics of the nonepileptic Wistar control rat with a well-validated model of absence epilepsy, the WAG/Rij rat, and demonstrated the effect of amygdala kindling on spike-and-wave discharges (SWDs) in the WAG/Rij group.

Methods: Electrodes were stereotaxically implanted into the basolateral amygdala of rats for stimulation and recording and into the cortex for recording. After a recovery period, the animals were stimulated at their afterdischarge thresholds. EEG was recorded to analyze SWDs and afterdischarge durations. The seizure severity was evaluated by using Racine's 5-stage scale.

Results: All nonepileptic control and four of seven WAG/Rij animals reached a stage 5 seizure state, whereas three animals failed to reach stage 3, 4, or 5 and stayed at stage 2 after application of 30 stimulations. Interestingly, WAG/Rij rats, resistant to kindling, demonstrated a significantly longer duration of SWDs on the first day of the experiment before kindling stimulation than did the kindled WAG/Rij animals. Additionally, the cumulative total duration and the number of SWDs after the kindling stimulation were statistically increased compared with SWDs before kindling stimulation.

Conclusions: The results of our study demonstrate that the progress of amygdala kindling is changed in rats with genetic absence epilepsy, perhaps as a consequence of the hundreds of daily SWDs. **Key Words:** Absence epilepsy rats—Spike-and-wave discharge—Generalized epilepsy—Partial epilepsy—Convulsion—GAERS.

Models of convulsive seizures and convulsive events offer unique opportunities for understanding the pathophysiology of epileptogenesis in animals and perhaps, by extrapolation, in humans. A recent kindling study in rats with genetic absence epilepsy from Strasbourg (GAERS) showed that GAERS failed to progress beyond stage 2, even after the maximum number of stimulations. Only nonepileptic control animals reached a stage 5 generalized convulsive seizure state. This suggests that GAERS are resistant to secondary generalization of limbic seizures during amygdala kindling (1). These results provide evidence for a thalamolimbic interaction and the involvement of limbic structures in absence epilepsy, even though this part of the brain circuitry is generally not involved in the expression of spike-and-wave discharges (SWDs) (2,3). SWDs are consistently recorded from the thalamus and the cortex, whereas no SWDs are recorded from the limbic structures including the amygdala in GAERS (3). Several thalamic nuclei are thought to have a neuromodulatory role in the physiology of limbic structures. Midline thalamic stimulation produces strong excitatory responses in the CA1 region of the hippocampus (4–6). Furthermore, a recent study suggests that circuits involving the midline thalamus and limbic structures are simultaneously activated in the early stages of seizure initiation (7).

Clinically, the occurrence of partial epilepsy has been reported to be less than 1% of the overall population with idiopathic generalized epilepsy (8,9). The mechanism underlying this rare coexistence of partial temporal lobe and idiopathic generalized epilepsies is not well understood, and the rat model can be seen as an opportunity for exploring the relationship.

To provide a more solid experimental basis for the uncommon coexistence of partial epilepsy and idiopathic generalized epilepsy, we studied the amygdala kindling

Accepted July 6, 2005.
Address correspondence and reprint requests to Dr. F.Y. Onat at Department of Pharmacology and Clinical Pharmacology, Marmara University, School of Medicine, 34668, Haydarpaşa, Istanbul, Turkey. E-mail: fonat@marmara.edu.tr

rate and afterdischarge characteristics in another model of genetic absence epilepsy, the WAG/Rij rat (10). The validity of the WAG/Rij rats as a model of absence epilepsy is supported by the pharmacologic response to the antiepileptic drugs (AEDs). Further support is provided by the prevalence of the SWDs during drowsiness and the existence of similar disturbances in perception of time during SWDs in humans and rats (11–15). Second, we aimed to examine whether the delayed development of amygdala kindling is indeed a property of rats with the genetic type of absence epilepsy or whether it is unique for GAERS. Third, the relation of the epileptogenesis induced by kindling to the numbers and the durations of the first type of SWDs was investigated in WAG/Rij animals. It was previously shown that first type of SWDs was associated with absence epilepsy in WAG/Rij rats (10,15,16).

MATERIALS AND METHODS

Experiments were carried out with nonepileptic Wistar control (n = 6) and WAG/Rij (n = 7) rats, aged 5 to 9 months. The animals were housed in a temperature-controlled room (20 ± 3°C) with a 12-h light–dark cycle. Animals were maintained in groups of four per cage before the stereotaxic surgery. After the surgery, animals were housed individually, one animal per cage, to prevent the problems in housing conditions due to the microconnector fixed on the skull and kindling-induced changes in behavior. All animals were allowed free access to commercial rat pellets and tap water. The experimental protocol was approved by the Animal Care and Use Committee of Marmara University (March 12, 2004).

Surgery

One week before the kindling experiments, the animals were anesthetized with ketamine [100 mg/kg, intraperitoneally (i.p.)] and chlorpromazine (0.5 mg/kg, i.p.). The head of each animal was placed in a stereotaxic instrument (Stoelting model 51600, Stoelting, Wood Dale, Ill., U.S.A.). The scalp was longitudinally incised, and the skull was leveled between lambda and bregma. Stainless steel electrodes, insulated except at the tip for stimulation and recording, were implanted bilaterally into the basolateral amygdala (BLA; 2.6 mm posterior, 4.8 mm lateral from bregma and 8.5 mm ventral). All coordinates were obtained from the stereotaxic atlas of Paxinos and Watson (17) with bregma as the reference point. Stainless steel screws, used for extradural ground and recording electrodes, were placed bilaterally in the skull over frontal and occipital cortex. Electrodes were connected by insulated wires to a microconnector for EEG recordings. All of the electrodes were fixed to the skull with dental acrylic. The animals were allowed to recover from surgery for ≥7 days before the first day of stimulation.

Kindling

On the day of the experiment, the animals were placed in Plexiglas cages. After an hour-long adaptation period, a baseline EEG was recorded for ≥30 min from the nonepileptic control and WAG/Rij rats. Then to determine the afterdischarge threshold, the right BLA of the rats was stimulated with an initial stimulation of 50 μA (monophasic, square-wave pulses of 80 Hz, each 1 ms in duration, for a total duration of 2 s). This was continued with 50-μA increments until an initial afterdischarge was obtained. Next, the animal was stimulated twice daily at the current afterdischarge threshold. Seizure stages observed after each stimulation were classified by using Racine's standard five-stage scale (18): stage 1, facial movements; stage 2, rhythmic head movements, head nodding; stage 3, unilateral forelimb clonus; stage 4, bilateral forelimb clonus and rearing; stage 5, falling and clonic convulsion. The animals were stimulated until they reached stage 5 seizure state. All the nonepileptic Wistar animals had at least two stage 5 seizures, but the data of 15 stimulations were given in the study. Additionally, because it is accepted that after the observation of a stage 5 seizure, animals will readily continue to have stage 5 seizures with the following stimulations, observation of stage 5 seizure was accepted as the end point of the study. If the animals did not reach stage 5, electrical stimulations were terminated after the 30th stimulation. That is, the maximum number of stimulations was 30. Minimal duration of afterdischarge activity was accepted as spike discharge lasting ≥2 s immediately after the stimulation (19).

Experimental protocol

Kindling was performed in nonepileptic Wistar control and WAG/Rij rats. Electrical activity of the stimulated region of the amygdala and cortex was amplified (through BioAmp ML 136) and recorded with a PowerLab 8S System running Chart v. 5, (ADI Instruments, Oxfordshire, U.K.) before and after each stimulation.

EEG analysis

EEGs in the nonepileptic Wistar control and WAG/Rij groups were recorded continuously for 1 hour before and after the kindling stimulations. The electrode placed into the right BLA was used for stimulations and afterdischarge recording; the electrode placed in contralateral BLA was used to record propagated afterdischarge activity. Afterdischarge duration was taken to be the total duration of spikes in the EEG recorded from right BLA electrode after the stimulation period. Afterdischarge durations recorded from the contralateral BLA reached the ipsilateral side after a few stimulations. Therefore afterdischarge durations from ipsilateral BLA were reported in the present study.

In the WAG/Rij rats, the first and second types of SWDs were determined as previously described (10,16). Because it has been reported that the first type of SWD is related to absence epilepsy (10,15,16), EEG analysis was performed

only for the first type of SWD in the present study. A SWD complex was identified as such if its duration was $\geq$1 s with a train of sharp spikes and slow waves (7.5–9 Hz) and an amplitude of at least twice the background amplitude of the EEG.

The cumulative total duration of SWDs, the number of SWD complexes, and the mean duration of SWD complexes were measured over 10-min periods. The cumulative total duration of the SWDs within a 20-min period on the first day of the experiment before the first kindling stimulation is expressed as "SWD duration on the first day of the experiment."

Histologic verification

After all experiments, the animals were decapitated to determine electrode placement. The brains were placed in a formalin/sucrose mixture, and 40-μm frozen sections were cut in a cryostat and stained with thionine. Only the animals with correct electrode placement were included in the study.

Data analysis

WAG/Rij rats reaching the stage 5 seizure state are referred as the kindled subpopulation of WAG/Rij animals. WAG/Rij rats displaying only stage 2 seizures and not having stage 3, 4, or 5 seizures are referred as the kindling-resistant subpopulation of WAG/Rij animals.

The results are expressed as mean $\pm$ SEM. Data were statistically evaluated by analysis of variance with repeated measures (ANOVA). A two-way ANOVA followed by the post hoc Bonferroni test was used to compare time–response curves (the kindling rate and afterdischarge durations) obtained from nonepileptic Wistar and WAG/Rij groups.

One-way ANOVA followed by the post hoc Dunnett test was used to analyze prestimulation and poststimulation

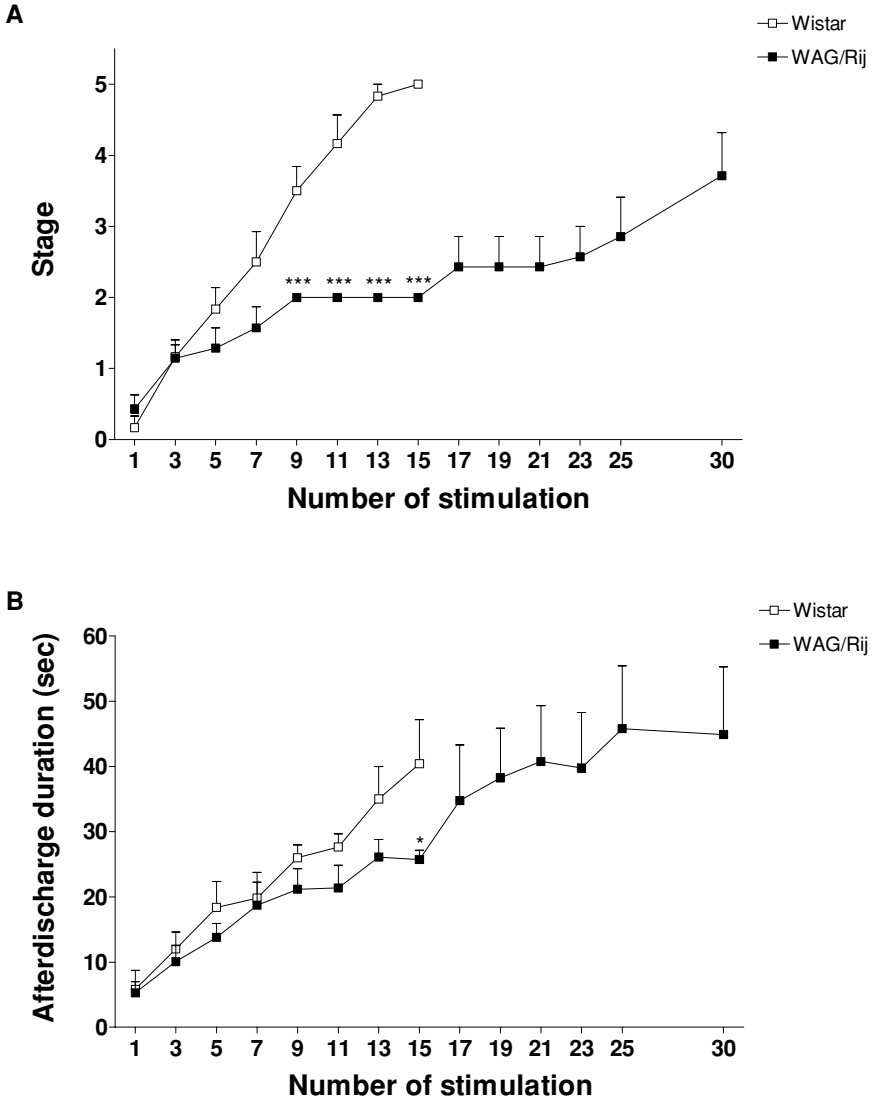

FIG. 1. Seizure stage **(A)** and duration of afterdischarge in the ipsilateral amygdala **(B)** of nonepileptic Wistar control (n = 6) and WAG/Rij (n = 7) rats. Data are expressed as mean $\pm$ SEM. Two-way analysis of variance was performed for the first 15 stimulations of both groups because all the animals in the control Wistar group reached stage 5 seizures and were not stimulated beyond the 15th stimulation. *$p < 0.05$, ***$p < 0.001$, significant differences between Wistar control and WAG/Rij groups.

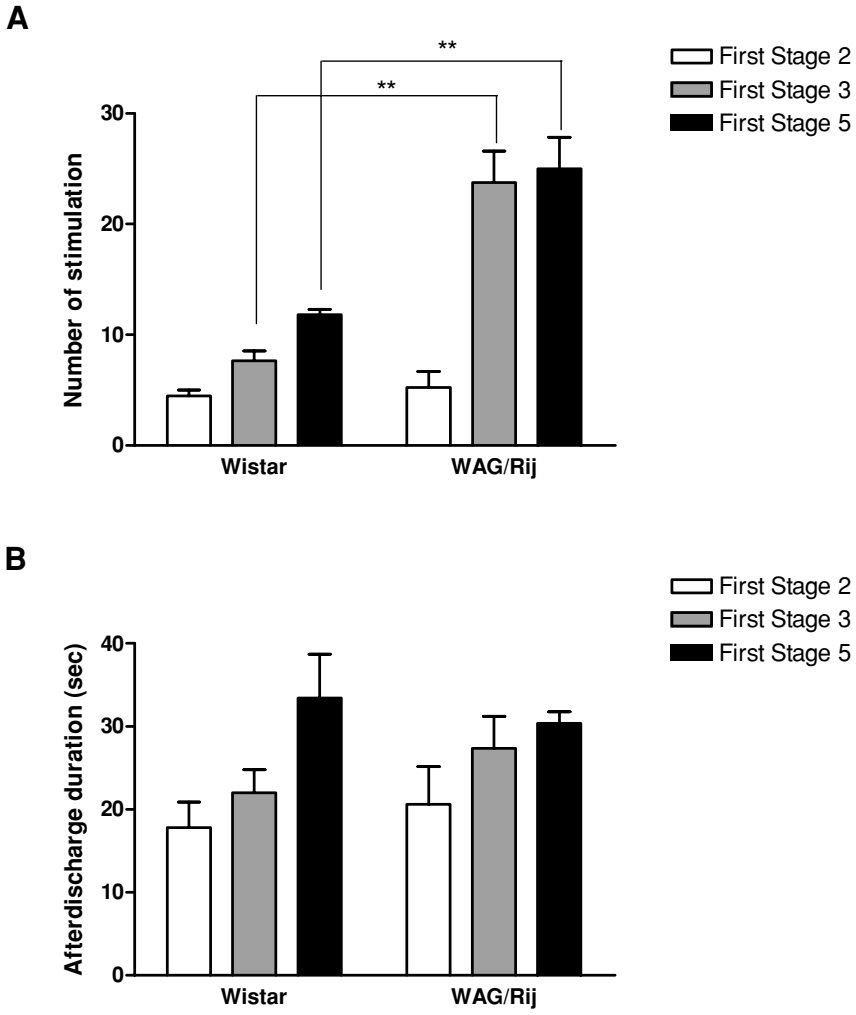

FIG. 2. A: The mean number of stimulations for the development of first stage 2, 3, and 5 seizure states of nonepileptic Wistar (n = 6) and kindled subpopulation of WAG/Rij (n = 4) animals. **B:** The mean duration of afterdischarges at first stage 2, 3, and 5 seizure states of nonepileptic Wistar (n = 6) and kindled subpopulation of WAG/Rij (n = 4). Data are expressed as mean ± SEM. $^{**}p < 0.01$, significant differences between Wistar control and WAG/Rij groups compared with the Mann–Whitney test.

cumulative total duration, number, and mean duration of SWDs within 10-min periods. The mean numbers of stimulations needed to reach the first stage 2, 3, and 5 seizure state were compared by the Mann–Whitney test. Student's *t* test was used to compare (a) the mean duration of afterdischarges during the first stage 2, 3, and 5 seizures; and (b) "SWD duration on the first day of the experiment" for kindled and kindling-resistant subpopulation of WAG/Rij rats. The level of statistical significance was considered to be $p < 0.05$.

RESULTS

The mean afterdischarge threshold for the Wistar control animals was 175 μA, and was 150 μA for the WAG/Rij rats. The amygdala kindling rates of nonepileptic Wistar and WAG/Rij rats are shown in Fig. 1. All of the nonepileptic control animals reached the stage 5 seizure state by the 14th stimulation. Of the seven WAG/Rij rats, four animals showed stage 5 seizures, but three failed to reach stage 3, 4, or 5 seizures and stayed at stage 2 after application of 30 stimulations (Fig. 1A). The two-way ANOVA with post hoc Bonferroni multiple comparison test showed a significant difference between the kindling rates of Wistar and WAG/Rij groups ($p < 0.001$). The mean number of stimulations for the development of the first stage 5 seizure was 11.8 ± 0.4 in the nonepileptic Wistar rats, whereas the mean number of stimulations for the kindled WAG/Rij animals was 25.0 ± 2.5 (Fig. 2A). Statistical analysis revealed a significant difference in the mean number of stimulations for the development of the first stage 3 and 5 seizures between the nonepileptic control and the kindled subpopulation of WAG/Rij rat groups ($p < 0.01$). Table 1 shows the kindling progression of the WAG/Rij rats. Afterdischarge threshold was 131.2 ± 18.3 μA for kindled WAG/Rij rats and 166.7 ± 23.6 μA for the kindling-resistant group. No significant difference was found between the afterdischarge threshold of kindled and kindling-resistant WAG/Rij rats.

Afterdischarge durations of nonepileptic Wistar and WAG/Rij groups are shown in Fig. 1B. The mean afterdischarge duration at stimulation 15 was 40.4 ± 6.1 s in

TABLE 1. *SWD durations and the number of stimulations required to reach first stage 2, 3, 4 and 5 seizures state for kindled and kindling-resistant subpopulations of WAG/Rij rats*

Animals		SWD duration on the first day (s)*	Number of stimulations required to reach				Total no. of stimulations
			Stage 2	Stage 3	Stage 4	Stage 5	
Kindled	WAG/Rij 3	3.8	9	16	–	17	17
	WAG/Rij 12	6.6	3	23	–	25	25
	WAG/Rij 9	12.6	6	29	–	30	30
	WAG/Rij 2	20.6	3	–	27	28	28
Kindling-resistant	WAG/Rij 11	33.6	4	–	–	–	30
	WAG/Rij 10	53.6	7	–	–	–	30
	WAG/Rij 7	62.0	8	–	–	–	30

*"SWD duration on the first day of experiment" is the mean cumulated total duration of the SWDs within 20-min period before stimulations on the first day of the experiment.

the nonepileptic Wistar and 25.7 ± 1.3 s in the WAG/Rij rats (Fig. 1B). Afterdischarge durations in the WAG/Rij group were shorter than those seen in nonepileptic Wistar animals. This difference was statistically significant at stimulation 15 when analyzed by two-way ANOVA with the post hoc Bonferroni multiple comparison test ($p < 0.05$). The mean duration of afterdischarges during the first stage 5 seizures in nonepileptic Wistar animals was 34.2 ± 4.4 sec and 30.3 ± 9.4 s in the kindled subpopulation of WAG/Rij rats (Fig. 2B).

SWD duration on the first day of the experiment was significantly shorter in the kindled subpopulation (10.9 ± 3.2 s) compared with that in the kindling-resistant subpopulation (49.7 ± 6.9 s) of WAG/Rij rats ($p < 0.01$) (Fig. 3).

The cumulative total duration of SWDs of WAG/Rij rats in the prestimulation period showed no significant changes for the 30 stimulations (Fig. 4A). The cumulative total duration of SWDs increased significantly for the (0–10 min) poststimulation period when compared with the prestimulation period. The cumulative total duration of SWDs did not differ significantly from the prestimulation values in the poststimulation (10–20 min) period. Amygdala-kindling stimulation significantly enhanced the number of SWDs in the EEGs of all WAG/Rij rats (Fig. 4B). The mean duration of one SWD complex was increased only during the last interval of electrical stimulations (Fig. 4C).

A few animals from both kindled and kindling-resistant subpopulations of WAG/Rij animals had the second type of SWDs (data not shown).

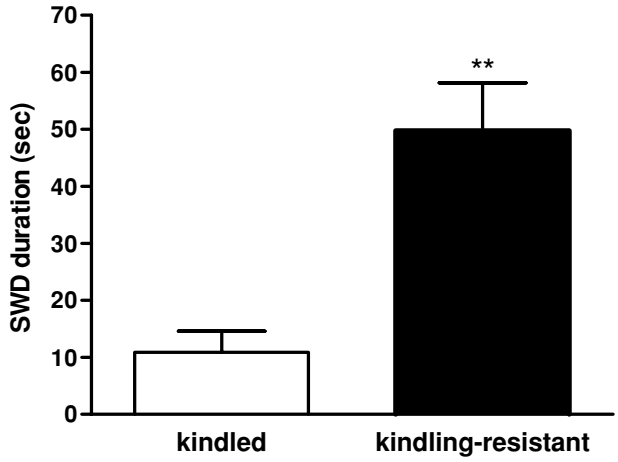

FIG. 3. Spike–wave discharge duration on the first day of the experiment of kindled (n = 4) and kindling-resistant (n = 3) subpopulations of WAG/Rij animals. Data are expressed as mean ± SEM. **$p < 0.01$, significant difference between groups compared with Student's *t* test.

DISCUSSION

In the present study, all of the nonepileptic control and four of seven WAG/Rij animals reached a stage 5 generalized convulsive seizure state. Of the four WAG/Rij rats, the mean number of stimulations needed for the development of stage 5 seizures was 2.1-fold higher than that for the nonepileptic control group. Of the seven WAG/Rij animals, three rats failed to reach stage 3, 4, or 5 seizures and stayed at stage 2 after the application of 30 stimulations. The failure to develop stage 5 seizures in three of seven WAG/Rij rats suggests a subpopulation of WAG/Rij rats that has a resistance to amygdala kindling. Comparable to the interindividual differences found in the present study, earlier reports showed that about 25% of the WAG/Rij rats showed sensitivity to audiogenic seizures (20), and 60% showed a second type of SWD (10,16,21). In our study, the second type of SWD was not found to be correlated with the rate of kindling in either the kindled or kindling-resistant subpopulations of WAG/Rij rats. Interestingly, the kindling-resistant subpopulation of WAG/Rij animals showed significantly longer durations of SWD compared with the kindled WAG/Rij rats. The intensity of SWDs in the WAG/Rij rats correlated negatively with the susceptibility to amygdala kindling. Moreover, GAERS in our previous study showed an apparently higher intensity of SWDs than did the WAG/Rij rats that displayed a lower intensity of epileptic activity. The complete resistance to kindling in GAERS and the presence of partial resistance in WAG/Rij could be explained by the difference of the

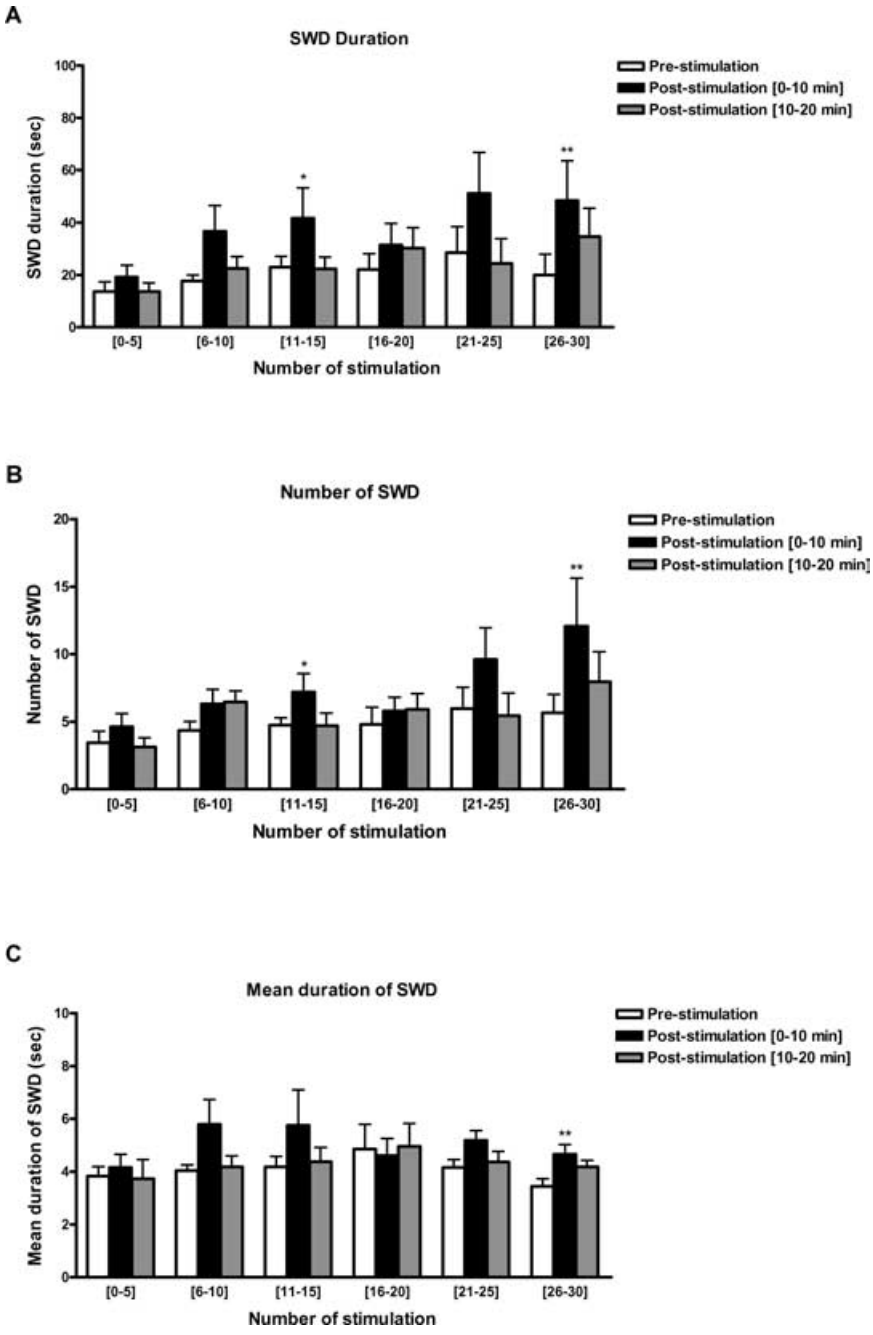

FIG. 4. The mean of cumulated total duration **(A)** and number **(B)** of spike-wave discharges (SWDs) before (prestimulation) and after (poststimulation) kindling electrical stimulations. The mean of the cumulative total duration and number of SWDs of five consecutive electrical stimulations in the WAG/Rij group ($n = 7$) were calculated for each animal and expressed as mean ± SEM; The mean duration of one SWD complex **(C)** is the ratio of SWD duration to the number of SWDs in five consecutive electrical stimulations. $^{*}p < 0.05$, $^{**}p < 0.01$, significant differences between prestimulation and poststimulation periods within the same stimulation interval compared with repeated measures of one-way analysis of variance followed by post hoc Dunnett test.

total duration of daily SWDs. However, direct comparative studies between GAERS and WAG/Rij rats have been limited. The possibility that the results in GAERS are specific to just the one model of absence epilepsy has now been excluded. Our findings indicate that resistance to kindling is more general and not limited to the GAERS.

It has been accepted that typical absence epilepsy is related to a predominance of inhibitory activity (22,23). In contrast to this, generalized convulsive and focal seizures are characterized by an excess of excitatory activity (24). The fact that in both of the rat models the resistance to kindling shows a relation to the total duration of SWDs suggests that the inhibitory mechanisms underlying the SWD activity may have an antagonistic effect on the process of kindling. Burchfiel et al. (25,26) described that kindling antagonism, which involves the concurrent, alternate stimulation of two limbic structures, represents an arrest of the normal kindling process in adult rats. The interaction between two developing kindling foci defines two critical transitions. The first gate, which controls "absolute antagonism," is from stage 1 and 2 to stage 3, and the second gate, which is involved in "relative antagonism," is from stage 3 to stages 4 and 5. In the kindled subpopulation of the WAG/Rij group animals in the present study,

stage 3 motor seizures were apparently delayed. This suggests that the mechanism involved in the delay of stage 3 seizures in WAG/Rij rats may be associated with the mechanisms underlying absolute antagonism.

The relation between the daily occurrence of SWDs and the resistance to kindling points to an interaction between the limbic system and the thalamic circuitry that is involved in the generation or spread of SWDs. Nanobashvili et al. (27) reported that costimulation of the thalamic reticular nucleus during hippocampal kindling stimulation reduced the number and the duration of generalized convulsions. They concluded that stimulation of the thalamic reticular nucleus suppressed limbic motor seizures in hippocampal kindling. This observation provided a new approach for seizure control in temporal lobe epilepsy. Similarly, in patients with partial complex seizures, a significant decrease in generalized tonic–clonic convulsions was observed in a long-term study with electrical stimulation of the centromedian thalamic nucleus. This result suggests that the propagation of epileptic activities involved the thalamic nuclei or the diffuse thalamic projection system or both (28).

An increase in total duration and particularly in the number of spontaneously occurring SWDs in the poststimulation (0–10 min) period was found to be associated with the kindling rate of WAG/Rij rats. The increase in the total duration and particularly in the number of SWDs after kindling stimulations suggests the activation of the cortical system by means of the mechanism involved in the onset of SWDs (29–31). Kindling with repeated electrical stimulations might gradually cause an increased excitability of cortex in the WAG/Rij group. Likewise, the cortical excitability was demonstrated to be different in WAG/Rij animals compared with the nonepileptic control rats. WAG/Rij animals exhibit the lowest threshold for the spread of epileptic activity into limbic structures in comparison with ACI and Wistar rats (32). In the present study, the mean baseline afterdischarge threshold of WAG/Rij rats was lower than that seen in Wistar nonepileptic control animals, although they were not statistically different. Those findings are consistent with our previous report, which shows that the mean baseline afterdischarge threshold for amygdala stimulation in Wistar control was higher than that seen in GAERS (33). All these results suggest that the excitability of the limbic system is increased in genetically absence epileptic rats, but that the process of epileptogenesis, as induced by kindling, is slowed.

One possible explanation underlying the difference between WAG/Rij rats and GAERS might be the kindling parameters (80 Hz instead of 60 Hz, 2-s duration of stimulation instead of 1 s). The development of kindling and motor seizures was previously shown to be dependent on the number of afterdischarges observed during the electrical stimulations rather than the intensity (34,35). Furthermore, Goddard stated in his original article (36), "sine wave, capacitatively coupled rectangular pulses, and biphasic rectangular pulses, at frequencies ranging from 20 to 200 pulse/s, were all found to be effective in eliciting the phenomenon." Therefore the difference observed in kindling resistance between the two strains cannot be attributed to the minor differences in kindling protocols used in the experiments.

In conclusion, no observation of stage 3–5 seizures in a subpopulation of WAG/Rij animals and a slow progression from stage 2 to stage 3 in the kindled subpopulation of WAG/Rij rats support the hypothesis that the mechanisms underlying generalized absence seizures may be responsible for the resistance to the secondary generalization of limbic seizures during amygdala kindling.

Acknowledgment: We thank Ray Guillery for his help in the final preparation of the manuscript. This study was supported by Turkish Scientific and Technical Research Council (TÜBİTAK; project number SBAG-1716) and Marmara University Scientific Research Committee (BAPKO). Ketamine and chlorpromazine were kindly provided by Eczacıbaşı A.Ş., Turkey. Electrodes were kindly provided by El-San Elektrik Gereçleri San. ve Tic. A.Ş, Turkey.

REFERENCES

1. Eşkazan E, Onat FY, Aker R, et al. Resistance to propagation of amygdaloid kindling seizures in rats with genetic absence epilepsy. *Epilepsia* 2002;43:1115–1119.
2. Marescaux C, Vergnes M, Depaulis A. Genetic absence epilepsy rats from Strasbourg: a review. *J Neural Trans* 1992;suppl 35:37–69.
3. Vergnes M, Marescaux C, Depaulis A. Mapping of spontaneous spike and wave discharges in Wistar rats with genetic generalized non-convulsive epilepsy. *Brain Res* 1990;523:87–91.
4. Dolleman-Van der Weel MJ, Witter MP. Projections from the nucleus reuniens thalami to the entorhinal cortex, hippocampal field CA1, and the subiculum in the rat arise from different populations of neurons. *J Comp Neurol* 1996;22:637–650.
5. Dolleman-Van der Weel MJ, Lopes da Silva FH, et al. Nucleus reuniens thalami modulate activity in hippocampal field CA1 through excitatory and inhibitory mechanisms. *J Neurosci* 1997;17:5640–5650.
6. Bertram EH, Zhang DX. Thalamic excitation of hippocampal CA1 neurons: a comparison with the effects of CA3 stimulation. *Neuroscience* 1999;92:15–26.
7. Bertram EH, Mangan PS, Zhang D, et al. The midline thalamus: alterations and a potential role in limbic epilepsy. *Epilepsia* 2001;42:967–978.
8. Nicolson A, Chadwick DW, Smith DF. The coexistence of idiopathic generalized epilepsy and partial epilepsy. *Epilepsia* 2004;45:682–685.
9. Koutroumanidis M, Hennessy MJ, Elwes RD, et al. Coexistence of temporal lobe and idiopathic generalized epilepsies. *Neurology* 1999;53:490–495.
10. van Luijtelaar EL, Coenen AM. Two types of electrocortical paroxysms in an inbred strain of rats. *Neurosci Lett* 1986;20:393–397.
11. van Luijtelaar EL, Van der Werf SJ, et al. Arousal, performance and absence seizures in rats. *Electroencephalogr Clin Neurophysiol* 1991;79:430–434.
12. van Luijtelaar EL, de Bruijn SF, Declerck AC, et al. Disturbances in time estimation during absence seizures in children. *Epilepsy Res* 1991;9:148–153.
13. Crunelli V, Leresche N. Childhood absence epilepsy: genes, channels, neurons and networks. *Nat Rev Neurosci* 2002;3:371–382.
14. Coenen AM, van Luijtelaar EL. Genetic animal models for absence epilepsy: a review of the WAG/Rij strain of rats. *Behav Genet* 2003;33:635–655.

15. Sitnikova E, van Luijtelaar G. Reduction of adrenergic neurotransmission with clonidine aggravates spike-wave seizures and alters activity in the cortex and the thalamus in WAG/Rij rats. *Brain Res Bull* 2005;64:533–540.
16. Midzianovskaia IS, Kuznetsova GD, Coenen AM, et al. Electrophysiological and pharmacological characteristics of two types of spike-wave discharges in WAG/Rij rats. *Brain Res* 2001;911:62–70.
17. Paxinos G, Watson N. *The rat brain in stereotaxic coordinates*, 4th ed. San Diego: Academic Press, 1998.
18. Racine J. Modification of seizure activity by electrical stimulation, II: motor seizure. *EEG Clin Neurophysiol* 1972;32:281–294.
19. Kelly ME, Staines WA, McIntyre DC. Secondary generalization of hippocampal kindled seizures in rats: examining the role of piriform cortex. *Brain Res* 2002;957:152–161.
20. Midzyanovskaya IS, Kuznetsova GD, Vinogradova LV, et al. Mixed forms of epilepsy in a subpopulation of WAG/Rij rats. *Epilepsy Behav* 2004;5:655–661.
21. Schridde U, van Luijtelaar G. The influence of strain and housing on two types of spike-wave discharges in rats. *Genes Brain Behav* 2004;3:1–7.
22. Danober L, Deransart C, Depaulis A, et al. Pathophysiological mechanisms of genetic absence epilepsy in the rat. *Prog Neurobiol* 1998;55:27–57.
23. Neidermeyer E. Primary (idiopathic) generalized epilepsy and underlying mechanisms. *Clin Electroencephalogr* 1996;27:1–21.
24. Engel J. Excitation and inhibition in epilepsy. *Can J Neurol Sci* 1996;23:167–174.
25. Applegate CD, Burchfiel JL. Microinjections of GABA agonists into the amygdala complex attenuates kindled seizure expression in the rat. *Exp Neurol* 1988;102:185–189.
26. Burchfiel JL, Applegate CD. Forebrain and brainstem mechanisms governing kindled seizure development: a hypothesis. In: Wada JA, ed. *Kindling 4*. New York: Plenum Press, 1990:93–107.
27. Nanobashvili Z, Chachua T, Nanobashvili A, et al. Suppression of limbic motor seizures by electrical stimulation in thalamic reticular nucleus. *Exp Neurol* 2003;181:224–230.
28. Velasco F, Velasco M, Jimenez F, et al. Centromedian nucleus stimulation for epilepsy: clinical, electroencephalographic, and behavioral observations. *Thalamus Re Systs* 2002;1:387–398.
29. Peeters BWMM, Spooren WPJM, van Luijtelaar ELJM, et al. The WAG/Rij rat model for absence epilepsy: anticonvulsant drug evaluation. *Neurosci Res Commun* 1998;2:93–97.
30. Seidenbecher T, Staak R, Pape H-C. Relations between cortical and thalamic cellular activities during absence seizures in rats. *Eur J Neurosci* 1998;10:1103–1112.
31. Meeren HK, Pijn JP, van Luijtelaar EL, et al. Cortical focus drives widespread corticothalamic networks during spontaneous absence seizures in rats. *J Neurosci* 2002;22:1480–1495.
32. Tolmacheva EA, van Luijtelaar G, Chepurnov SA, et al. Cortical and limbic excitability in rats with absence epilepsy. *Epilepsy Res* 2004;62:189–198.
33. Onat FY, Eşkazan E, Aker R. Experimental absence versus amygdaloid kindling. In:Corcoran M, Moshe S, eds. *Kindling 6*. New York: Springer, 2005:37–47.
34. Racine RJ. Modification of seizure activity by electrical stimulation, I: after-discharge threshold. *Electroencephalogr Clin Neurophysiol*. 1972;32:269–279.
35. Moshe SL, Ludvig N. Kindling. In: Pedley TA, Meldrum BS, eds. *Recent advances in epilepsy*. New York: Churchill Livingstone, 1988:21–44.
36. Goddard GV. Development of epileptic seizures through brain stimulation at low intensity. *Nature* 1967;214:1020–1021.