PATTERN ANALYSIS OF DRUG RESPONSES MAY UNMASK FALSE NON-RESPONDERS

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INTRODUCTION

The WAG/Rij rat is recognized as a genetic animal model of human absence epilepsy[1]. WAG/Rij rats spontaneously exhibit spike-wave discharges (SWDs) in the EEG with a mean incidence of about 15/hour[1]. This model has an excellent predictive validity[1].
Although being an inbred strain, there exists quite some inter-individual variation in the spontaneous incidence of SWDs[2] as well as in the response to drugs. When the response data after administration of a drug are within the data of the control group, the question is whether this can be considered as biological variation in drug response or the subject should be considered a non-responder. In order to answer this question, we propose to analyze the dynamics of the response rather than the absolute data pre- or post drug administration.
As a sample drug the anti-epileptic drug vigabatrin, which increases GABA concentrations by inhibiting GABA transaminase[3], was used. It is known to cause an increase in SWD activity in WAG/Rij rats[4]. This effect develops slowly and reaches a maximum after 4 hours[5,6]. We analyzed the dynamics of the drug response for each individual animal.

METHODS

Sixteen male one year old WAG/Rij rats (mean weight 337g; SE 16,5g) were used. A tripolar electrode was implanted in each animal under isoflurane anaesthesia, with one electrode placed in the frontal region (2.0, 3.5), one placed parietally (-6.0, 4.0) and a ground electrode placed above the cerebellum.
After surgery the animals were housed individually and kept on a reversed light-dark 12-12h cycle with lights on at 8h pm. They were allowed to recover for at least two weeks.
Baseline EEGs were recorded for one hour before 500 mg/kg vigabatrin was administered intraperitoneally to half of the animals and 12.5 ml/kg saline to the other half. Vigabatrin was dissolved in 40 mg/ml water to obtain a solution isotonic to saline.
After administration the EEG was recorded for four hours. Recording of the total period started at 10.30 AM.
The EEG was recorded between 1 Hz and 100 Hz and at a sample rate of 512 Hz using Windaq (DATAQ Instruments, Akron, OH) and was stored for offline analysis. The incidence of the SWDs was determined and, to describe the dynamics of the response, the equation: Cumulative Incidence = $A \bullet (Time)^B$ was fitted to the data.

RESULTS AND DISCUSSION

During the baseline recording of one hour, the mean number of SWDs was 18 (95% confidence interval (CI) 13-22; range 1-31). The data of the individual animals are given in Table 1. The animals were divided in two groups such that the baseline incidence was not significantly different (figure 1, left panel, unpaired two-tailed t-test t=1.30, df=14; p= 0.12). In the recorded period, the total number of SWDs (figure 1, right panel) was significantly higher in vigabatrin injected animals (mean 353; 99% CI 134-571) than in controls (mean 75; 99% CI 50-99)(t=4.43, df=14; p<0.001). This increase of SWDs after vigabatrin is in accordance with our earlier reports[4].
When we look at the absolute data for the individual animals (Table 1), it is noticeable that one of the animals, V3, showed a total of 83 SWDs in the 5 hours measurement, which is outside the 99% CI of the vigabatrin treated group and within the 99% CI of the control group. The question rose whether this animal behaved like the control animals thus as a non-responder, or whether there was evidence that it did respond to the drug. We therefore analyzed the dynamics of cumulative incidences of the individual animals. The cumulative SWD incidence for each animal can be seen in figure 1, middle panel.

Table 1. The quantitative results of the response of the individual vigabatrin treated (V) and control (C) animals during the baseline hour and the total recorded period. The coefficient of variation for each of the parameters can be found in the bottom row.

Controls				*Vigabatrin*			
Animal no	*Baseline (# SWD in 1 hour)*	*Total (# SWD in 5 hours)*	*Exp b (fit: ax^b)*	*Animal no*	*Baseline (# SWD in 1 hour)*	*Total (# SWD in 5 hours)*	*Exp b (fit: ax^b)*
C1	22	62	0.63	V1	21	339	2.5
C2	20	100	1.1	V2	24	304	2.1
C3	19	58	0.62	V3	1	83	1.9
C4	24	75	0.72	V4	8	444	2.7
C5	10	42	0.86	V5	21	611	3.4
C6	26	90	0.77	V6	16	533	2.5
C7	12	77	0.78	V7	8	163	3.7
C8	31	93	0.77	V8	24	348	2.4
%CV	34%	26%	19%		57%	50%	23%

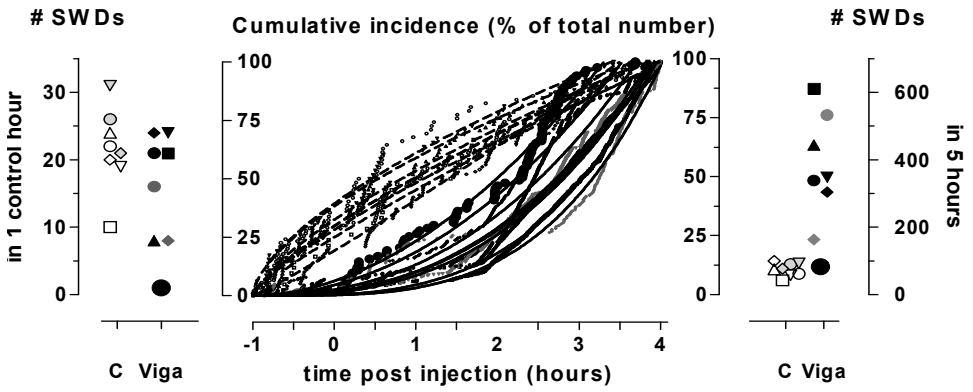

Figure 1. Graphic representation of the quantitative results and the dynamic pattern in response to vigabatrin of the individual animals. C stands for the control animals and Viga denotes the vigabatrin treated animals. Left panel: number of SWDs recorded during the baseline hour. Right panel: total number of SWDs in 5 hours recording. The bold black dots are the data of the animal that was questioned to be a responder. Middle panel: cumulative SWD incidence, with the total number that is depicted in the right panel being 100%. Fits of the control animals are represented with dotted lines; fits of treated animals with a continued line.

There is a clear difference in the dynamics of SWD incidence between controls and vigabatrin treated animals. This is visible in the exponents of the equation fitted to the data (the b-values). These exponents differed significantly (control mean 0.78, CI: 0.59-0.96; vigabatrin mean 2.7, CI: 1.9 - 3.4; $t = 8.29$, $df=14$; $p<0.0001$). The value of the exponent of the animal that was questioned to be a responder was within the 99% CI of its group now, although it had the lowest exponent (1.9). Moreover, the % CV (coefficient of variation, SD/mean) of the treated animals is greatly reduced: from 50 % when considering the raw incidence data to 23 % for the parameter describing the dynamics. The parameters describing the dynamics of the drug response appear to be more indicative for the drug response then the raw incidence data.

Judged only by incidence, which is in the same range as controls, animal V3 could easily be regarded as a non-responder. When taking into consideration the remarkably similar dynamics of the cumulative incidence curve compared with the other vigabatrin treated animals however, considering this animal a non-responder can be excluded. The fits on the data of the vigabatrin treated animals all show concavity down whereas all fits on the controls show concavity up (except one animal who showed a straight line with an exponent not different from unity (1.1)). The concavity down, that is an increase in incidence in time, is in line with the delayed effects well known of the irreversible GABA transaminase inhibitor vigabatrin[3], whereas the concavity up, that is a decrease in incidence towards the end of the dark period, of the control animals is in line with the circadian rhythmicity described in earlier studies[7]. In another study in which large individual differences in response to an anticonvulsant drug were found[8], rats with no or moderate reduction in seizure frequency after treatment were considered non-responders. The present study shows that drawing such conclusions based on only absolute values may lead to incorrect exclusion of the subjects and thus is not recommendable. We showed that it is not unlikely that low responders come from the same population as the others and should not be excluded.

Also in another long-term study[9] response patterns to antidepressant drugs were analyzed in patients over a six-week period. It was found that substantially more patients receiving active than placebo medication displayed treatment response patterns. The study emphasizes the benefit of identification of a distinctive pattern of clinical response to an active drug for both research and clinical applications. Our results support this conclusion.

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CHRONIC SLEEP RESTRICTION GRADUALLY DESENSITIZES THE BRAIN SEROTONIN 1A RECEPTOR SYSTEM

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INTRODUCTION

In our 24h society, frequently-disrupted and restricted sleep is a rapidly increasing problem that may contribute to the development of diseases such as depression[1,2]. One of the proposed neurobiological mechanisms underlying depression is a disturbance in the brain's serotonergic neurotransmission, particularly a desensitization of the serotonin (5-HT) 1A receptor system[3,4]. However, a relationship between chronic sleep loss and changes in serotonin receptors has not been established. Therefore, in the present study we experimentally tested the hypothesis that chronic sleep restriction leads to desensitization of the serotonin 1A receptor system.

METHODS

Sleep restriction

Adult male Wistar rats were subjected to a protocol of chronic sleep restriction allowing them only 4h of undisturbed rest per day at the beginning of the light phase, their normal resting phase[5]. For the remainder of the time, animals were kept awake by placing them in slowly rotating wheels driven by an engine at constant speed (0.4 m/min). Since rats normally sleep about 10 to 12h per day, the 4h of rest would not be sufficient to fully recover from the 20h of forced wakefulness.

Forced activity control

Since the procedure of sleep deprivation included mild forced locomotion, we performed an additional experiment to establish whether effects of sleep restriction were partly due to forced activity rather than sleep loss per se. A second group of rats was subjected to a schedule of forced activity in the same drums that were used for the sleep restriction. These new animals were forced to walk at double speed for half the time (0.8 m/min for 10h per day). In other words, the housing conditions were the same and the animals covered the same distance as the sleep-restricted rats, however, they had to walk at a higher intensity and had more time to sleep (14h of rest per day versus 4h in the sleep-restricted animals).

Serotonin 1A sensitivity

To examine the effect of chronic sleep restriction on serotonin 1A receptor sensitivity, we measured the physiological response to a subcutaneous injection with the serotonin 1A agonist (±)-8-hydroxy-2-(di-*n*-propyl-amino) tetralin hydrobromide (8-OH-DPAT). This drug causes an acute hypothermic response that can be used as an indicator of central 5-HT_{1A} neurotransmission, as has been shown in rats as well as in humans[6,7]. In depressed patients, this serotonin 1A mediated hypothermia is attenuated, in accordance with other evidence of

decreased 5-HT_{1A} signaling[3,4]. In the present study, we applied radio telemetry with intraperitoneally implanted transmitters to record the 5-HT_{1A} mediated drop in body temperature (Data Sciences, St. Paul, USA). The hypothermic response to a standard injection of a 8-OH-DPAT (0.25 mg/kg body weight) was measured after 2 and 8 days of restricted sleep or forced activity.

Glucocorticoid levels

It has been reported that serotonin 1A receptor sensitivity can be attenuated by stress and elevated levels of glucocorticoids[8,9]. We therefore sought to determine whether our sleep restriction protocol might attenuate 5-HT_{1A} receptor sensitivity by increased levels of stress hormones. Blood samples were collected from the tail to measure effects of sleep restriction and forced activity on plasma levels of corticosterone. The blood samples were taken on the first and the seventh day of the protocol, thereby not interfering with the 8-OH-DPAT challenges on day 2 and 8.

RESULTS AND DISCUSSION

The injection of 8-OH-DPAT caused a drop in body temperature of approximately 2^{o}C within 20-30 min. The temperature returned to baseline levels about 90 min after the injection (Figure 1). In rats that were sleep-restricted for 2 days, the hypothermic response was not different from control animals that were allowed unrestricted sleep (Figure 1A), but after 8 days of restricted sleep 5-HT_{1A}-mediated response was significantly attenuated (Figure 1B). These results suggest that sleep restriction gradually desensitizes the 5-HT_{1A} receptor system. Contrary to the sleep-restricted animals, the rats subjected to chronic forced activity at a higher intensity did not show significant changes in the temperature response to 8-OH-DPAT (Figure 1C and D).

In the sleep-restricted animals, corticosterone levels were not significantly elevated at the end of their daily 20h sleep deprivation session, neither after 1 day nor after 7 days of chronic sleep restriction. It thus appears that the sleep disruption procedure was not particularly stressful for the rats. In contrast, the animals that were subjected to the forced activity protocol showed elevated levels of corticosterone at the end of their activity sessions (ANOVA $p<0.05$; data not shown). Together these results suggest that sleep restriction attenuates 5-HT_{1A} receptor sensitivity by a mechanism that does not involve glucocorticoids and that is independent of stress and forced activity.

SUMMARY AND CONCLUSIONS

The present study aimed to make a link between two sets of observations: one, the observation that sleep problems may be associated with increased sensitivity to psychopathology[2]; and two, the observation that mood disturbances are associated with decreased serotonergic neurotransmission[3,4]. The data show that experimental restriction of sleep in rats gradually desensitizes the serotonin 1A receptor system and, thus, changes the brain in a direction that is similar to what is seen in affective disorders. These findings provide a link between chronic sleep loss and sensitivity for disorders that are associated with deranged serotonergic neurotransmission.

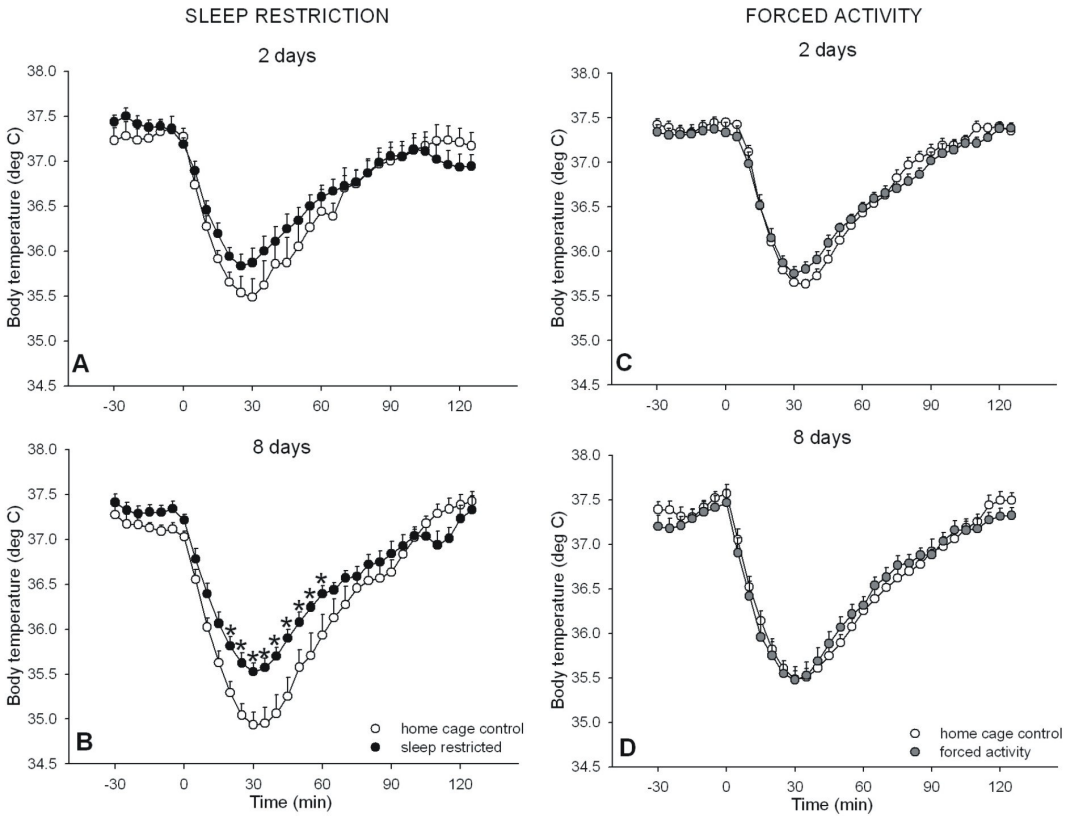

Figure 1. Chronic sleep restriction gradually desensitizes serotonin 1A receptors in the brain. [A and B] The hypothermic response to 8-OH-DPAT after 2 or 8 days of restricted sleep (on each day, n=8 for sleep restriction, n=6 for control). After 8 days of restricted sleep the 5-HT_{1A} mediated response was significantly attenuated (repeated measures ANOVA with posthoc t-test: * $p<0.05$).[C and D] The hypothermic response to 8-OH-DPAT after 2 or 8 days of forced activity at double speed for half the time (on each day, n=8 for forced activity and n=8 for control). No significant differences between animals subjected to forced activity and home cage controls.

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