Sleep, 18(4):252–256

Epilepsy and Sleep

Sleep Deprivation and Spike-Wave Discharges in Epileptic Rats

W. H. I. M. Drinkenburg, A. M. L. Coenen, J. M. H. Vossen and E. L. J. M. van Luijtelaar

Department of Psychology, University of Nijmegen, The Netherlands

Summary: The effects of sleep deprivation were studied on the occurrence of spike-wave discharges in the electroencephalogram of rats of the epileptic WAG/Rij strain, a model for absence epilepsy. This was done before, during and after a period of 12 hours of near total sleep deprivation. A substantial increase in the number of spike-wave discharges was found during the first 4 hours of the deprivation period, whereas in the following deprivation hours epileptic activity returned to baseline values. Immediately after termination of deprivation, a decrease in the number of spike-wave discharges parallelled a rebound of rapid eye movement (REM) sleep and deep non-REM sleep. An initial increase in epileptic activity has also been reported during sleep deprivation of humans. This initial increase as well as the epileptogenic effects during the course of the sleep deprivation and during the recovery period after sleep deprivation can be interpreted in terms of changes in sleep–wake states. Although the epilepsy-provoking mechanisms are not yet understood, an explanation is suggested based on changes of transitions between sleep–wake states and shifts in level of synchronization. **Key Words:** Epilepsy—Sleep deprivation—Sleep–wake states—Spike-wave discharges—WAG/Rij rats.

Sleep deprivation is an effective method of provoking epileptic discharges in patients (1,2). A basic question, however, remains how sleep–wake regulation and epileptogenesis are linked. It is unclear whether the provocative effects of sleep deprivation are caused by changes in the amounts of sleep–wake states or by an intermediate factor that produces a lowering of the threshold for the occurrence of paroxysmal activity (3–5). Examination of the changes in both epileptic activity and sleep–wake states as a result of sleep deprivation may help to clarify the epileptogenic mechanisms of sleep deprivation.

Despite the apparent sensitivity of several types of generalized epilepsy to sleep deprivation, only a few studies have investigated the effects of sleep deprivation in animal models. Shouse (6) found in cats that a 24-hour period of sleep deprivation enhances experimentally induced myoclonic absence seizures in all sleep–wake states. She suggested that sleep loss induces brain hyperexcitability in all states of vigilance. Peeters et al. (7) selectively deprived epileptic rats of rapid eye movement (REM) sleep and reported a decrease in the number of epileptic discharges during and after sleep deprivation. They held an increase in tonic arousal induced by REM sleep deprivation responsible for the modulation of epileptic activity.

The present study describes the effects of depriving rats of both REM and non-REM sleep on epileptic activity. Sleep–wake states and epileptic discharges were analyzed before, during and following a 12-hour period of almost total sleep deprivation. This question was addressed in a genetic model for absence epilepsy, the WAG/Rij strain of rats, of which all animals spontaneously show trains of spike-wave discharges in the cortical electroencephalogram (EEG). These spike-wave discharges have been extensively evaluated, and these studies showed that the WAG/Rij model could be regarded as a valid model for human absence epilepsy. Therefore, this model is useful for studying the relationship between sleep–wake states and spike-wave discharges (8,9).

Accepted for publication January 1995.
Address correspondence and reprint requests to W. H. I. M. Drinkenburg, NICI, Department of Psychology, University of Nijmegen, P.O. Box 9104, 6500 HE Nijmegen, The Netherlands.

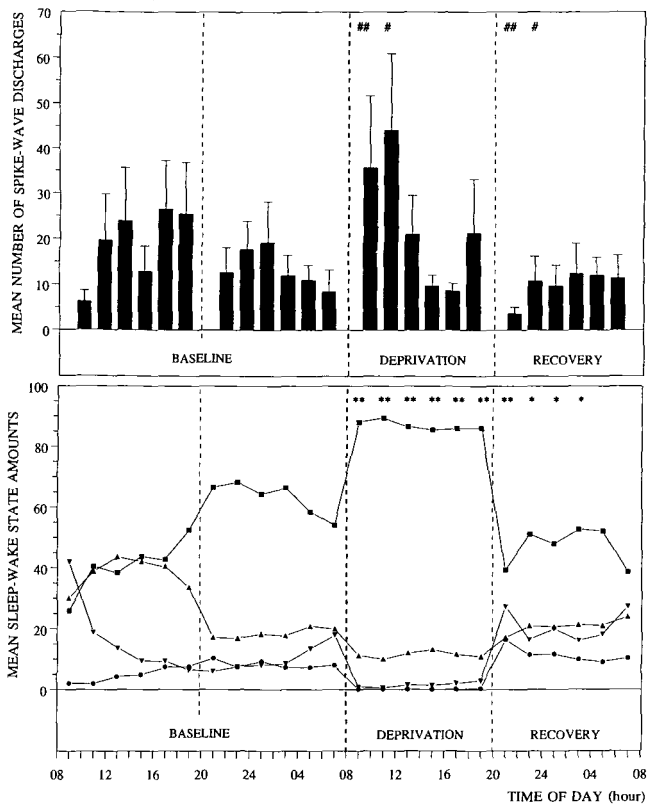

FIG. 1. Mean number of spike-wave discharges (top window) and mean sleep–wake state amounts in percentage of total recording time (bottom window) are given for successive 2-hour periods of the baseline period, the deprivation period and the recovery period, respectively. The mean number of spike-wave discharges and standard error of the mean are indicated in the bar graph. In the line graph light non-REM sleep is indicated by ▲, deep non-REM sleep by ▼, REM sleep by ● and wakefulness by ■. $^{\#\#}p < 0.02$ and $^{\#}p < 0.05$ are for Wilcoxon matched-pairs signed-ranks test for differences in number between experimental and corresponding baseline 2-hour periods; $^{**}p < 0.02$ and $^{*}p < 0.05$ are for Wilcoxon matched-pairs signed-ranks test for differences in percentage of total sleep (combined REM sleep, light non-REM sleep and deep non-REM sleep) between experimental and corresponding baseline 2-hour periods.

METHODS

Eight adult male rats of the WAG/Rij strain, with ages of about eight months each and weights between 288 and 377 g were used. Under deep anesthesia (Nembutal, 60 mg/kg body weight, i.p.), rats were equipped with a tripolar EEG electrode set (Plastics One MS-333/2-A) and a bipolar electromyographic (EMG) electrode set (MS 303/71). With the skull surface placed horizontally, placement of the EEG electrodes was done at coordinates A 2.0, L 3.5 for the frontal electrode and A −6.0, L 4.0 for the parietal electrode. The reference EEG electrode was located over the cerebellum, and EMG electrodes were subcutaneously placed over the dorsal neck muscles. Following surgery, rats were singly housed and maintained on a 12:12 hour light–dark cycle with bright white lights on at 8 a.m. Animals had ad libitum access to standard laboratory food and water.

Electroencephalogram and EMG signals were amplified and filtered by an Elema-Schönander polygraph, allowing EEG frequencies between 0.5 and 70 Hz and EMG frequencies between 27 and 700 Hz to pass. Signals were recorded on magnetic tape (SE 7000) and printed out on chart paper with a paper speed of 1.0 cm/second. In addition, signals were analyzed on line by means of an automatic sleep–wake classification system to determine the various sleep–wake states of the rat such as wakefulness, REM sleep, light non-REM sleep and deep non-REM sleep (10). The sleep–wake state of the animal was determined for subsequent epochs of 5 seconds duration. Spike-wave discharges were scored by visual inspection of the EEG, according to criteria elaborated earlier (8).

Animals were allowed to recover from the operation for 2 weeks and habituated to the experimental setting for 48 hours. Starting at 8 a.m., a baseline registration of EEG and EMG was made for 24 hours. Subsequently, animals were deprived of total sleep by shaking their cages as soon as sleep onset was detected. Determination of sleep onset was based on visual detection of slow waves of non-REM sleep or theta activity associated with REM sleep. This was verified by observation of the animal. To awaken the animal upon detection of sleep onset, the experimenter started to shake the cage with a fixed intensity in a remote-controlled way from an adjacent room. Shaking continued till the animal was clearly aroused. Sleep deprivation was imposed for 12 hours from 8 a.m. until 8 p.m. during the light period, which is the main sleep period for rats. Thereafter, recording of the animals was continued for the next 12 hours until 8 a.m. In this period the animals could recover from the sleep deprivation.

The amounts of sleep–wake states, determined by the automatic sleep–wake classification system, were expressed as a percentage of recording time during baseline, deprivation and recovery. The efficacy of the sleep deprivation procedure was analyzed by comparing amounts of sleep–wake states during successive 2-hour deprivation periods with amounts obtained from the corresponding 2-hour baseline periods. Furthermore, sleep–wake states occurring during the recovery and during the baseline period were also compared. In the same way, comparisons were made in the number of spike-wave discharges. Intraindividual differences in sleep–wake state amounts and in epileptic activity between corresponding periods were statistically analyzed by means of the non-parametric Wilcoxon matched-pairs signed-ranks test. All values are means of eight subjects and are given with standard errors of the mean (SEM).

TABLE 1. *Sleep–wake state presence and epileptic activity, both expressed as a percentage of the corresponding 2-hour baseline period*[a]

Time	SWD	REM	l-NREM	d-NREM	Awake
Deprivation period					
08–10	553.8 (107.3)**	0.0 (0.0)**	33.7 (9.1)**	0.8 (0.5)**	382.5 (57.3)**
10–12	246.1 (45.3)*	5.6 (5.6)*	24.0 (5.5)**	3.5 (2.4)**	228.7 (16.7)**
12–14	134.3 (30.0)	0.0 (0.0)**	26.6 (4.6)**	17.0 (6.8)**	268.0 (40.0)**
14–16	138.7 (39.5)	0.0 (0.0)**	29.7 (5.2)**	20.7 (5.4)**	205.5 (14.3)**
16–18	61.3 (19.4)	1.8 (1.8)**	30.1 (6.7)**	42.8 (27.8)*	233.0 (31.6)**
18–20	111.1 (52.1)	11.5 (8.5)**	32.7 (6.5)**	57.7 (17.8)	173.0 (17.2)**
Recovery period					
20–22	35.2 (8.2)**	203.7 (40.0)**	110.3 (2.0)	549.7 (112.4)**	60.0 (8.2)**
22–24	47.3 (12.8)*	206.1 (53.7)	154.4 (36.6)	396.6 (102.4)*	80.5 (11.6)*
00–02	64.3 (21.0)	137.0 (21.1)	119.7 (15.4)	353.8 (102.8)**	75.5 (10.4)*
02–04	88.5 (23.6)	164.1 (35.7)	124.6 (13.8)	236.3 (45.0)	81.1 (7.3)*
04–06	119.8 (37.3)	124.7 (17.3)	109.6 (16.5)	166.1 (32.7)	91.1 (12.1)
06–08	242.2 (100.0)	143.2 (21.8)	131.4 (12.7)	167.3 (31.2)	73.2 (13.3)

[a] Baseline values were set at 100% for each subject. For subsequent 2-hour periods, percentages and standard error of means (between brackets) are given with respect to the mean number of spike-wave discharges (SWD) as well as with respect to the percentages of recording time for REM sleep (REM), light non-REM sleep (l-NREM), deep non-REM sleep (d-NREM) and waking (Awake). All conditions consist of eight animals.

[b] ** $p < 0.02$ and * $p < 0.05$ are for Wilcoxon matched-pairs signed-ranks test for within-subject comparison of absolute percentages of the experimental 2-hour period with corresponding baseline 2-hour period (see also Fig. 1).

RESULTS

The percentage of each sleep–wake state was calculated during baseline and deprivation treatment for periods of 2 hours. As compared to baseline values, all 2-hour deprivation periods contained significantly less non-REM and REM sleep. Results of the 2-hour periods of the baseline period, the deprivation period and the recovery period are presented in Fig. 1. In order to determine the overall efficacy of the sleep deprivation procedure, the total amounts of REM sleep and non-REM sleep were calculated. Sleep was registered for 59% of total time during the light period of the baseline, whereas the amount of sleep decreased to 13% of the light period of the deprivation phase. This reduction is highly significant ($Z = -2.37$, $p < 0.018$).

In the recovery period, a marked rebound of REM sleep and in particular of deep non-REM sleep was found during the first 2 hours (Table 1: $Z = -2.52$, $p < 0.012$, $Z = -2.52$, $p < 0.012$, respectively). The increase in deep non-REM sleep was also present during the second 2-hour period after termination of sleep deprivation ($Z = -2.37$, $p < 0.018$). To facilitate the overview of the deprivation-induced effects, in Table 1 the values of the deprivation and recovery period are additionally expressed as a percentage of the corresponding 2-hour baseline period values, which were all set to 100%.

An increase in the number of spike-wave discharges compared to the corresponding baseline hours was evident during the first 4 hours of the deprivation period (Table 1: $Z = -2.52$, $p < 0.012$, $Z = -2.20$, $p < 0.028$, successively). After this initial increase, the mean number of spike-wave discharges gradually returned to baseline levels (Fig. 1). During the recovery period, a decrease in the number of spike-wave discharges compared to corresponding baseline numbers was found for a period up to 4 hours after the end of the deprivation ($Z = -2.52$, $p < 0.012$, $Z = -2.10$, $p < 0.036$, successively).

DISCUSSION

Sleep deprivation produced an increase in epileptic discharges during the first 4 hours of deprivation. After this initial increase, the number of spike-wave discharges gradually returned to baseline levels. The first hours of the post-deprivation recovery period showed a decrease in the occurrence of spike-wave discharges compared to baseline. The numbers of discharges again returned to baseline values during the remaining hours of the recovery period.

In contrast to studies in humans on the epileptogenic effects following sleep deprivation (1–5), data obtained in humans during sleep deprivation are scarce (11,12). Rodin et al. (12) reported an increase in epileptic-like paroxysms during sleep deprivation, and this increase was limited to the first part of the sleep deprivation period. In the present study, an increase of epileptic activity was also found only during the first part of the deprivation period. Furthermore, a reduction of epileptic activity was initially found during the recovery period. The majority of clinical studies investigated the effects of sleep deprivation after its termination. Although there is debate on whether activation is most

prominent during waking or during sleeping, activation of typical absence spike-waves has been reported to be most conspicuous after the initial sleep rebound, when the patient is inactive but awake (13). Considering the differences between man and rat with respect to sleep organization, it is awkward to compare recovery results between both species.

The question regarding the epilepsy-provoking mechanisms has not yet been solved. It has been suggested that sleep deprivation causes its epileptogenic effect by changing amounts of sleep–wake states (11). In earlier studies in spontaneously epileptic rats, it was indeed confirmed that epileptic activity and sleep–wake states have a distinct relationship. Spike-wave discharges preferably occur during passive wakefulness and light non-REM sleep, but seldom during active wakefulness, deep non-REM sleep and REM sleep (14,15). As a consequence, changes in the duration and the distribution of the several sleep–wake states may predict the amount of spike-wave activity during sleep deprivation. In the first hours of sleep deprivation that start at the beginning of the light or sleep period of the rat, a high occurrence of wakefulness is enforced. Because at that time the rats seemed drowsy but had a waking EEG, the animals were most likely in a state of passive wakefulness where spike-wave activity is frequent. This increase in passive wakefulness may therefore account for the initial increase in spike-wave discharges during deprivation. Nevertheless, after this initial increase the amount of spike-wave activity drops to baseline levels while an increased percentage of wakefulness is maintained. It is considered that cumulative waking time increases, and this cumulation will increase sleep propensity and sleepiness (3). When sleep propensity increases, it reaches a level at which animals are difficult to arouse. In that situation, the activation needs to be so fierce and intensive that rats become wide awake shortly, but nonetheless fall into a deep sleep again immediately thereafter. It is not surprising that the occurrence of spike-wave discharges is then no longer favored. During the recovery period high percentages of sleep–wake states such as deep non-REM and REM sleep occur that are not favorable for the occurrence of spike-wave discharges. This can explain the low incidence of epileptic activity after deprivation that is even lower than during the corresponding baseline period.

In our opinion in all situations the same epilepsy-modulating mechanism is working. From neurophysiological studies it appears that epilepsy susceptibility is maximal during transitions and shifts in vigilance (16). Epileptic paroxysms have been found to occur preferentially in periods in which sleep-inducing and arousing mechanisms are in competition and unstable levels of brain synchronization prevail (17,18). In addition, transitions during intermediate levels of vigilance have been reported to be favorable for the occurrence of spike-wave discharges (14,15). We suggest that the epileptogenic effects of sleep deprivation can be adequately explained by changes in shifts between levels of brain synchronization. Although a detailed study of such transitions was beyond the scope of the present experiment, we hypothesize that in the first hours of deprivation the number of shifts sharply increases as a result of drowsiness. Furthermore, we hold the view that after this initial period sleep propensity becomes so high that rats when falling asleep pass the paroxysm-sensitive, intermediate states of vigilance so quickly that there is less opportunity for epileptic discharges to occur. Often, a short spindle-like phenomenon is then seen in this fast transition to the production of large slow sleep waves. The results of the recovery period can be interpreted in an analogous way. Evidence exists that during recovery sleep the level of synchronization is even higher than during normal slow wave sleep (19). Indeed, the decrease in epileptic activity is parallelled especially by rebound deep non-REM sleep. Future studies, aimed at describing the shifts in synchronization during state transitions, may substantiate this proposal.

Acknowledgements: The biotechnical assistance of Thijs Janssen is gratefully acknowledged. We thank Lique Coolen and Gerdy Konings for their helpful participation. We also thank Mark Leiblum for his linguistic comments. This research was supported by a grant from the Netherlands Organization for Scientific Research (NWO 560-258-043).

REFERENCES

1. Logothetis J, Milonas I, Bostantzopoulou S. Sleep deprivation as a method of EEG activation. *Eur Neurol* 1986;25, Suppl 2:134–40.
2. Broughton RJ. Sleep and sleep deprivation studies in epilepsy. In: Wada JA, Ellingson RJ, eds. *Clinical neurophysiology of epilepsy EEG handbook,* revised series, vol. 4. Amsterdam: Elsevier Science Publishers B.V., 1990:89–119.
3. Veldhuizen R, Binnie CD, Beintema DJ. The effect of sleep deprivation on the EEG in epilepsy. *Electroencephalogr Clin Neurophysiol* 1983;55:505–12.
4. Klingler D, Trägner H, Deisenhammer E. The nature of the influence of sleep deprivation on the EEG. In: Degen R, Rodin EA, eds. *Epilepsy, sleep and sleep deprivation,* 2nd edition. Amsterdam: Elsevier Science Publishers B.V., 1991:231–4.
5. Pratt KL, Mattson RH, Weikers NJ, Williams R. EEG activation of epileptics following sleep deprivation: a prospective study of 114 cases. *Electroencephalogr Clin Neurophysiol* 1968;24:11–5.
6. Shouse MN. Sleep deprivation increases susceptibility to kindled and penicillin seizure events during all waking and sleep states in cats. *Sleep* 1988;11:162–71.
7. Peeters BWMM, Van Luijtelaar ELJM, Coenen AML. Absence epilepsy in rats is reduced by deprivation of REM sleep. *Physiol Behav* 1989;46:115–9.
8. Coenen AML, Drinkenburg WHIM, Inoue M, Van Luijtelaar ELJM. Genetic models of absence epilepsy, with emphasis on the WAG/Rij strain of rats. *Epilepsy Res* 1992;12:75–86.
9. Coenen AML, Drinkenburg WHIM, Peeters BWMM, Vossen

JMH, Van Luijtelaar ELJM. Absence epilepsy and the level of vigilance in rats of the WAG/Rij strain. *Neurosci Biobehav Rev* 1991;15:259–63.
10. Van Luijtelaar ELJM, Coenen AML. An EEG averaging technique for automated sleep–wake stage identification in the rat. *Physiol Behav* 1984;33:837–41.
11. Beck U, Wenzel D, Sauer M. Selective deprivation of sleep in pycnoleptic children. *Arch Psychiatr Nervenkr* 1977;223:107–15.
12. Rodin EA, Luby ED, Gottlieb JS. The electroencephalogram during prolonged experimental sleep deprivation. *Electroencephalogr Clin Neurophysiol* 1962;14:544–51.
13. Jovanović UJ. General considerations of sleep and sleep deprivation. In: Degen R, Rodin EA, eds. *Epilepsy, sleep and sleep deprivation,* 2nd edition. Amsterdam: Elsevier Science Publishers B.V., 1991:205–15.
14. Drinkenburg WHIM, Coenen AML, Vossen JMH, Van Luijtelaar ELJM. Spike-wave discharges and sleep–wake states in rats with absence epilepsy. *Epilepsy Res* 1991;9:218–24.
15. Gralewicz S, Łuczak C, Tomas T, Wiaderna D. Spontaneous seizure electrocortical activity in rats: relation to arousal level. *Acta Neurobiol Exp* 1994;54:19–31.
16. Speckmann E-J, Elger CE. The neurophysiological basis of epileptic activity: a condensed overview. In: Degen R, Rodin EA, eds. *Epilepsy, sleep and sleep deprivation,* 2nd edition. Amsterdam: Elsevier Science Publishers B.V., 1991:1–7.
17. Halász P. Sleep, arousal and electroclinical manifestations of generalized epilepsy with spike wave pattern. In: Degen R, Rodin EA, eds. *Epilepsy, sleep and sleep deprivation,* 2nd edition. Amsterdam: Elsevier Science Publishers B.V., 1991:43–71.
18. Terzano MG, Parrino L, Anelli S, Halász P. Modulation of generalized spike-and-wave discharges during sleep by cyclic alternating pattern. *Epilepsia* 1989;30:772–81.
19. Dijk D-J, Brunner DP, Borbély AA. EEG power density during recovery sleep in the morning. *Electroencephalogr Clin Neurophysiol* 1991;78:203–14.