Alteration of behavior in mice by muscimol is associated with regional
electroencephalogram synchronization

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Section Editor: Dr. Minoru Kimura

Abbreviations

EEG: electroencephalogram; EMG: electromyogram; IR: infra red; NREM sleep: non rapid-eye movement sleep; RW: running wheel; SWA: slow wave activity.

Abstract

We tested the hypothesis that the effects of GABAergic agonists on behavior and the electroencephalogram (EEG) result from an increased regional synchronization in cortical circuits. The relationship between regional EEG topography, EEG synchronization and alteration of behavior was investigated by administering male C57BL/6 mice (n=7) a high, 3 mg/kg i.p. dose of muscimol, a selective GABA_A agonist. Parietal and frontal cortical EEG, electromyogram, infra red- and running wheel-activity were recorded for 3 h before and 9 h after injection.

Muscimol consistently elicited biphasic behavioral changes. Initially, it induced a catalepsy-like state lasting 96.0 ± 12.4 min. This state was followed by a hyperactivity period of 49.7 ± 5.4 min, during which the mice engaged in vigorous wheel running. During catalepsy, the EEG exhibited high amplitude waves which showed a consistent phase relationship between the frontal and parietal derivation. Moreover, the typical regional differences between the EEG spectra of the two derivations were abolished, and a redistribution of EEG power towards lower frequencies (<3 Hz) occurred in both derivations. In contrast, during hyperactivity the parietal EEG was dominated by theta-activity (7-9 Hz), which is typical for running behavior, while high amplitude slow waves, resembling the normal NREM sleep EEG pattern, predominated in the frontal EEG.

The data indicate that the GABAergic system is involved in the regulation of cortical synchronization of neuronal activity and suggest a link between regional EEG synchronization and behavioral states.

Keywords: sleep; topography; EEG spectral analysis; GABA_A agonist; sedation; catalepsy.

Introduction

Electrical activity of the brain is determined by specific interactions between billions of excitatory and inhibitory neurons of the neocortex and subcortical structures. All major oscillations in the electroencephalogram (EEG), namely delta, theta, spindle and gamma frequencies, arise from synchronous neuronal activity in the neocortex, thalamus, hippocampus and other brain structures, where GABAergic interneurons play an important role (Steriade et al., 1990, von Krosigk et al., 1993, Steriade and Amzica, 1998, Bland et al., 1999, Chapman and Lacaille, 1999).

Regional EEG differences are present during waking and sleep in humans and rodents. These differences are enhanced after prolonged wakefulness and are thought to reflect use-dependent processes (Huber et al., 2000, Vyazovskiy et al., 2000, Vyazovskiy et al., 2006). Regional EEG dynamics during sleep have not only a functional but also a morphological basis. Thus, EEG recordings in acallosal mice which have enhanced neocortical connectivity in the rostral part of the hemisphere due to the Probst bundle, showed higher frontal EEG power at approximately 1 Hz at the expense of the remaining delta frequencies (Vyazovskiy and Tobler, 2005). Based on such results we proposed that there is a link between local changes of EEG power and regional neuronal synchronization (Vyazovskiy and Tobler, 2005).

The level of regional synchronization might be an important factor underlying vigilance. In contrast to waking, during which cortical activity is activated, behavioral states characterized by high EEG synchrony, such as non-rapid eye movement (NREM) sleep or absence seizures, are accompanied by reduced responsiveness, immobility and loss of consciousness. Neurophysiological mechanisms underlying the relationship

between regional synchronization and the behavioral state are unresolved, though there is considerable evidence that GABA-mediated inhibition might be involved.

GABAergic agonists elicit sedation and sleep (Nelson et al., 2002, Reynolds et al., 2003, Mohler, 2006, Rudolph and Mohler, 2006), but the neurophysiologic mechanisms underlying their hypnotic properties remain unknown (Nelson et al., 2002). A large body of evidence suggests that the sedative and/or hypnotic action of GABA_A agonists is associated with increased cortical synchronization in the low EEG frequencies. Thus, in rats muscimol induced sedation and catalepsy (reviewed in DeFeudis, 1980) and an increase of low EEG frequencies (2-6 Hz) in NREM sleep (Lancel et al., 1996, Lancel et al., 1997). These findings are consistent with EEG alterations found in an absence epilepsy-prone rat strain (WAG\Rij), where icv muscimol induced a dose-dependent abnormal EEG pattern consisting of slow waves and spikes which persisted for several hours (Peeters et al., 1989). Bilaterally synchronous spike-waves appeared after systemic administration of muscimol in the cat (Fariello et al., 1981), while in the baboon *Papio papio*, muscimol induced spike-wave complexes (Pedley et al., 1979) similar to the EEG changes elicited by 5 mg/kg muscimol in patients suffering from Huntington's disease (Shoulson et al., 1978). Other behavioral effects of muscimol were prolongation of propofol-induced anesthesia in mice (Irifune et al., 2003), and a dose-dependent increase in the duration of spontaneous petit mal-like seizures in the rat (Vergnes et al., 1984). Taken together, these observations suggest that neuronal synchronization, underlying the generation of low EEG frequencies is markedly increased after administration of potent GABA_A receptor agonists.

We tested the hypothesis that behavioral alterations, such as sedation and reduced locomotion elicited by GABA_A agonists, are accompanied by changes in regional EEG topography and EEG synchronization in low frequencies. We used muscimol because it is a powerful GABA_A agonist and tested its effects on behavior and the EEG in mice.

Experimental procedures

Animals. The experiments were performed in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and were approved by the Cantonal Veterinary Office of Zurich. All efforts were made to minimize the number of mice used and their suffering. Male C57BL/6 mice ($n=7$) (Harlan Netherlands, B.V.) were operated at 12.6 ± 0.2 weeks (weighing 25.5 ± 0.6 g at surgery). The mice were kept individually in Macrolon cages (36 x 20 x 35 cm), equipped with running wheels (Vyazovskiy et al., 2006) to investigate voluntary locomotor activity. Food and water were available *ad libitum*, and the mice were maintained on a 12 h light - 12 h dark cycle (daylight type fluorescent tubes, 58 W, approximately 30 lux) at a constant ambient temperature (22-24°C).

Surgery. Stainless steel electrodes (0-80 x 1/8; Plastics One® Inc., Roanoke, Virginia, USA) for EEG recording were implanted epidurally under deep anesthesia (ketamin 87 mg/kg – xylazin 13 mg/kg, i.p.) over the right frontal cortex (2 mm anterior to bregma, 1.5 mm lateral to the midline; Paxinos and Franklin, 2001), and over the right parietal cortex (3 mm posterior to bregma, 2 mm lateral to midline) and as reference over the cerebellum (~ 2 mm posterior to lambda, on the midline). The electrodes were connected to stainless steel wires and fixed to the skull with dental cement. Two gold wires (diameter 0.2 mm) were inserted into the neck muscles to record the electromyogram (EMG). After surgery the mice were connected by a fine cable to the amplifier via a swivel, and remained connected during the entire experiment. At least 3 weeks were allowed for recovery and adaptation to the recording conditions.

Data acquisition. The EEG and EMG signals were amplified (amplification factor approx. 2000), conditioned by analogue filters (high-pass filter: -3 dB at 0.016 Hz; low-pass filter: -3 dB at 40 Hz, < -35 dB at 128 Hz) sampled with 256 Hz, digitally filtered (EEG: low-pass FIR filter 25 Hz; EMG: band-pass FIR filter 20-50 Hz) and stored with a resolution of 128 Hz. Consecutive 4-s epochs were subjected to a Fast Fourier Transform routine and EEG power density was computed for 4-s epochs in the frequency range of 0.25-35.0 Hz (frequencies above 35 Hz were not analyzed).

Experimental protocol and data analysis. The experimental group of mice, implanted with EEG electrodes (n=7, see below) was injected i.p. with vehicle and after at least 4 days, with 3 mg/kg muscimol. This design was chosen to avoid the potential long-term effects of muscimol on the EEG, precluding a reliable comparison with the effects of vehicle on behavior and the EEG. Muscimol (Tocris Cookson Ltd., Bristol, UK) was dissolved in physiological pyrogen-free saline (0.9% NaCl; vehicle). Injections occurred 3 h after light onset.

The 3 mg/kg dose was chosen based on a behavioral pilot study, where the effects of two doses (2 mg/kg i.p., n=14, and 1 mg/kg, n=4,) were evaluated. Both doses reduced locomotor activity within 15 min post-injection. All mice were sedated, with eyes open and displaying an extended, rigid body posture and elongated tail, behaviors typical for a catalepsy-like state. The effect lasted between 40-150 min, recovery occurring more rapidly after the lower dose (30-60 min). In a few mice, muscimol induced myoclonic jerks of the hind limbs, as described by Menon and Vivonia, 1981.

Furthermore, we assessed in $n=5$ of the 14 mice receiving 2 mg/kg muscimol, the ability to respond to multimodal sensory stimuli (tapping the cage once) applied every 15 min during catalepsy. Arousal responses such as head or ear movements or a body jolt were observed in all mice. Since the aim of the study was to test the hypothesis that behavioral changes are related to the regional EEG pattern, we chose 3 mg/kg to make sure that reliable, prolonged catalepsy and hyperactivity phases would be induced.

Continuous EEG, time-lapse video recordings, infrared (IR) - and running-wheel (RW)-activity recordings were obtained ($n=7$). RW-revolutions and activity counts were integrated over consecutive 1-min epochs and stored on a computer, as described previously (Deboer and Tobler, 2000; Chronobiology Kit, Stanford Software System, Stanford, California). Vigilance states were visually scored for 4-s epochs on the basis of the raw parietal EEG, slow-wave activity (SWA, EEG power between 0.75-4.0 Hz) and the EMG (Tobler et al., 1997). In the first 1-2 h after muscimol administration the waking and NREM sleep EEG were sometimes indistinguishable. Vigilance states were then determined based on the EMG, IR-activity and the behavioral scoring of the video tapes. Epochs containing EEG artifacts in either derivation were visually identified and excluded from spectral analysis of both derivations (7.7 ± 1.4 % of 12 h).

EEG wave and cross-correlation analysis. The incidence and amplitude of EEG waves were investigated in the 3 h before and after injection of muscimol. EEG half-waves were defined based on zero-crossings of the EEG signal after band-pass filtering of delta frequencies (0.5-4.0 Hz; stop band edge frequencies 0.1 and 8.0 Hz, respectively). Wave incidence was the number of peaks detected in the filtered signal

within pre-defined amplitude ranges. The amplitude of EEG half-waves was assessed by computing the maximal or minimal voltage (positive and negative half-waves, respectively) between two consecutive zero-crossings. Half-waves shorter than 0.1 seconds and $< 10 \mu\text{V}$ were excluded.

An estimate of the cross-correlation between the frontal and parietal EEG time series was computed based on 4-s epochs (sampling rate 128 Hz) with Matlab function `xcorr`. The sequences of correlation coefficients were obtained for all 4-s epochs during muscimol induced catalepsy and for all 4-s epochs in NREM sleep of the corresponding time interval after vehicle. Subsequently, for each 4-s epoch the time difference between lag = 0 of the correlation and the time lag corresponding to the highest correlation value within a 1-s window below or above the lag = 0 was determined.

Before computing averages and for statistical comparisons Fisher's z-transformation was applied to correlation values. The software package Matlab (The Math Works, Inc., Natick, MA, USA) was used for data and signal analysis.

Statistics. The effects of muscimol and vehicle on vigilance states and EEG spectra were compared within each treatment by rANOVAs with factors 'pharmacological treatment' (muscimol and vehicle) and 'time interval', followed by two-tailed paired *t*-tests. The relationship between the duration of the hyperactivity phase or RW intensity and the subsequent level of SWA in NREM sleep was assessed by linear correlation analyses (Pearson). SAS (SAS Institute, Inc., Cary, NC, USA) was used for statistical analysis.

Results

Biphasic behavioral effects of 3 mg/kg muscimol

Muscimol elicited in all mice a particular order of behaviors associated with specific EEG patterns (Fig. 1). An initial activation lasting 13.3 ± 1.1 min was characterized by active movement (IR counts/min: 8.7 ± 2.4) and short running bouts (RW counts/min: 9.4 ± 2.6). This phase was followed by a long-lasting suppression of motor activity (IR counts/min: 0.09 ± 0.05 ; SEM) and subsequent hyperactivity (IR counts/min: 11.5 ± 3.3). Based on the behavioral observations, the inactivity phase was defined as catalepsy. This state lasted 96 min (± 12.4 ; SEM, range 55-157 minutes). The hyperactivity phase was dominated by vigorous wheel running (RW revolutions/min: 17.9 ± 2.6) lasting 49.7 min (± 5.4 ; range: 28-69 min). The duration of catalepsy and the intensity of wheel running during hyperactivity were negatively correlated ($n=7$; $r=-0.82$, Pearson's correlation coefficient, $p=0.02$). The behavior of the $n=7$ EEG mice, and the $n=14 + 4$ mice of the pilot experiment, changed as a function of the dose. As the dose was increased, inactivity (catalepsy) and hyperactivity was prolonged. Such a biphasic effect was never elicited by the vehicle. Thus, vehicle injection elicited a significantly longer initial activation than muscimol, 35.3 ± 3.1 min ($p<0.001$), and lower IR-activity (4.4 ± 1.1 counts/min, $p=0.08$) and RW-activity (4.7 ± 1.1 counts/min, $p=0.06$). During the time interval corresponding to hyperactivity after muscimol, locomotor activity after vehicle was markedly lower: IR-activity 0.05 ± 0.03 ($p<0.05$); RW-activity: 0.0 ± 0.0 ($p<0.001$).

Major EEG alterations after administration of muscimol

The main EEG feature during catalepsy was a regularly recurring high-voltage wave pattern on a low-voltage background (Fig. 2). These waves were similar in both derivations and occurred synchronously. As a result, the EEG spectrum during catalepsy did not show the regional differences between the frontal and parietal derivation observed during sleep after vehicle (Fig. 3). EEG power < 3 Hz was enhanced during muscimol-induced catalepsy in both derivations, while power > 4 Hz (parietal) or > 8 Hz (frontal) up to 35 Hz was below the values of NREM sleep after vehicle (Fig. 3, top panels).

During hyperactivity, slow waves resembling NREM sleep slow waves dominated the frontal EEG (Fig. 2), resulting in a major increase in EEG power in the slow-wave range compared to the parietal derivation (Fig. 3, bottom panels). In contrast, the parietal EEG exhibited a pronounced peak in the theta frequency range which is characteristic of running behavior (Vyazovskiy et al., 2006).

A “physiological” sleep pattern with a NREM-REM sleep alternation was re-established approximately 2.5 h after the muscimol injection corresponding to the end of the hyperactivity phase (Fig. 4). SWA in NREM sleep was dramatically enhanced immediately after the muscimol-induced hyperactivity. This was the case for both derivations, but especially for the frontal EEG (Fig. 4, bottom panels). The SWA increase in the first hour after the hyperactivity phase (expressed relative to the pre-injection level) was unrelated to the duration of the hyperactivity phase or to the amount of RW-activity (correlation coefficients for duration of hyperactivity: parietal, $r=0.12$, $p=0.8$; frontal, -0.31 , $p=0.5$; RW-activity: parietal, $r=0.41$, $p=0.4$; frontal, -0.36 , $p=0.4$).

The phase relationship between slow waves in the frontal and parietal EEG was enhanced after muscimol

A striking finding was the synchronous occurrence of high-amplitude waves within both derivations during catalepsy (Fig. 2). We therefore investigated the amplitude distribution and phase-relationship of EEG waves between the two EEG derivations. During catalepsy, high amplitude half-waves were more abundant than during pre-injection NREM sleep in both derivations (Fig. 5). During the muscimol-induced catalepsy slow-waves in the frontal EEG showed a stable phase relationship to those of the parietal EEG in all 7 mice (one individual is shown in Fig. 6 A). To assess this relationship quantitatively and to enable comparison with baseline and post-vehicle NREM sleep two analyses were performed.

First, cross-correlation functions of frontal versus (vs) parietal 4-s EEG epochs were computed during catalepsy as well as during NREM sleep belonging to the corresponding time interval following vehicle injection. Several peaks appeared on the cross-correlation function during catalepsy (3 peaks of high correlation can be seen in the illustration of a single 4-s epoch in Fig. 6 B) indicating a slow periodic occurrence of synchronous waves after muscimol. In contrast, such synchronous oscillatory activity was weak during NREM sleep (Fig. 6 B, vehicle). In most 4-s epochs the highest correlation was observed within $\text{lag}=0 \pm 1$ s. To assess the strength of the correlation, we compared the averaged highest correlation coefficients of all 4-s NREM sleep epochs after vehicle with the average computed for catalepsy epochs. The correlation between the frontal and the parietal derivation was significantly higher during catalepsy

compared to NREM sleep (muscimol induced catalepsy: positive: $r = 0.30 \pm 0.01$, negative: $r = -0.28 \pm 0.01$; NREM sleep after vehicle: positive: $r = 0.24 \pm 0.01$, negative: $r = -0.25 \pm 0.01$; $p < 0.05$ both for the comparison of positive and negative r -values; paired t -test after Fisher z -transformation).

To investigate the phase relationship between the frontal and parietal waves in more detail, the distribution of time-lags of the maximal value of the cross-correlation function was computed (Fig. 6 D). After muscimol a single peak was apparent at -0.065 s, indicating that on average slow waves in the parietal derivation were delayed relative to the frontal waves. In contrast, after vehicle as well as during baseline NREM sleep before the injection (not shown), the distribution of the lags was bimodal with peaks at $\text{lag} \pm 0.125$ s. Thus, a subset of frontal EEG waves occurred consistently before or after the waves in the parietal EEG, indicating that slow waves originated in some cases in the frontal regions while in others they originated in the parietal derivation. A considerable number of waves occurred simultaneously in both derivations ($\text{lag} = 0$). A redistribution towards an increased number of short lags at the expense of longer lags was apparent after muscimol compared to vehicle (Fig. 6 D).

Secondly, because muscimol induced typical high amplitude slow waves (see Fig. 2 and 6 A), we performed a single wave analysis to determine the shortest time difference between the peaks of high-amplitude waves ($>100\mu\text{V}$) of the frontal and parietal EEG (Fig. 6 C). The mean difference in occurrence of the frontal and parietal waves (independently of whether there was an advance or a delay), was after muscimol shorter than after vehicle (positive half-wave peaks by 26.0 ± 2.5 % SEM; negative half-wave peaks by 26.9 ± 2.5 %).

We next investigated whether during muscimol-induced catalepsy the frontal high-amplitude waves occurred consistently earlier than in the parietal derivation. Positive half-wave peaks of the frontal waves on average preceded the positive peaks of the parietal derivation by 83.2 ± 9.2 ms. In contrast, after vehicle the time difference between wave occurrence showed considerable variability (10.4 ± 16.6 ms) and was significantly smaller (muscimol vs vehicle: $p < 0.01$, paired t -test), indicating that after muscimol the phase relationship between the EEG waves of the two derivations was more stable.

Discussion

We investigated the association between regional EEG topography and EEG synchronization with behavioral states after peripheral administration of a high dose of the GABA_A agonist muscimol. This model was well suited because muscimol consistently elicited a succession of two behavioral states: a profound initial motor depression, resembling a catalepsy-like state, followed by a highly activated state characterized by robust wheel running.

We tested the hypothesis that these two pharmacologically induced behavioral states are associated with a distinct regional EEG pattern. Specifically, we expected that in analogy to other quiescent states, such as sleep, the muscimol induced sedation would be associated with increased regional EEG synchronization. Consistent with our hypothesis, we found that during the initial catalepsy-like state, regional EEG differences were virtually abolished. This was a result of the strikingly similar frontal and parietal

EEG pattern. The high-amplitude waves dominating the EEG during this state, showed a tighter phase-relationship between the regions. Taken together, it seems that the sedative effects of GABA_A agonists and GABA_A modulators are related to an increased global synchrony of cortical neuronal activity. These results are consistent with the increased EEG power in the slow-wave range, high-amplitude synchronous EEG waves or spikes observed in the rat (Peeters et al., 1989, Lancel et al., 1996, Lancel et al., 1997), in cats (Fariello et al., 1981), and in baboon *Papio papio* (Pedley et al., 1979).

The catalepsy-like phase, characterized by immobility and abolition of regional EEG differences, was followed by a hyperactivity phase during which the mice consistently engaged in wheel running. The cortical EEG pattern between the frontal and parietal derivation became remarkably dissociated (Fig. 2 and 3). Thus, the parietal EEG exhibited high levels of theta-activity, typical for running (Vyazovskiy et al., 2006), while the frontal slow waves resembled the NREM sleep EEG (Fig. 2). The succession of the depressed and hyperactivity phase could result from a disbalance between excitatory and inhibitory mechanisms.

Muscimol elicited a dramatic increase of SWA during both the catalepsy and hyperactivity phase. However, during sleep following the hyperactivity phase, SWA was still massively above the levels (a 2-3 fold increase) encountered at the beginning of the light period (Fig. 4). While the high SWA level at light onset is the consequence of the considerable amount of spontaneous waking towards the end of the dark period, the remarkable muscimol-induced SWA increase occurred after only a short period of hyperactivity. In contrast to the well-known correlation between waking and SWA (Huber et al., 1999, Franken et al., 2001, Vyazovskiy et al., 2006), the level of SWA at

the onset of the recovery phase and the duration of the preceding hyperactivity phase were not correlated. The high SWA level immediately after the hyperactivity phase when NREM-REM sleep cycles reappeared (Fig. 4) and its progressive decline to pre-injection levels, suggests that normal physiological sleep is necessary to dissipate the sleep pressure which apparently accumulated, despite the muscimol induced slow waves. Consistent with this interpretation, the time course of SWA during recovery after muscimol induced hyperactivity resembles recovery after sleep deprivation.

Neuronal mechanisms underlying high-amplitude synchronous waves induced by muscimol or by THIP, another GABA_A agonist (Vyazovskiy et al., 2005) are unknown. Inhibitory connections play a crucial role in the generation and synchronization of activity in the thalamocortical network (McCormick, 1992, Kim et al., 1997). It is possible that high-amplitude waves induced by GABA_A agonists arise from the same basic phenomenon - the slow cortical oscillation - as slow waves during sleep (Steriade et al., 1993b, Steriade and Amzica, 1998). The synchronous occurrence of high-amplitude slow waves across cortical areas elicited by muscimol is consistent with recent data suggesting an important role of inhibitory neurons in the large-scale synchronization of the slow oscillation (Volgushev et al., 2006). In addition to the intracortical synchronizing mechanisms, subcortical structures might play an important role in the generation of the EEG pattern after muscimol and THIP. Thus the stimulation of the pedunculopontine tegmental nucleus or locus coeruleus blocked cyclic hyperpolarization-depolarization components of the slow oscillations, leading to EEG activation, thereby increasing power in faster frequencies and lowering power between 0-8 Hz (Steriade et al., 1993a). Our results are consistent with the notion that EEG synchronization is associated with

high values of SWA. Muscimol injection led to a redistribution of spectral EEG power towards lower EEG frequencies at the expense of higher frequencies above 4-8 Hz. Muscimol may act both in the neocortex, where it plays a role in synchronization of the slow cortical oscillation, and at subcortical structures which maintain the cortex in an activated state.

In conclusion, we demonstrate that muscimol abolished regional differences and enhanced phase-relationship between EEG waves in the two cortical regions. These results suggest that GABAergic mechanisms play an important role in regional cortical synchronization. In addition, the global level of synchrony in neocortical networks might determine behavioral states.

Acknowledgements

We gratefully acknowledge Dr. P. Achermann for his advice in data analysis. This study was supported by the Swiss National Science Foundation grants (3100A0-100567; 3100.062112.00), PBZHB-106264, EU LSHM-CT-2005-518189, and the European Union Marie Curie grant MCRTN-CT-2004-512362.

References

- Bland, B. H., Oddie, S. D. and Colom, L. V., 1999. Mechanisms of neural synchrony in the septohippocampal pathways underlying hippocampal theta generation. *J Neurosci.* 19, 3223-3237.
- Chapman, C. A. and Lacaille, J. C., 1999. Cholinergic induction of theta-frequency oscillations in hippocampal inhibitory interneurons and pacing of pyramidal cell firing. *J Neurosci.* 19, 8637-8645.
- Deboer, T. and Tobler, I., 2000. Running wheel size influences circadian rhythm period and its phase shift in mice. *J Comp Physiol [A]*. 186, 969-973.
- DeFeudis, F. V., 1980. Physiological and behavioral studies with muscimol. *Neurochem Res.* 5, 1047-1068.
- Fariello, R. G., Golden, G. T. and Black, J. A., 1981. Activating effects of homotaurine and taurine on corticoreticular epilepsy. *Epilepsia.* 22, 217-224.
- Franken, P., Chollet, D. and Tafti, M., 2001. The homeostatic regulation of sleep need is under genetic control. *J Neurosci.* 21, 2610-2621.
- Huber, R., Deboer, T. and Tobler, I., 1999. Prion protein: a role in sleep regulation? *J Sleep Res.* 8 Suppl 1, 30-36.
- Huber, R., Deboer, T. and Tobler, I., 2000. Topography of EEG dynamics after sleep deprivation in mice. *J Neurophysiol.* 84, 1888-1893.
- Irifune, M., Takarada, T., Shimizu, Y., Endo, C., Katayama, S., Dohi, T. and Kawahara, M., 2003. Propofol-induced anesthesia in mice is mediated by gamma-aminobutyric acid-A and excitatory amino acid receptors. *Anesth Analg.* 97, 424-429.

- Kim, U., Sanchez-Vives, M. V. and McCormick, D. A., 1997. Functional dynamics of GABAergic inhibition in the thalamus. *Science*. 278, 130-134.
- Lancel, M., Cronlein, T. A. and Faulhaber, J., 1996. Role of GABAA receptors in sleep regulation. Differential effects of muscimol and midazolam on sleep in rats. *Neuropsychopharmacology*. 15, 63-74.
- Lancel, M., Faulhaber, J., Schiffelholz, T., Mathias, S. and Deisz, R. A., 1997. Muscimol and midazolam do not potentiate each other's effects on sleep EEG in the rat. *J Neurophysiol*. 77, 1624-1629.
- McCormick, D. A., 1992. Neurotransmitter actions in the thalamus and cerebral cortex. *J Clin Neurophysiol*. 9, 212-223.
- Menon, M. K. and Vivonia, C. A., 1981. Muscimol-induced myoclonic jerks in mice. *Neuropharmacology*. 20, 441-444.
- Mohler, H., 2006. GABA(A) receptor diversity and pharmacology. *Cell Tissue Res*. 326, 505-516.
- Nelson, L. E., Guo, T. Z., Lu, J., Saper, C. B., Franks, N. P. and Maze, M., 2002. The sedative component of anesthesia is mediated by GABA(A) receptors in an endogenous sleep pathway. *Nat Neurosci*. 5, 979-984.
- Paxinos, G. and Franklin, B. J., 2001. The mouse brain in stereotaxic coordinates. Academic Press, San Diego.
- Pedley, T. A., Horton, R. W. and Meldrum, B. S., 1979. Electroencephalographic and behavioral effects of a GABA agonist (muscimol) on photosensitive epilepsy in the baboon, *papio papio*. *Epilepsia*. 20, 409-416.

- Peeters, B. W., van Rijn, C. M., Vossen, J. M. and Coenen, A. M., 1989. Effects of GABA-ergic agents on spontaneous non-convulsive epilepsy, EEG and behaviour, in the WAG/RIJ inbred strain of rats. *Life Sci.* 45, 1171-1176.
- Reynolds, D. S., Rosahl, T. W., Cirone, J., O'Meara, G. F., Haythornthwaite, A., Newman, R. J., Myers, J., Sur, C., Howell, O., Rutter, A. R., Atack, J., Macaulay, A. J., Hadingham, K. L., Hutson, P. H., Belelli, D., Lambert, J. J., Dawson, G. R., McKernan, R., Whiting, P. J. and Wafford, K. A., 2003. Sedation and anesthesia mediated by distinct GABA(A) receptor isoforms. *J Neurosci.* 23, 8608-8617.
- Rudolph, U. and Mohler, H., 2006. GABA-based therapeutic approaches: GABAA receptor subtype functions. *Curr Opin Pharmacol.* 6, 18-23.
- Shoulson, I., Goldblatt, D., Charlton, M. and Joynt, R. J., 1978. Huntington's disease: treatment with muscimol, a GABA-mimetic drug. *Ann Neurol.* 4, 279-284.
- Steriade, M. and Amzica, F., 1998. Coalescence of sleep rhythms and their chronology in corticothalamic networks. *Sleep Res Online.* 1, 1-10.
- Steriade, M., Amzica, F. and Nunez, A., 1993a. Cholinergic and noradrenergic modulation of the slow (approximately 0.3 Hz) oscillation in neocortical cells. *J Neurophysiol.* 70, 1385-1400.
- Steriade, M., Gloor, P., Llinas, R. R., Lopes de Silva, F. H. and Mesulam, M. M., 1990. Report of IFCN Committee on Basic Mechanisms. Basic mechanisms of cerebral rhythmic activities. *Electroencephalogr Clin Neurophysiol.* 76, 481-508.
- Steriade, M., Nunez, A. and Amzica, F., 1993b. A novel slow (< 1 Hz) oscillation of neocortical neurons in vivo: depolarizing and hyperpolarizing components. *J Neurosci.* 13, 3252-3265.

- Tobler, I., Deboer, T. and Fischer, M., 1997. Sleep and sleep regulation in normal and prion protein-deficient mice. *J Neurosci.* 17, 1869-1879.
- Vergnes, M., Marescaux, C., Micheletti, G., Depaulis, A., Rumbach, L. and Warter, J. M., 1984. Enhancement of spike and wave discharges by GABA-mimetic drugs in rats with spontaneous petit-mal-like epilepsy. *Neurosci Lett.* 44, 91-94.
- Volgushev, M., Chauvette, S., Mukovski, M. and Timofeev, I., 2006. Precise long-range synchronization of activity and silence in neocortical neurons during slow-wave oscillations. *J Neurosci.* 26, 5665-5672.
- von Krosigk, M., Bal, T. and McCormick, D. A., 1993. Cellular mechanisms of a synchronized oscillation in the thalamus. *Science.* 261, 361-364.
- Vyazovskiy, V., Borbely, A. A. and Tobler, I., 2000. Unilateral vibrissae stimulation during waking induces interhemispheric EEG asymmetry during subsequent sleep in the rat. *J Sleep Res.* 9, 367-371.
- Vyazovskiy, V. V., Kopp, C., Bosch, G. and Tobler, I., 2005. The GABAA receptor agonist THIP alters the EEG in waking and sleep of mice. *Neuropharmacology.* 48, 617-626.
- Vyazovskiy, V. V., Ruijgrok, G., Deboer, T. and Tobler, I., 2006. Running wheel accessibility affects the regional electroencephalogram during sleep in mice. *Cereb Cortex.* 16, 328-336.
- Vyazovskiy, V. V. and Tobler, I., 2005. Regional differences in NREM sleep slow-wave activity in mice with congenital callosal dysgenesis. *J Sleep Res.* 14, 299-304.

Figure legends

Figure 1. Representative example of slow wave activity (SWA, EEG power between 0.75-4.0 Hz) of the parietal and frontal derivation, EMG variance and locomotor activity (infra red: IR and running wheel: RW) for the 4 h following administration of muscimol (3 mg/kg, i.p.). Bars in the top three panels represent 1-min median values (slow-wave activity and EMG variance). Four phases are delimited by vertical lines within panel 4 (IR) based on the presence/absence of IR-activity: (a) initial activation, (b) catalepsy, (c) hyperactivity and (d) recovery.

Figure 2. Examples of EEG (parietal and frontal derivation) and EMG traces in NREM sleep and waking before muscimol injection, and during the muscimol-induced catalepsy and hyperactivity phase (after injection). Filter settings for the EEG: high-pass filter: -3 dB at 0.016 Hz; low-pass filter: -3 dB at 40 Hz. Catalepsy is characterized in both derivations by sporadic waves on a low voltage background and a low EMG. During the hyperactivity phase, theta-activity is evident in the parietal derivation, while slow waves predominate in the frontal cortex.

Figure 3. Top: EEG power density in NREM sleep computed for the time interval after vehicle (left) corresponding to the catalepsy interval (right). Bottom: EEG power density during waking after vehicle injection and during the hyperactivity phase. Mean values (n=7). Horizontal lines below the curves indicate frequency bins that differed significantly between the derivations, as well as the effect of treatment (paired *t*-tests on log-transformed values, $p < 0.05$).

Figure 4. Time course of vigilance states and slow wave activity (SWA, EEG power between 0.5-4.0 Hz) in NREM sleep after sleep onset (five consecutive 1-h intervals) following vehicle injection or after the muscimol-induced hyperactivity phase. SWA is represented as % of mean SWA in NREM sleep computed for 5 h after vehicle injection (frontal (F), parietal (P) derivation). Data are mean values ($\pm$ SEM; n=7). Triangles above the curves: effect of treatment in each derivation. Triangles below the curves: comparison between the frontal and the parietal EEG (post-hoc paired *t*-tests when significance was reached after rANOVA, factor 'treatment' or interaction 'treatment x '1-h interval', $p < 0.05$). Orientation of triangles indicates the direction of deviation from power in the parietal EEG. Note different y-axis scaling for vehicle and muscimol.

Figure 5. Amplitude of half-wave distribution (band-pass filter of delta frequencies (0.5-4 Hz)) in NREM sleep before the muscimol injection compared to muscimol-induced catalepsy expressed as percentage of total number of half-waves within the corresponding NREM sleep, or catalepsy. Mean values (logarithmic scale; SEM; n=7) for the parietal and frontal derivation. Half-waves shorter than 0.1 sec and $< 10 \mu V$ were excluded. The number of half-waves was computed for 50 μV -bins, and plotted at bin midpoint expressed as percentage of the total number of half-waves. Triangles indicate differences between the curves ($p < 0.05$, Sidak test) and their orientation the direction of the difference.

Figure 6. A: Representative examples of the NREM sleep EEG after vehicle and during muscimol induced catalepsy (4-s epochs). B: Cross-correlation function (frontal vs parietal EEG) for the epochs illustrated in A. The earlier occurrence of the frontal waves can be seen in the negative lag of the highest correlation value. C: Single-wave analysis of temporal relationship between frontal and parietal high-amplitude waves: mean shortest time difference between the peaks of high-amplitude waves ($>100\mu\text{V}$) of the frontal and parietal derivation occurring during NREM sleep after vehicle (V) and muscimol-induced catalepsy (M). Mean values (SEM; $n=7$). Triangles indicate significant difference between treatment ($p<0.05$, paired t -test). D: Distribution of time-lags (mean $\pm$ SEM; $n=7$ mice. Expressed as percentage of total number of analyzed 4-s epochs, i.e. total number of lags) of the highest correlation coefficient within a 1-s window below or above the lag = 0 during catalepsy (muscimol) and NREM sleep (vehicle). Triangles above indicate time-lag bins (resolution 1/16 s) where treatments differed significantly and triangle orientation indicates the direction of difference from vehicle (paired t -test, $p<0.05$ after significance in ANOVA, interaction factors 'treatment' x 'time-lag').