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# PHASIC EVENTS IN NORMAL AND EPILEPTIC RATS: TIME OF DAY EFFECTS

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## INTRODUCTION

The function of sleep spindles is by and large unknown. It has been proposed that sleep spindles may serve to protect the brain from incoming arousing events, when non-REM sleep is still not deep yet and fragile<sup>1</sup>. In the last few years, evidence has been collected in humans as well as in rats that two types of sleep spindles can be discriminated, based on topographic and frequency criteria<sup>2</sup>. Topographically and pharmacologically different types of sleep spindles have also been described in rats<sup>3,4</sup>. Anterior sleep spindles were enhanced after the administration of benzodiazepines and barbiturates and reduced after the administration of clonidine, while posterior sleep spindles were aggravated by clonidine. In humans it was proposed that only high frequency sleep spindles are under homeostatic control, while both types of sleep spindles (high and low spindle activity) are controlled by circadian factors<sup>5</sup>. In the present experiment it was investigated whether also in rats the two types of sleep spindles but also other phasic events such as paroxysmal spike-wave discharges (SWDs), type I and II<sup>6</sup> are influenced by time of day, a first indication for circadian control. This was investigated in two types of rats (ACI and WAG/Rij) and at two ages (4 and 6 months). This allowed us to investigate additionally whether the four types of phasic events are also under genetic control and whether aging affected circadian control of sleep spindles.

## METHODS

*Subjects* Thirty-five male WAG/Rij and ACI rats were used. The rats were group housed until surgery, maintained on 12 hr light/dark cycle (white light on at 07.00), and given free access to water and food. Four groups were used: WAG/Rij rats four months old (W4, n=7, body weight at surgery 250-281 g); WAG/Rij six months old (W6, n=9, weighing 261-332 g); ACI rats four months (A4, n=10, 205-239 g); ACI six months old (A6, n=9, weighing 267-325 g). Experiment was approved by the Ethical Committee on Animal Experimentation of Nijmegen University.

*Surgery* For recording of EEG, two chronic electrode sets were implanted in each rat (Plastics One, USA) under deep Isoflurane anesthesia. The following coordinates of Paxinos and Watson rat brain atlas were used: one bipolar electrode set was implanted into the frontal cortex (coordinates with skull surface flat and bregma 0-0: AP -2.0; ML 1.9-2.1), two electrodes of the tripolar electrode set was placed into the parietal/occipital region of the cortex (AP 5.0; ML 2.2), the other, which served as ground, was placed over the cerebellum. After implantation of electrodes rats were housed individually with free access to water and food. The length of recovery period was 7-10 days.

*EEG recording and analysis.* One day prior to start of recording procedure, each rat was put into the recording cage for adaptation. The EEG was monitored and recorded using CODAS

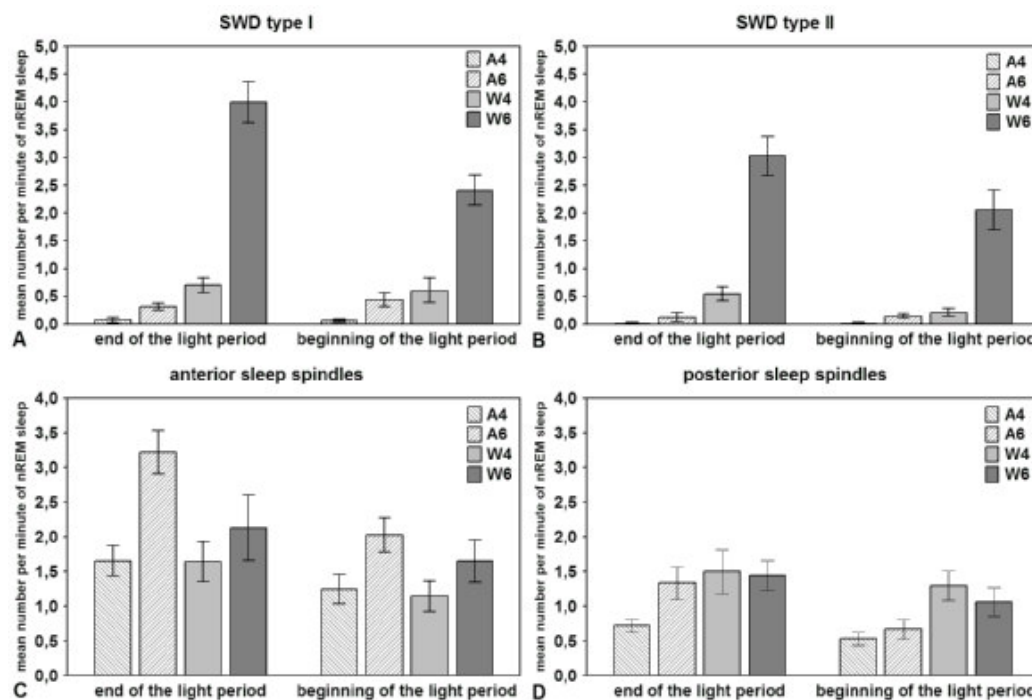
system (DATAQ Instruments, Inc., Akron, OH, USA) during 24 hours. The EEG was also passed through a 7-11 Hz bandpass filter. We have calculated the number of sleep spindles and SWD's per minute of nonREM sleep during a two representative sleep cycles in both the 3<sup>rd</sup> hour of the light and in the 11<sup>th</sup> hour of the light since the sleep cycle turned out to be dependant on time of day. The digitized EEG was stored on hard disk for off-line analysis. Analyzed was the spindle density per minute non-REM sleep in sleep recorded at the beginning and end of the light period. Detection of sleep spindles and SWD's was performed using previously developed software (Friso Westerhuis) followed by visual inspection and verification of extracted events.

*Statistical analyses.* Statistical treatment and analyses included the calculation of descriptive statistics for the number of anterior and posterior sleep spindles and two types of SWD's, type I is generalized, type II more local and can be found at the posterior part of the neocortex. In order to estimate the effects of time of day, age and strain on phasic events, the data were analysed with a MANOVA with strain and age as a between and time of day as a between group subject factor.

## RESULTS

The obtained values are presented in Fig. 1.

**Figure 1.** Mean number and s.e.m. of 4 types of phasic event in 4 groups of rats for the end and beginning of the light period. Significant differences between 6 months old WAG/Rij (W6) group in comparison with 4 months old WAG/Rij (W4), six (A6) and four (A4) months old ACI rats (all at  $p < .001$ ) were found for SWD type I and II. Age effects were found for SWD type I and II and anterior sleep spindles. Strain effects for SWD type I and II.



The outcomes of the MANOVA showed a significant time of day effect for all 4 variables: there were always more phasic events at the end of the light period than at the beginning ( $5.59 < F < 9.20$ ,  $df 1,132$ ,  $0.05 > p < 0.01$ ). There were also strain effects and age effects for posterior sleep spindles (WAG/Rij > ACI), age effects for anterior sleep spindles (old > young), strain (WAG > ACI) and age (old > young) effects for type II SWD and interactions between strain and age (older WAG > any other groups) and strain and time of day (WAG/Rij more at end of day than any other group), and finally, strain, age and all possible interactions between time of day, age and strain for type I SWD. The post-hoc tests showed that this was due to a high number of SWD type I and II specifically at the end of the light period on the 6 months old WAG/Rij rats.

## DISCUSSION

The results of the present study show all type of phasic oscillations, frontal and occipital sleep spindles and the two type of spike-wave discharges showed time of day effects, with more phasic events in the period in which light-non-REM sleep dominates. During the earlier hours of sleep, deep slow wave sleep dominates and the inverse relation between deep slow wave sleep and phasic activity was often reported<sup>4</sup>. Our finding, time of day effects, confirms and extend human data, which showed circadian effects of different types of spindle activity. The circadian distribution of SWDs was also known both from human data obtained in patients with generalized absence seizures<sup>7</sup>, we established earlier that the number of SWD type I was also distributed over the 24 hour day, almost no SWDs at the beginning of the light period, a slow increase and a maximum during the 4-5 hrs of the dark period<sup>8</sup>. Different amounts of SWD type II over the day were not previously studied.

The dominance of sleep spindle activity in the later hours might be expected based on the inverse relationship between delta and spindle activity, it is not very well understandable with the assumed function of sleep spindles. A predominance of sleep spindles (and other phasic events) during the later hours of the sleep cycle when the need for deep slow wave sleep and the continuity of sleep has been largely fulfilled, does not favor a protective function of sleep by sleep spindles during the latter hour of the sleep period.

WAG/Rij and ACI rats do not differ in the density (number of sleep spindles per minute of non-REM sleep) of anterior sleep spindles, only in the number of posterior sleep spindles. Little is known about the origin of posterior sleep spindles, it can be speculated that a circuitry that is involved in posterior sleep spindles, most likely a circuitry involving the reticular thalamic nucleus, some specific thalamic nuclei and the occipital cortex, might be involved (Sitnikova, personal communication). The anterior sleep spindles are evoked in the thalamo-cortical network, in which the lateral thalamus, including the reticular thalamic nucleus is involved. There are no differences in properties of these spindle types in the two strains. Earlier we could not find differences in the microstructure of the RTN in these two strains<sup>9</sup>.

The interaction of time of day with strain for type II SWD and age and strain for type I SWD indicates the high prevalence for these events mainly in WAG/Rij at specific the latter hours of the light phase. It also points to the predominance for phasic events to occur in older WAG/Rij rats at the latter hours of the light period sleep period. Interestingly, at this hour old WAG/Rij rats showed a reduction of the sleep wake cycle<sup>10</sup>. We propose that this reduction in the length of the sleep cycle is due to the high density of SWD type I and to some extend type II at this hour of the day.

## CONCLUSIONS

Different genotype of rats have two types of sleep spindles. These sleep spindles and two types of SWD are more prevalent during the end of the light period and might be under circadian control. The occurrence of SWD interferes with the length of the sleep cycle.

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