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# The effects of vigabatrin on spike and wave discharges in WAG/Rij rats

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**Summary** The effects of vigabatrin, which increases GABA concentrations by inhibiting GABA transaminase, on spike and wave discharges (SWDs) in the electroencephalogram of WAG/Rij rats were studied. Vigabatrin increased the incidence and duration of the SWDs, suggesting a quantitative $GABA_A$ergic involvement in the mechanism(s) underlying the starting and stopping of an ongoing SWD. Also, vigabatrin decreased the SWD peak frequency, suggesting an important role of $GABA_B$ in the mechanism(s) underlying the peak frequency of the SWDs. Vigabatrin gradually changed the course of the hazard rates of the SWD durations, suggesting a qualitative GABAergic role in the mechanism(s) underlying the stopping of an ongoing SWD.


## Introduction

Absence or ''petit mal'' seizures are stereotyped generalised seizures with a highly characteristic electroencephalographic and behavioural pattern. The absence epileptic human electroencephalogram (EEG) as well as that of animal models of absence epilepsy are characterised by generalised spike and wave discharges (SWDs) that are accompanied by a brief decrease in consciousness. The WAG/Rij rat, an inbred strain of rats, is recognised as an animal model of human absence epilepsy (Van Luijtelaar and Coenen, 1986; Coenen and van Luijtelaar, 2003). For the development of new antiepileptic drugs, information about the mechanisms underlying SWDs is essential. By means of observing the effects of alterations in the neurotransmitter systems on various parameters of the SWDs, as can be obtained by pharmacological manipulations, more can be learned about these underlying mechanisms.

Gamma-aminobutyric acid (GABA) is generally recognized to be implicated in the occurrence and control of SWDs (Crunelli and Leresche, 2002). The GABAergic

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neurotransmission system contains two major receptor types, the $GABA_A$ and $GABA_B$ receptors. The $GABA_A$ receptor is an ionotropic receptor with a chloride channel, whereas the $GABA_B$ receptor is a metabotropic receptor coupled to calcium and potassium channels (Bowery et al., 2002). It has been shown that both $GABA_A$- and $GABA_B$-ergic drugs modulate the SWDs (Vergnes et al., 1984; Peeters et al., 1989; Duysens et al., 1991; Marescaux et al., 1992; Budziszewska et al., 1999; Kaminski et al., 2001; Bouwman et al., 2003, 2004).

Interestingly, it has been suggested that not the absolute level of GABA but more specifically the balance between $GABA_A$ and $GABA_B$ conductances is essential for the control of SWDs (Destexhe, 1999; Destexhe and Sejnowski, 2003). From a computational model it is predicted that that the different properties of the SWDs are differentially affected by this balance (Destexhe, 1999). In particular, shifting this balance more towards $GABA_B$ activities is predicted to decrease the peak frequency of the SWDs (Destexhe, 1999). Relatively high $GABA_A$ conductances are predicted to be mainly responsible for increasing the number of oscillation cycles; corresponding to the duration of a SWD in vivo (Destexhe, 1999).

In vivo, the balance between $GABA_A$ and $GABA_B$ conductances can be easily altered by pharmacological manipulations. Vigabatrin is an antiepileptic drug that irreversibly inhibits GABA transaminase one of the major enzymes implicated in GABA degradation (Hammond and Wilder, 1985), and thus increases GABA concentrations. Since the efficacy of GABA for the $GABA_A$ and $GABA_B$ receptors is different (Sodickson and Bean, 1996), and the regional distribution of these receptors differs throughout the brain (Chu et al., 1990; Gottesmann, 2002), the balance between $GABA_A$ and $GABA_B$ conductances will be altered by vigabatrin administration.

The rise of GABA concentration as a result of vigabatrin injection has a slow onset (Hammond and Wilder, 1985; Valdizan et al., 1999; Tong et al., 2000) which allows for investigation of gradual changes in the balance between $GABA_A$ and $GABA_B$. Earlier, we demonstrated that vigabatrin gradually lowers the peak frequency of type II SWDs, indicating that it is indeed likely to gradually shift the balance more towards $GABA_B$ neurotransmission (Bouwman et al., 2003). Since type II SWDs may differ in underlying mechanisms with the what are considered to be typical absence epileptic SWDs type I (Midzianovskaia et al., 2001), in the present study we aim to demonstrate the SWD modulating effects of vigabatrin on the type I SWDs.

Recently, we have shown that hazard rate analysis is a useful tool for investigating the mechanisms underlying SWDs (Maris et al., 2006). Using this analysis we demonstrated that vigabatrin decreases the instantaneous chance of a SWD to stop, creating a condition favouring development of long seizures (Maris et al., 2006). Whether these qualitative changes also have a gradual time course, remains to be determined.

Further investigation on the effects of vigabatrin on SWDs might provide additional knowledge on the underlying mechanisms involved in the control of the absence epileptic SWDs. Therefore, in the present study, the effects of vigabatrin on SWDs in the EEG of WAG/Rij rats were studied, with specific focus on the development over time.

## Methods

This study was performed in accordance with the guidelines of the European Community for the use of experimental animals. Approval of the local Ethical Committee for Animal Studies was obtained (KUN-DEC 2001-34).

### Animals

Male WAG/Rij rats ($n = 16$), 1-year-old, with a mean body weight of 331 g (S.E. = 4 g), were used. The rats were maintained on a reversed 12-h light:12-h dark cycle with lights on at 20:00 h. The rats had ad libitum access to food and water.

### Surgery

Isoflurane anaesthesia was used during the implantation of a tripolar EEG electrode (Plastics One MS-332/2-A) on the cortical surface: one electrode on the frontal region (coordinates with skull surface flat and bregma zero-zero: A2.0 L3.5) and a second one in the parietal region (A-6.0 L4.0). The reference electrode was placed over the cerebellum. The rats were allowed to recover from surgery for 28 days before experimentation began.

### Drugs

Either saline (0.9%) or 500 mg/kg of vigabatrin (Yamanouchi Pharma B.V., The Netherlands) dissolved in saline (0.9%) in a volume of 2 ml/kg was administered via i.p. injection.

### Procedure

The rats were habituated to the experimental setting during 30 min per day on 2 successive days before experimentation. Experimental sessions were performed between 9:00 and 16:00 h. At 9:00 h the rats were given saline or vigabatrin. On experimental days, four rats were placed in separate recording cages (19 cm × 19 cm × 40 cm) and connected with leads through swivels to an amplifier and a computer-based data acquisition system (Dataq Instruments, OH, USA). The EEG signals in a bandwidth between 0.1 and 100 Hz, were sampled with a frequency of 512 Hz.

### Analysis

Offline, the SWDs were detected by visual inspection based on the criteria for standard type I spike and wave discharges as proposed by Van Luijtelaar and Coenen (1986). For the recordings of the 7th hour, only data of five of the eight rats in the vigabatrin group could be analysed, since the SWDs during this period in the other three rats could not be distinguished anymore from background EEG. The EEG, both background and SWDs, of these three rats was severely modified by vigabatrin, which made it impossible to reliably determine transitions between background and epileptic EEG. SWD incidence, duration and SWD peak frequency were determined per block of 1 h. The SWD peak frequency was determined from the frequency spectrum of the first 2 s of each SWD. The frequency spectrum was obtained by tapering the signal with a Hanning window and subsequently applying a Fast Fourier Transform.

Statistical analyses on the SWD incidence, duration and peak frequency were performed, using a repeated measures Analysis of Variance for each dependent variable, treating time as a within subjects (rats) factor and treatment as a between subjects (rats) factor ($\alpha = 0.05$). To describe the Time × Treatment interactions, post hoc, independent samples *T*-tests were performed in SPSS Version 11.5 for Windows (SPSS Inc., Chicago, IL, USA). If the within sample variances were significantly different (as assessed by means of Levene's