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Role of $GABA_A$ Receptors in Sleep Regulation

Differential Effects of Muscimol and Midazolam on Sleep in Rats

Marike Lancel, Ph.D., Tatjana A. M. Crönlein, and Johannes Faulhaber, Ph.D.

To assess the influence of the γ-aminobutyric acid $(GABA)_A$ receptor on sleep and sleep EEG, rats were injected intraperitoneally with vehicle, two doses of muscimol (0.2 and 0.4 mg/kg), a selective $GABA_A$ agonist, and midazolam (3 mg/kg), a benzodiazepine-$GABA_A$ agonist. EEG and EMG recordings were made for 6 or 8 hours. Muscimol dose-dependently increased the amount of nonrapid eye movement sleep (nonREMS) and REMS. The higher dose of muscimol enhanced EEG activity over almost the entire frequency range (0.5–25 Hz), including delta (0.5–4 Hz) and sigma (11–16 Hz) activity, within nonREMS and in the frequencies over 10 Hz within REMS. Midazolam also increased the amount of nonREMS. However, most of the other effects of midazolam contrasted the effects of muscimol: midazolam decreased REMS, reduced low frequency (≤11 Hz) EEG activity within nonREMS, and enhanced the activity in higher frequencies during both nonREMS and REMS. These data demonstrate the involvement of $GABA_A$ receptors in the regulation of sleep-wake behavior as well as in the generation of spindles and delta waves during nonREMS. The effects of these two $GABA_A$ agonists indicate that activation of different binding sites on the $GABA_A$ receptor complex differentially affect sleep states and sleep EEG.

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KEY WORDS: *Muscimol; Midazolam; GABA; $GABA_A$ receptor; Sleep; EEG spectral analysis; Rat*

γ-Aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the central nervous system (CNS) of mammalian species. It interacts with two receptor types, the $GABA_A$ and $GABA_B$ receptors, which are present in nearly all neurons in the CNS. The $GABA_A$ receptor is a macromolecular protein composed of a receptor-gated chloride channel, a GABA recognition site, and various distinct modulatory binding sites for benzodiazepines, picrotoxin, barbiturates, and anesthetic steroids (for review, see Deutsch et al. 1992; Macdonald and Olsen 1994). Activation of the $GABA_A$ receptor complex by GABA increases membrane conductance for anions, especially for chloride ions, usually resulting in hyperpolarization. Ligands interacting with the modulatory sites influence the efficacy of GABA in promoting the chloride channel opening.

In view of their inhibitory effects, it is not surprising that $GABA_A$ receptors play an essential role in the generation of nonrapid eye movement sleep (nonREMS). Benzodiazepine-$GABA_A$ agonists (Gaillard et al. 1973; Johnson et al. 1979; Borbély et al. 1985; Dijk et al. 1989; Mendelson and Martin 1990; Lancel et al. 1994), as well as nonbenzodiazepine-$GABA_A$ agonists like zopiclone (Trachsel et al. 1990; Kim et al. 1993), zolpidem (Brunner et al. 1991), the β-carboline abecarnil (Coenen et al. 1992), and the endogenous steroid THDOC (Mendelson et al. 1987) all reduce nonREMS latency and increase the amount of nonREMS. Studies in which the EEG within nonREMS was analyzed with spectral analysis demonstrated that $GABA_A$ agonists systematically reduce low-

From the Max-Planck Institute of Psychiatry, Clinical Institute, Munich, Germany.

Address correspondence to Marike Lancel, Max-Planck Institute of Psychiatry, Clinical Institute, Department of Neuroendocrinology, Kraepelinstrasse 2, 80804 Munich, Germany.

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frequency activity (≤10 Hz) and enhance spindle activity in humans (Gaillard et al. 1973; Johnson et al. 1979; Borbély et al. 1985; Dijk et al. 1989; Trachsel et al. 1990; Brunner et al. 1991; Kim et al. 1993) and higher-frequency activity (≥13 Hz) in rats (Lancel et al. 1994). $GABA_A$ agonists also affect REMS: Except for THDOC, all the compounds mentioned above have been shown to increase REMS latency and decrease the amount of REMS and, in so far as examined, promote high frequency EEG activity in humans (Borbély et al. 1985) and rats (Lancel et al. 1994). These data strongly suggest that different types of $GABA_A$ agonists typically promote a nonREMS state that is characterized by the frequent occurrence of spindles and the infrequent appearance of slow waves (delta waves).

Spindles are composed of rhythmic waves of 11 to 16 Hz, with a waxing and waning amplitude. The appearance of spindles on a background of intermediate-frequency, low-amplitude EEG signals conventionally marks nonREMS onset. Spindle oscillations are triggered in the GABAergic reticular thalamic nucleus (RE) (Steriade et al. 1985, 1987), when cholinergic input subsides, which disinhibits the RE neurons. During spindling RE cells impose cyclical inhibitory postsynaptic potentials (IPSPs) on thalamocortical neurons, thereby de-inactivating low-threshold calcium currents, underlying the rebound low-threshold calcium spikes (LTSs) and associated bursts of action potentials. These are transferred to the cortex, where they induce excitatory postsynaptic potentials (EPSPs) in the frequency range of spindles (Steriade and Deschênes 1988). The excitation generated in thalamocortical and cortical neurons is projected back to the RE and facilitates the spindle oscillation (Steriade et al. 1991). Delta waves are slow-frequency signals (0.5–4 Hz) with high amplitudes that occur during deeper nonREMS states. When membrane potentials are sufficiently negative, thalamic and cortical neurons generate delta oscillations. Intracellular recordings show that these oscillations result from the critical interplay between two voltage-dependent currents: the hyperpolarization-activated Na^+/K^+ inward rectifying current (Ih) and the low-threshold calcium current (It), which underlies the rebound LTS (McCormick and Pape 1990). Recent electrophysiological experiments showed that a progressive hyperpolarization of membrane potential (Vm) switches the state of thalamocortical neurons from the "relay mode," characteristic for wakefulness and REMS, into a "spindle mode" and ultimately into a "delta mode" (Steriade et al. 1991; Nuñez et al. 1992).

In the light of these electrophysiological findings, one would expect that, depending on the induced degree of Vm hyperpolarization, $GABA_A$ agonists promote nonREMS predominated either by spindles or by delta waves. The fact that the earlier mentioned compounds consistently increase a nonREMS state with high spindling activity, may indicate that $GABA_A$ receptors are not involved in the genesis of delta oscillations. To investigate the role of the $GABA_A$ receptor complex in the regulation of sleep and sleep EEG in more detail, we administered muscimol to rats, a drug that selectively interacts with the GABA binding site on the $GABA_A$ receptor. The effects on sleep-wake behavior and sleep EEG were examined and compared to the changes induced by midazolam, a benzodiazepine-agonistic modulator of $GABA_A$ receptors.

MATERIALS AND METHODS

Experimental Procedure

Sixteen adult, male Wistar rats (Charles River Laboratories, Germany) were used for the experiments. The rats were housed individually in a ventilated, sound-attenuated Faraday room under a 12-hour light/12-hour dark schedule (lights on from 8.30 to 20.30 h, 50–120 lux) at an ambient temperature of 21 to 23°C. Food and water were available ad libitum.

Under deep inhalation halothan (Hoechst) anesthesia, rats were implanted with four stainless steel screws for epidural EEG recording (electrode positions: frontal cortex 3.9 A, ± 2 L and occipital cortex 6.4 P, ± 4 L relative to the bregma), and two stainless steel wires were inserted under the neck muscles for EMG recording. The leads were connected to a socket, which was fixed on the skull by dental acrylic cement. At least 2 weeks were allowed for recovery from surgery and 4 days to adapt to the recording conditions.

Experiment 1. Eight rats, with a body weight of 290 to 370 g at the beginning of the experiment, were subjected to four experimental conditions in random order. Each condition consisted of an 8-hour recording period, beginning at lights on, preceded by a 12-hour dark period control recording. The time between the conditions was at least 3 days. The experimental conditions were: (1) no injection (baseline); (2) injection of vehicle (0.4 ml/kg pyrogen-free saline); (3) 3 mg/kg midazolam (Hoffmann-La Roche, Germany) and (4) 0.2 mg/kg muscimol [Sigma, Germany; dissolved in pyrogen-free saline (0.5 mg/ml)].

Experiment 2. Eight rats, with a body weight ranging from 270 to 470 g at the start of the experiment, were subjected to two experimental conditions in random order. Each condition started with a 12-hour dark period control recording, followed by a 6-hour experimental recording, beginning at light onset. The time between the experiments was at least 2 days. The experimental conditions were: (1) injection of vehicle (1 ml/kg pyrogen-free saline), and (2) 0.4 mg/kg muscimol [dissolved in pyrogen-free saline (0.4 mg/ml)]. All injections were given intraperitoneally (IP) at 8.30 h. The experimental

protocols were approved by the local commission for animal welfare.

Recording

The EEG signal was derived from a frontal electrode and the contralateral occipital electrode. The remaining occipital electrode served as ground. All signals were transmitted by cable. The EEG and EMG signals were amplified and filtered (EEG, high pass 0.3 Hz and low pass 29 Hz, 48 dB/oct; EMG, high pass 16 Hz and low pass 3000 Hz, 6 dB/oct). Both the EEG and the rectified and integrated EMG were digitized with a sampling rate of 64 Hz. The EEG recordings were subjected to an on-line fast Fourier transform routine (cosine taper) run on a Macintosh Quadra 900. A power spectrum was computed for the frequencies between 0.5 and 25.5 Hz (0.5-Hz bins in the 0.5 to 4.5-Hz frequency range and 1-Hz bins for the higher frequencies) for 2-s windows. Power spectra were averaged over 10-s epochs. An off-line program displayed the 10-s epochs of raw EEG and of rectified and integrated EMG on screen for the manual scoring of the states wakefulness, nonREMS, and REMS (see Neckelmann and Ursin 1993 for scoring criteria). All data were scored by Marike Lancel.

Data Analysis and Statistics

For both vehicle conditions the time course of the vigilance states over 2-hour intervals was analyzed by means of a repeated-measures analysis of variance [ANOVA, General Linear Model Procedures, Greenhouse Geisser correction (SAS/STAT 1987)]. Differences in the amount of the vigilance states between vehicle and other experimental conditions were tested with a two-factor repeated-measures ANOVA, with the factors time (2-hour intervals) and condition (vehicle versus another condition).

For each experimental condition nonREMS latency (arbitrarily defined as the 20th epoch of nonREMS), REMS latency (arbitrarily defined as the third epoch of REMS), the average length of nonREMS episodes (computed over all nonREMS episodes, interruptions $\leq$10 s were allowed), and REMS episodes, as well as the number of episode occurrences, were determined. These data were analyzed with a two-sided, paired *t*-test.

For nonREMS, average EEG power densities were computed per 2-hour interval and for REMS, to avoid missing values, per 4-hour interval (experiment 1) or 6-hour interval (experiment 2). For standardization these values were expressed relative to the average power density within the same frequency band and vigilance state recorded during the preceding 12-hour control dark period and were then log-transformed. For the vehicle condition of experiment 1, a separate analysis was made of the time course of state-specific EEG power density by subjecting the data of each frequency band and each sleep state to a one-factor repeated-measures ANOVA. Differences between vehicle and other conditions were tested with a two-factor repeated-measures ANOVA, with the factors time (2- or 4-hour intervals) and condition, and for REMS in experiment 2 (6-hour intervals) only with the factor condition.

For the analysis of changes in the intra-episodic time course of delta activity and sigma activity, all nonREMS episodes that were preceded by two or more 10-s epochs of wakefulness and lasted at least ten 10-s epochs were selected from the first 4 hours of all experimental conditions. Delta activity (0.5–4 Hz) and sigma activity (11–16 Hz) were computed for each epoch of the selected episodes. The values were averaged over the selected episodes and then expressed in percent of the average delta and sigma activity within nonREMS during the preceding 12-hour control dark period. Thereafter, these values were log-transformed. The data of the last epoch of wakefulness and the first 9 epochs of nonREMS were used in the analysis. For the vehicle conditions of both experiments the time course of intra-episodic delta and sigma activity was analyzed by means of a one-factor repeated-measures ANOVA. Differences between vehicle and other conditions were tested by entering the data in a two-factor repeated-measures ANOVA, with the factors time (10-s epochs) and condition. Post hoc testing was done by means of two-sided, paired *t*-tests.

RESULTS

Duration of the Vigilance States

The analysis of the vehicle condition of experiment 1 revealed a significant effect only of the factor time for REMS (see Table 1 for 2-hour data and statistics). Pairwise comparison of the amount of REMS between successive 2-hour intervals showed a significant increase from interval 3–4 to interval 5–6 ($p < .009$). During baseline the amounts and distribution of the vigilance states were similar to those for vehicle (Table 1, Figure 1). Midazolam significantly promoted nonREMS over the 8-hour recording period (Figure 1) because of a marked increase during interval 3–4 (Table 1). It significantly decreased REMS over the 8-hour period (Figure 1). The decrease occurred mainly during interval 1–2, although a tendency toward a decrease was still present during interval 3–4 ($p < .06$) (Table 1). Midazolam also affected the distribution of REMS. Comparison of successive 2-hour intervals revealed significant increases in REMS over intervals 1–2, 3–4, and 5–6. Muscimol (0.2 mg/kg) significantly reduced wakefulness during the 8-hour recording period (Figure 1), an effect primarily due to a decrease during the second 2-hour interval (Table 1). For the vehicle condition of experiment 2 a time

Table 1. Vigilance States (% of recording time per 2-hour interval)

Condition	State	1–2	3–4	5–6	7–8	Time	Condition[a]	Time by Condition[a]
VEH	Wake	39.3	41.3	25.7	34.4	NS	—	—
		(11.2)	(10.6)	(14.6)	(10.9)			
	NonREMS	53.9	49.3	60.2	52.7	NS	—	—
		(9.6)	(9.6)	(10.2)	(8.9)			
	REMS	6.8	9.4	14.2	12.9	$p < .0009$	—	—
		(2.8)	(5.4)	(5.7)	(5.6)	$F = 11.2$		
BAS	Wake	40.2	38.3	30.3	31.4	NS	NS	NS
		(15.1)	(13.6)	(11.2)	(10.0)			
	NonREMS	53.4	51.7	56.9	55.5	NS	NS	NS
		(13.6)	(10.5)	(10.0)	(7.8)			
	REMS	6.4	10.1	12.8	13.1	$p < .0001$	NS	NS
		(3.3)	(4.8)	(5.7)	(4.3)	$F = 21$		
MID	Wake	42.5	34.2	22.3	34.1	$p < .01$	NS	NS
		(11.8)	(11.4)	(6.8)	(11.4)	$F = 5.2$		
	NonREMS	57.4	61.5*	64.2	51.3	NS	$p < .03$	NS
		(11.7)	(9.9)	(5.7)	(7.8)		$F = 7.9$	
	REMS	0.2***	4.4	13.6	14.5	$p < .0001$	$p < .03$	$p < .01$
		(0.2)	(3.6)	(4.8)	(6.6)	$F = 26.9$	$F = 7.6$	$F = 6.2$
MUS (0.2 mg/kg)	Wake	39.9	27.1**	31.7	26.2	NS	$p < .04$	NS
		(20.4)	(5.7)	(13.4)	(8.7)		$F = 6.4$	
	NonREMS	53.7	59.5	55.2	58.3	NS	NS	NS
		(17.5)	(8.1)	(10.3)	(6.8)			
	REMS	6.4	13.3	13.2	15.5	$p < .0001$	NS	NS
		(4.4)	(3.8)	(5.0)	(5.9)	$F = 14.9$		
						df (3,21)	df (1,7)	df (3,21)
VEH	Wake	47.7	31.5	28.6		NS		
		(17.4)	(16.5)	(11.9)				
	NonREMS	45.1	55.2	56.3		NS		
		(13.8)	(12.3)	(9.6)				
	REMS	7.2	13.3	15.1		$p < .004$		
		(3.8)	(4.8)	(3.9)		$F = 8.6$		
MUS (0.4 mg/kg)	Wake	33.2**	36.0	23.7		NS	$p < .005$	NS
		(9.4)	(11.5)	(9.8)			$F = 15.9$	
	NonREMS	57.2**	50.0	58.4		NS	$p < .03$	NS
		(7.6)	(8.8)	(6.6)			$F = 7.7$	
	REMS	9.5	14.0	18.0		$p < .0001$	$p < .05$	NS
		(2.2)	(3.2)	(4.1)		$F = 18.1$	$F = 5.6$	
						df (2,14)	df (1,7)	df (2,14)

Abbreviations: VEH = vehicle, BAS = baseline; MID = midazolam, and MUS = muscimol.

Distribution of the vigilance states over 2-hour intervals ($n = 8$). SD values are in parentheses, and the results of the two-factor repeated-measures ANOVA run on the 2-hour interval values (NS = nonsignificant). Post hoc testing was done by means of a two-sided, paired t-test, and significant differences are indicated by $^*p < .05$, $^{**}p < .01$ and $^{***}p < .001$.

[a]Condition, vehicle versus another condition.

effect was found for REMS. In this group of rats a significant increase in REMS occurred from interval 1–2 to interval 3–4 ($p < .03$). Muscimol (0.4 mg/kg) affected all vigilance states. During the 6-hour recording period wakefulness was significantly reduced, whereas nonREMS and REMS levels were above those for the vehicle (Figure 1). The changes in wakefulness and nonREMS mainly occurred during the first 2-hour interval (Table 1). The increase in REMS spread over all 2-hour intervals and never reached statistical significance.

Sleep Latency, Frequency and Average Duration of the Sleep Episodes

The baseline data did not differ in any respect from the vehicle data (Table 2). Midazolam significantly shortened nonREMS latency and lengthened REMS latency. Furthermore, it increased the number of nonREMS episodes as a tendency ($p < .06$) and significantly reduced the frequency of REMS episodes. Under the muscimol (0.2 mg/kg) condition fewer nonREMS episodes oc-

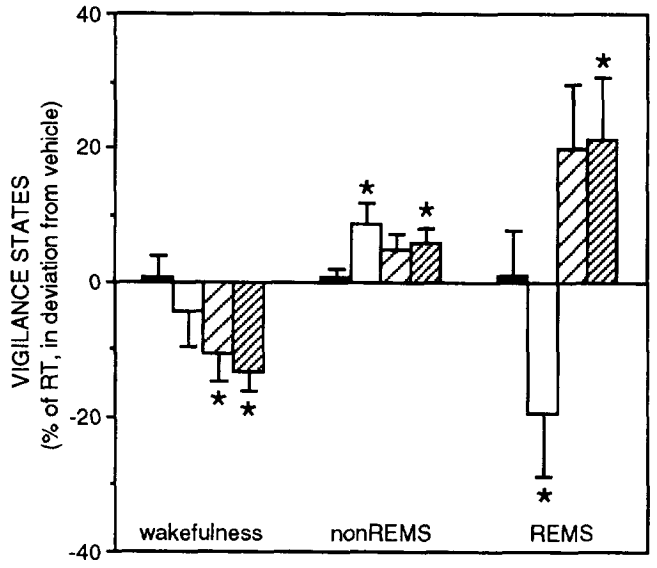


Figure 1. Duration of the vigilance states during the 8-hour recording period for baseline (*solid bars*), midazolam (*open bars*), and muscimol 0.2 mg/kg (widely striped bars), and during the 6-hour recording period for muscimol (0.4 mg/kg) (narrowly striped bars). The data are expressed as deviation from vehicle (% time vehicle − % time other condition), (n = 8, ± SEM). Significant differences from vehicle are indicated by *($p < .05$, two-sided, paired t-test). RT, recording time.

curred, but the average duration was significantly increased. Muscimol (0.4 mg/kg) also tended to lengthen the duration of the nonREMS episodes ($p < .1$).

EEG Power Density within nonREMS and REMS

In Figures 3A and 3B average EEG power spectra of the vehicle condition of experiment 1 are plotted per 2-hour interval for nonREMS and per 4-hour interval for REMS. The data are expressed relative to the values of the first interval. For nonREMS, a significant effect of the factor time ($p < .05$) emerged for all frequency bands, except 20 Hz. Low-frequency EEG activity (≈ ≤9 Hz) declines markedly across the 8-hour recording period, whereas EEG activity in the higher frequencies tends to increase after an initial small decrease. For REMS a significant time effect was found for some frequency bands. Compared to the first 4-hour interval, EEG activity within these frequencies slightly increases during the second 4-hour interval. The baseline data did not differ from those for vehicle. ANOVAs run on nonREMS-specific EEG activity during midazolam and vehicle administration revealed significant condition effects for 3 to 10 Hz and 15 to 25 Hz and significant interaction effects between condition and time for the frequencies between 2.5 to 11 Hz and 13 to 25 Hz. Over the 8-hour recording period low-frequency EEG activity was generally reduced, whereas high-frequency activity was markedly enhanced (Figure 2A). Reductions in low-frequency EEG activity were limited to interval 1–2, whereas enhancements in high-frequency activity were still present during interval 3–4 (Figure 3C). For REMS significant condition effects emerged for the frequency regions 4 to 6Hz, 11 to 14 Hz, and 18 to 25 Hz, and significant interaction effects were found for 4 Hz and for the frequency bands from 19 to 25 Hz. Over the 8-hour recording period EEG activity was enhanced in all these frequency bands (Figure 2B). The enhancements were most prominent during interval 1–4 but still significant during interval 5–8 (Figure 3D). The analysis of EEG activity within nonREMS under the muscimol (0.2 mg/kg) condition revealed hardly any significant condition effect (Figure 2C). However, significant interaction ef-

Table 2. Sleep Latency and Episode Parameters

	VEH (8 h)	BAS (8 h)	MID (8 h)	MUS (0.2 mg/kg, 8 h)	VEH (6 h)	MUS (0.4 mg/kg, 6 h)
NonREMS						
Latency (minutes)	32.6	31.0	11.2*	38.7	29.5	24.4
	(17.9)	(15.3)	(4.5)	(28.9)	(13.5)	(8.3)
Epidose duration (minutes)	2.6	2.7	2.6	3.0*	2.9	3.2
	(0.5)	(0.3)	(0.6)	(0.3)	(0.4)	(0.3)
Episode frequency	103.0	100.1	114.7	92.3*	65.7	63.2
	(16.7)	(15.9)	(25.3)	(13.3)	(7.5)	(9.1)
REMS						
Latency (minutes)	57.2	57.8	160.8**	60.4	67.0	49.5
	(23.3)	(21.7)	(49.9)	(30.6)	(32.4)	(15.7)
Episode duration (minutes)	1.3	1.3	1.4	1.5	1.5	1.8
	(0.4)	(0.3)	(0.2)	(0.3)	(0.4)	(0.4)
Episode frequency	38.5	39.4	29.1*	40.4	28.7	27.6
	(12.4)	(11.6)	(9.3)	(13.1)	(7.1)	(5.3)

Abbreviations: VEH = vehicle, BAS = baseline, MID = midazolam, MUS = muscinol.
The data represent mean values (n = 8). SD values are in parentheses. Differences from vehicle were tested with a two-sided, paired t-test and are indicated by *$p < .05$.

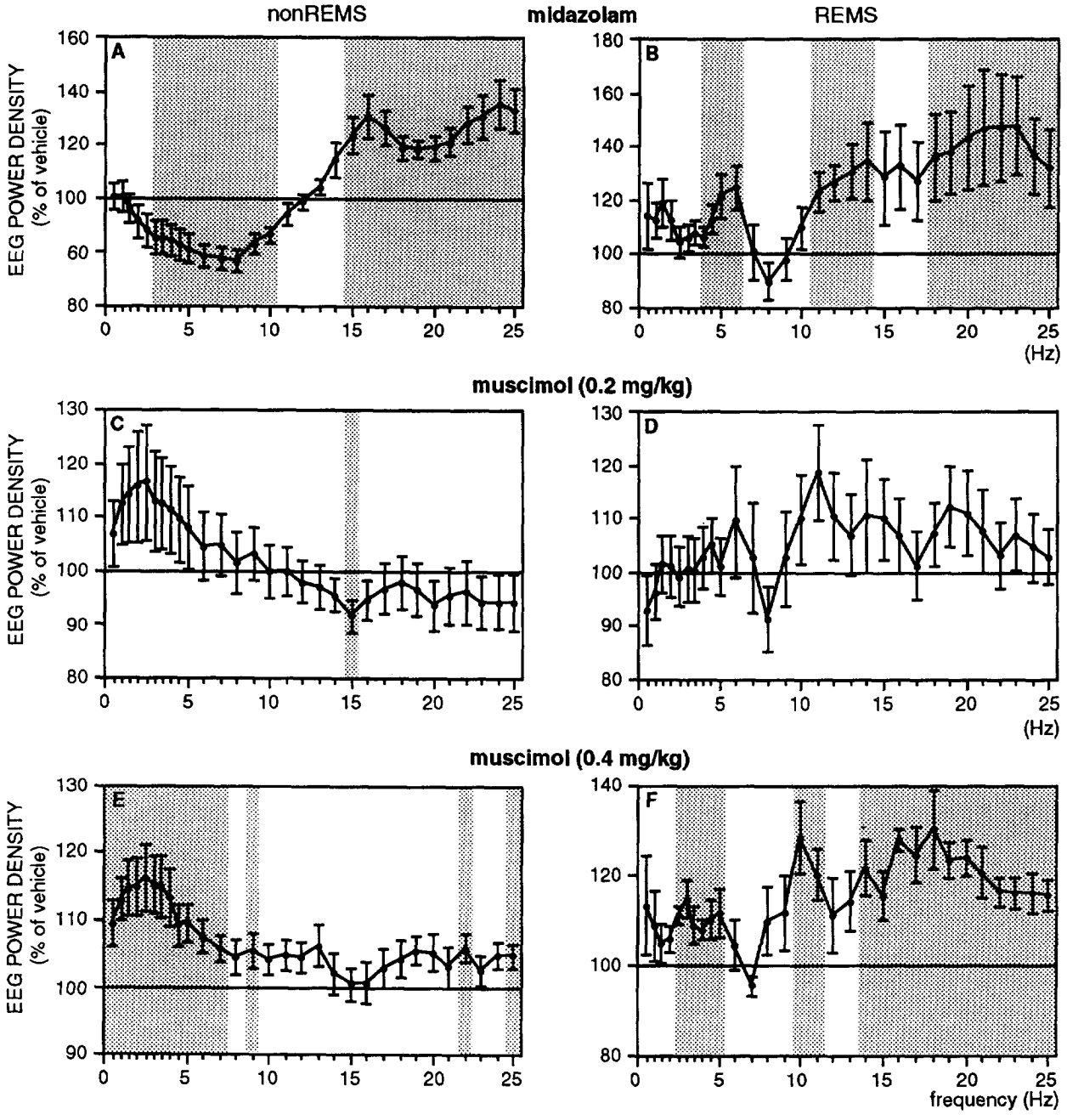


Figure 2. EEG power densities within nonREMS (left panels) and REMS (*right panels*) over the 8-hour recording period for midazolam (A, B) and muscimol 0.2 mg/kg (C, D) and over the 6-hour recording period for muscimol 0.4 mg/kg (E, F). Curves connect mean values (n = 8, ± SEM). For each animal and each frequency band average power densities were computed, normalized (see Materials and Methods), and then expressed in percent of the average power densities of the corresponding frequency and vigilance state of vehicle. *Shaded areas* indicate the frequencies for which the ANOVA run on the 2-hour, log-transformed values revealed a significant effect of the factor condition.

fects between condition and time emerged for 0.5 Hz and 2 to 4.5 Hz. Small, nonsignificant enhancements during the first three 2-hour intervals result in a steeper decline of low-frequency EEG activity in the course of the 8-hour recording period than during vehicle (Figure 3E). For REMS no significant effects emerged (Figures 2D, 3F). For nonREMS under muscimol (0.4 mg/kg) condition effects were observed for 0.5 to 7 Hz, 9 Hz, 22 Hz, and 25 Hz and interaction effects for 9 Hz, 11 to 12 Hz, 15 Hz, 17 Hz, and 20 to 25 Hz. Over the 6-hour recording period EEG activity was enhanced in all these frequencies, most prominently in the delta frequency range (Figure 2E). The enhancements were limited to interval 1–2 (Figure 3G). For REMS significant enhance-

ments were observed for 2.5 to 5 Hz, 10 to 11 Hz, and 14 to 25 Hz (Figure 2F).

Development of Delta and Sigma Activity within nonREMS Episodes

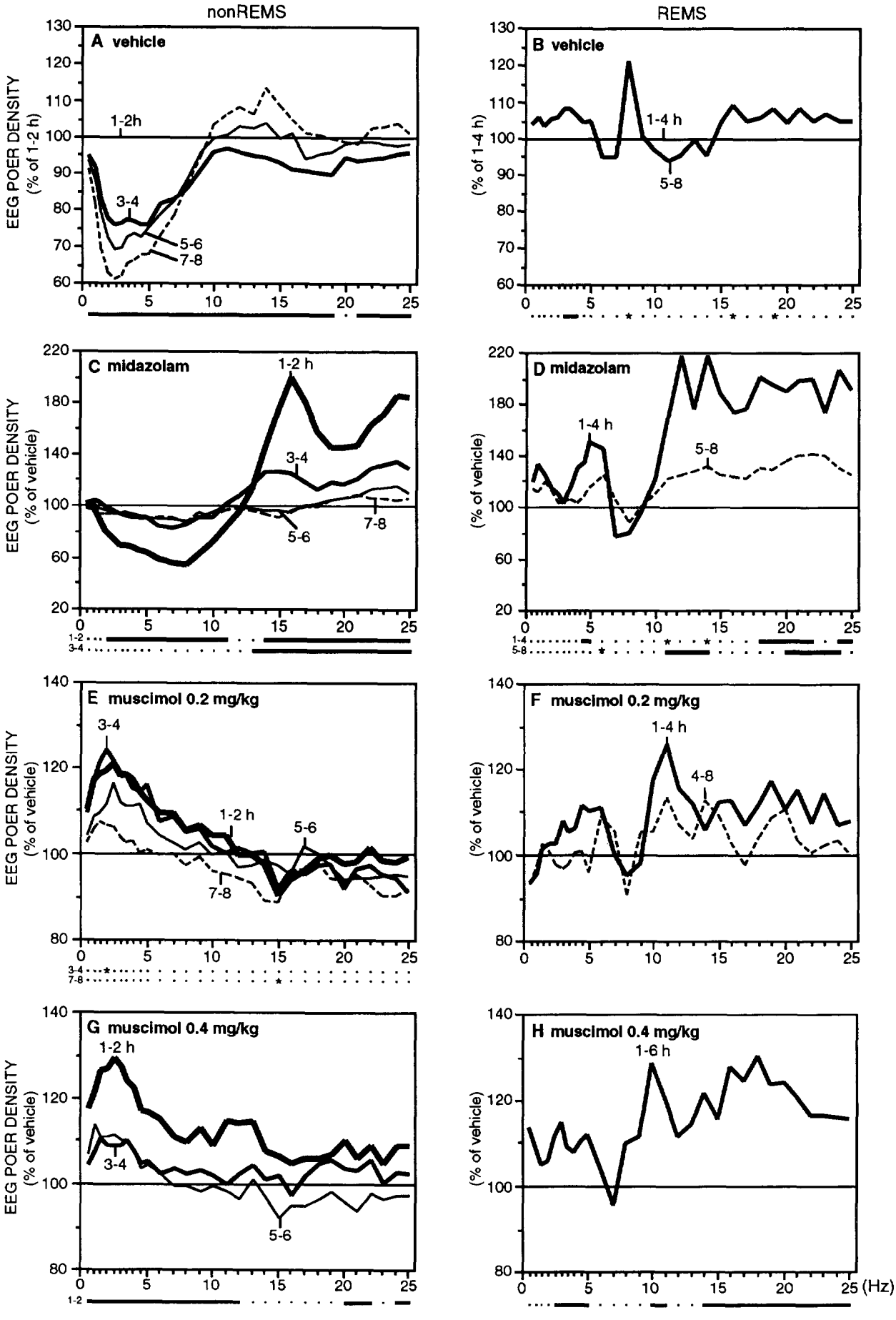
The ANOVA run on the log-transformed data of the two vehicle conditions revealed significant effects of the factor time for both frequency bands [experiment 1, delta: $F_{(9,63)} = 82.2$, $p < .0001$ and sigma: $F_{(9,63)} = 39.0$, $p < .0001$; experiment 2, delta: $F_{(9,63)} = 118.4$, $p < .0001$ and sigma: $F_{(9,63)} = 48.8$, $p < .0001$], reflecting a rising trend (Figures 4A, 4B). The temporal development of delta and sigma activity during baseline did not differ from that for vehicle. ANOVAs run on the midazolam and vehicle data showed significant condition effects for delta and sigma activity [delta activity: $F_{(1,7)} = 41.0$, $p < .0004$; sigma activity: $F_{(1,7)} = 20.2$, $p < .003$]. Delta activity was similar to vehicle activity during wakefulness preceding nonREMS (Figure 4A). Delta activity rose during the transition from wakefulness to nonREMS but was below vehicle during all nine epochs of nonREMS. In contrast, sigma activity was slightly increased during the last epoch of wakefulness and markedly enhanced during all nine epochs of nonREMS. Muscimol (0.2 mg/kg) did not significantly affect the intra-episodic development of delta and sigma activity. For muscimol (0.4 mg/kg) a significant condition effect emerged for delta activity [$F_{(1,7)} = 13.7$, $p < .008$]. Delta activity was unchanged during wakefulness, but enhanced during all epochs of nonREMS (Figure 4B). For sigma activity a significant condition and interaction effect was found [condition: $F_{(1,7)} = 16.6$, $p < .005$ and condition by time: $F_{(9,63)} = 2.9$, $p < .04$]. Sigma activity was slightly enhanced during the last epoch of wakefulness and increased more rapidly during the transition from wakefulness to nonREMS. In the course of the nonREMS episode the enhancement of sigma activity decayed and during epochs 8 and 9 sigma activity was similar to vehicle activity.

DISCUSSION

The present data demonstrate prominent effects of muscimol and midazolam, both $GABA_A$ agonists, on the duration of the sleep states and sleep state-specific EEG. This study also shows that $GABA_A$ agonists that interact with different binding sites of the $GABA_A$ receptor complex affect sleep and sleep EEG differentially.

Muscimol

In accordance with an earlier study (Mendelson and Martin 1990), both doses of muscimol were devoid of a nonREMS latency-reducing effect (Table 2). Muscimol increased the amount of nonREMS and REMS dose dependently (Table 1, Figure 1). The promotion of both sleep states was mainly associated with a lengthening of the nonREMS and REMS episodes (Table 2). Thus, muscimol increases the time spent sleeping and sleep maintenance. In previous studies muscimol did not influence the amount of sleep (Mendelson and Martin 1990; Mendelson and Monti 1993). The lower doses (0.05 and 0.1 mg/kg) and the short registration time of 2 hours may explain this discrepancy. Muscimol also altered EEG activity within nonREMS in a dose-dependent manner. The lower dose of muscimol induced slight enhancements in delta activity (Figures 2C, 3E). The higher dose strongly enhanced the EEG activity in the 0.5 to 12 Hz frequency range and in some higher frequencies during the first 2 hours (Figure 3G). The largest enhancements occurred in the delta frequencies. Because delta activity within nonREMS is normally maximal during the first hours of the light period (Borbély et al. 1984; Franken et al. 1991; Lancel et al. 1994), as is the case in our vehicle data (Figure 3A), a further increase of 20% to 30% denotes very high absolute delta activity. Although in earlier studies on muscimol no EEG spectral analysis was performed, mention has been made of hypersynchronous EEG activity (Mendelson and Martin 1990; Mendelson and Monti 1993). The observation that awakening thresholds increase with higher levels of delta activity (Neckelmann and Ursin 1993) indicates that muscimol potently intensifies nonREMS. The present study demonstrates the involvement of $GABA_A$ receptors in the genesis of delta waves. Apparently, the selective interaction of muscimol with $GABA_A$ receptors hyperpolarizes the Vm of thalamic and/or cortical neurons into the range where delta oscillations are potentiated. It has been postulated that the hyperpolarization induced by $GABA_A$ receptors is not negative enough to trigger delta oscillations (Benington and Heller 1995). However, in intact animals thalamic neurons have a resting Vm of about −60 mV (Paré et al. 1991; Steriade et al. 1991; Nuñez et al. 1992), and $GABA_A$ inhibition readily attains levels below −74 mV (Paré et al. 1991). Such a hyperpolarization may well suffice to de-inactivate LTSs to about 90% (Jahnsen and Llinás 1984). The neurophysiological finding that an increase in hyperpolarization of Vm switches thalamocortical cells from spindle oscillations toward delta oscillations (Steriade et al. 1991, 1993; Nuñez et al. 1992) implies a mutual exclusion between spindles and delta waves. Thalamic EEG recordings in cats and cortical EEG recordings in humans during normal sleep and after sleep deprivation (SD) indeed showed an inverse relation between sigma/spindle activity and delta activity in the course of nonREMS episodes (Lancel et al. 1992; Dijk et al. 1993). The muscimol-induced enhancement of delta activity is not associated with a parallel attenuation of sigma activity: the analysis of 2-hour averages (Figure


nonREMS
REMS
A vehicle
B vehicle
C midazolam
D midazolam
E muscimol 0.2 mg/kg
F muscimol 0.2 mg/kg
G muscimol 0.4 mg/kg
H muscimol 0.4 mg/kg
EEG POER DENSITY (% of 1-2 h)
(% of 1-4 h)
EEG POER DENSITY (% of vehicle)
(% of vehicle)
1-2h
3-4
5-6
7-8
1-4 h
5-8
1-2 h
4-8
1-6 h
(Hz)

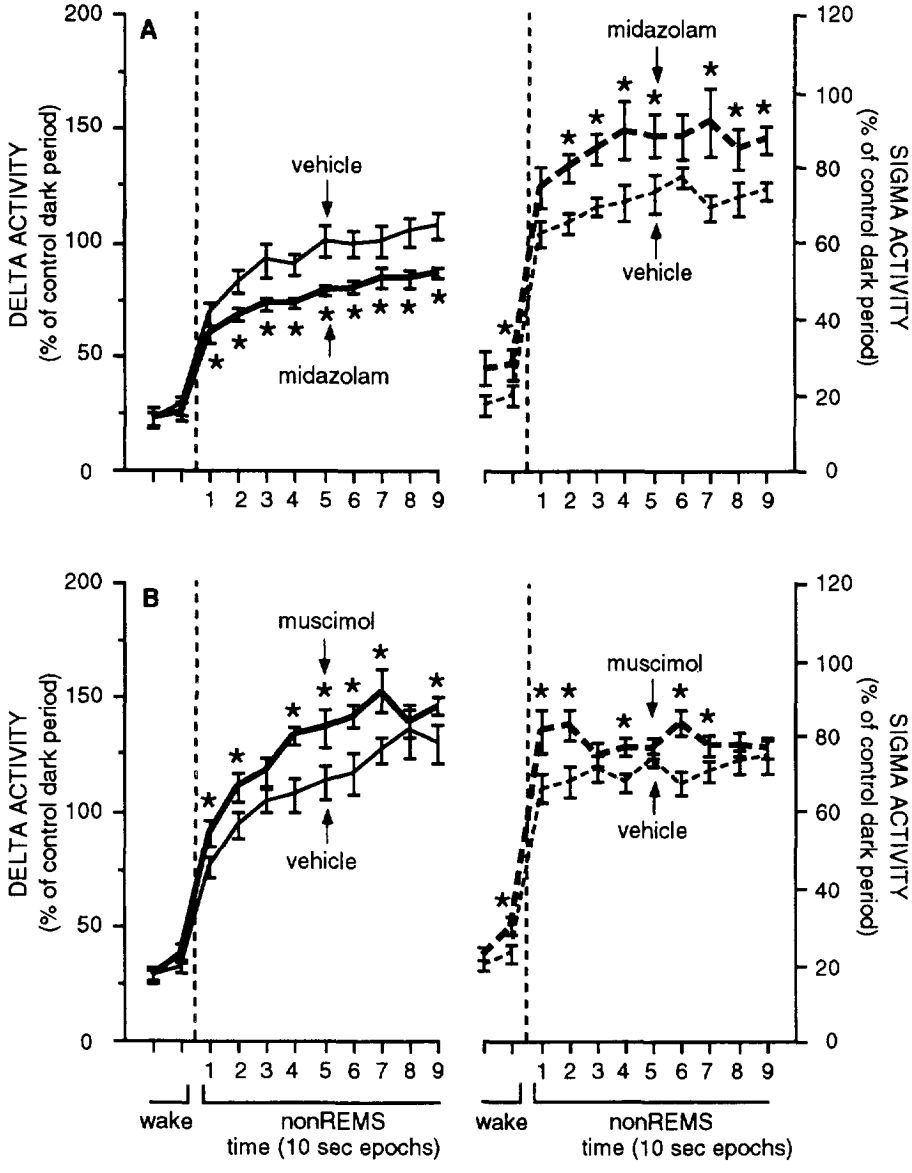


Figure 4. Average time course of delta and sigma activity across the last two 10-s epochs of wakefulness preceding nonREMS and the first ten 10-s epochs of nonREMS during the first 4 hours of the vehicle and midazolam condition (A) and of the vehicle and muscimol 0.4 mg/kg condition (B). The curves connect mean values (n = 8, ± SEM). The data of each animal were normalized by expressing the delta and sigma activities in percent of the average delta and sigma power density within nonREMS during the preceding 12-hour control dark period. Statistics were performed on the log-transformed values. Significant differences between corresponding values of vehicle and other conditions are indicated by * ($p < .05$, two-sided, paired t-test).

3E), as well as the analysis on a smaller time scale (Figure 4B), reveal a simultaneous enhancement of delta and sigma activity. Possibly, muscimol potentiates delta oscillations in the cortex and spindle oscillations in the thalamus (Steriade, personal communication). EEG activity within REMS was also dose-dependently affected by muscimol. After the higher dose of muscimol enhancements emerged in the higher delta frequencies and in the frequencies around 10 Hz and above 14 Hz (Figures 2D, 3H). The prominent enhancements in high-frequency activity are especially reminiscent of the effects of benzodiazepines on REMS-specific EEG (Borbély et al. 1985; Lancel et al. 1994) and suggest a common $GABA_A$ agonistic modulation of neuronal activity during REMS.

All muscimol-induced changes in the amount of sleep, sleep continuity, and nonREMS-EEG enhance-

Figure 3. EEG power densities within nonREMS (*left panels*) for each 2-hour interval and within REMS (*right panels*) for each 4-hour interval or over 6 hours during the vehicle condition of experiment 1 (A, B), midazolam (C, D), muscimol 0.2 mg/kg (E, F) and muscimol 0.4 mg/kg (G, H). Curves connect mean values (n = 8). For each animal, interval and frequency band EEG power density was first normalized and then expressed relative to a reference (100%). For vehicle, the reference is the value of the same sleep state and frequency band during the first interval and for the other conditions, the reference is the value of the corresponding time interval during vehicle. Statistics were done on log-transformed data. Dots below the abscissa refer to frequency bands. *Solid bars* and * on the dotted lines indicate significant interval effects for the vehicle condition (one-factor repeated-measures ANOVA) and significant differences between vehicle and other conditions ($p < .05$, two-sided, paired t-test).

ments over almost the entire frequency range, with peak values in the 2 to 3-Hz region that diminish across time, as well as the increase in rise rate and maximal level of delta activity within nonREMS episodes are qualitatively similar to the effects of SD in the rat (Borbély et al. 1979, 1984; Lancel and Kerkhof 1989; Franken et al. 1991). The present data document for the first time that muscimol produces sleep with the characteristics of "natural" sleep. In this regard pharmacological stimulation of the GABA-binding site on $GABA_A$ receptors may have therapeutic interest in sleep disturbances.

Midazolam

Midazolam shortened nonREMS latency and increased the amount of nonREMS (Tables 1, 2, Figure 1). These are well-known hypnotic effects of benzodiazepines (Gaillard et al. 1973; Johnson et al. 1979; Borbély et al. 1985; Dijk et al. 1989; Mendelson and Martin 1990; Lancel et al. 1994). The increase in nonREMS was brought about by the occurrence of more nonREMS episodes (Table 2), which suggests that midazolam does not promote sleep maintenance in the rat. Simultaneously, midazolam strongly suppressed the occurrence of REMS episodes during the first 4 hours (Tables 1 and 2). A temporary inhibition of REMS has repeatedly been reported for benzodiazepines (Borbély et al. 1985; Dijk et al. 1989; Mendelson and Martin 1990; Lancel et al. 1994). Midazolam also affected sleep state-specific EEG power densities. Within nonREMS low-frequency EEG activity ($\leq$11 Hz) was reduced and high-frequency activity ($\geq$13 Hz) markedly enhanced (Figures 2A, 3C). The analysis of the intra-episodic time course of delta and sigma activity showed that in the course of the nonREMS episodes delta activity remained at a lower level, whereas sigma activity was persistently higher than vehicle activity (Figure 4A). These data are in accordance with the hypothesized inverse relation between delta activity and sigma activity. The midazolam-induced decrease in delta activity and elevation in sigma activity agree with earlier reports on benzodiazepines (Gaillard et al. 1973; Johnson et al. 1979; Borbély et al. 1985; Dijk et al. 1989; Lancel et al. 1994). Benzodiazepines possibly stimulate spindle oscillations and as a consequence inhibit the genesis of delta oscillations. For instance, the benzodiazepine agonistic modulation of $GABA_A$ receptor functioning might enhance the removal of inactivation of LTSs, resulting in large rebound burst discharges of thalamocortical and cortical neurons, which are transferred back to and excite the RE. More likely, benzodiazepines may primarily depress delta activity and thereby enable spindling. It has recently been shown that pentobarbital, a $GABA_A$ agonistic barbiturate, abolishes delta oscillations, although it hyperpolarizes the Vm of thalamic cells into the range optimal for the occurrence of delta oscillations (Nuñez et al. 1992). The authors hypothesized that the barbiturate-induced increase in membrane permeability might unbalance the necessary interaction between the currents Ih and It. Similar mechanisms may underlie the effects of benzodiazepines. In accordance with a previous study (Lancel et al. 1994), midazolam also changed the EEG activity within REMS. EEG activity in the 3.5 to 5-Hz frequency and most prominently in the frequencies $\geq$11 Hz were enhanced during the entire 8-hour recording period (Figures 2B, 3D). These alterations are comparable with the effects of a short SD in rats (Tobler and Borbély 1990). Because midazolam initially suppresses the occurrence of REMS, one might argue that a slight increase in the pressure for REMS underlies these effects. The observation that muscimol affects REMS-specific EEG activity similarly does not necessarily make this explanation unlikely, considering the fact that muscimol increased the total amount of REMS, an effect usually observed after SD (Borbély et al. 1984; Tobler and Borbély 1990; Franken et al. 1991).

Muscimol versus Midazolam

The present study demonstrates that muscimol and midazolam have strikingly different effects on sleep architecture and sleep EEG. Various explanations for the observed discrepancies exist. For example, they have different effects on $GABA_A$ receptor functioning: Muscimol probably binds to all GABA-recognition sites on the $GABA_A$ receptor and thereby evokes the opening of chloride channels of longer opening durations that are usually opened at higher GABA concentrations (for review, see Macdonald and Olsen 1994). In contrast, benzodiazepines enhance the binding of GABA to a low-affinity binding site, resulting in an increase of chloride current by increasing the frequency of channel opening, without altering the open duration (for review, see Macdonald and Olsen 1994). Furthermore, muscimol is a poor substrate for uptake mechanisms and may therefore evoke a more tonic hyperpolarization than GABA itself. In addition, $GABA_A$ receptors are composed of a combination of at least five different polypeptide subunits (α, β, γ, δ, ρ) with various subunit subtypes. Thus, multiple isoforms of $GABA_A$ receptors exist, each with its own physiological and pharmacological properties and distribution in the CNS (for review, see Deutsch et al. 1992; Kuriyama et al. 1993; Macdonald and Olsen 1994). It is highly likely that muscimol activates all functional $GABA_A$ receptors, whereas benzodiazepines only enhance activation of certain types of $GABA_A$ receptors, each with its own regional distribution (for review, see Deutsch et al. 1992; Macdonald and Olsen 1994).

The present muscimol data indicate a role of $GABA_A$ receptors in the induction and/or maintenance of the sleep states and in the genesis of both spindles and

delta waves within nonREMS. It has recently been shown that the thalamic administration of $GABA_B$ antagonists enhances light nonREMS and reduces deep nonREMS in cats (Juhász et al. 1994). Thus, both fast and slow IPSPs induced by $GABA_A$ and $GABA_B$ receptors, respectively, are involved in the occurrence and/or generalization of slow oscillations in Vm of thalamic and cortical neurons during nonREMS. Furthermore, the present study revealed that muscimol and midazolam, both called $GABA_A$ agonists, have strikingly different effects on sleep states and sleep EEG. These data suggest that the influence of compounds interacting with the $GABA_A$ receptor complex critically depend on the receptor site to which they bind. Because muscimol exclusively binds to the GABA recognition site of the $GABA_A$ receptor, its effects on sleep may represent physiological $GABA_A$ agonistic effects. In contrast, benzodiazepines, barbiturates, and several endogenous steroids bind to modulatory binding sites of the $GABA_A$ receptor. At least concerning their effects on sleep, they may not have the working mechanism nor the working profile of a $GABA_A$ agonist.

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REFERENCES

Benington JH, Heller HC (1995): Restoration of brain energy metabolism as the function of sleep. Prog Neurobiol 45:347–360

Borbély AA, Neuhaus HU (1979): Sleep-deprivation: Effects on sleep and EEG in the rat. J Comp Physiol 133:71–87

Borbély AA, Tobler I, Hanagasioglu M (1984): Effect of sleep deprivation on sleep and EEG power spectra in the rat. Behav Brain Res 14:171–182

Borbély AA, Mattmann P, Loepfe M, Strauch I, Lehmann D (1985): Effect of benzodiazepine hypnotics on all-night sleep EEG spectra. Human Neurobiol 4:189–194

Brunner D, Dijk DJ, Münch M, Borbély AA (1991): Effect of zolpidem on sleep and sleep EEG spectra in healthy young men. Psychopharmacology 104:1–5

Coenen AML, Stephens DN, van Luijtelaar, ELJM (1992): Effects of the β-carboline abecarnil on epileptic activity, EEG sleep and behavior in rats. Pharmacol Biochem Behav 42:401–405

Deutsch SI, Mastropaolo J, Hitri A (1992): GABA-active steroids: Endogenous modulators of GABA-gated chloride ion conductance. Clin Neuropharmacol 15:352–364

Dijk DJ, Beersma DGM, Daan S, van den Hoofdakker RH (1989): Effects of seganserin, a 5-HT antagonist, and temazepam on human sleep stages and EEG power spectra. Eur J Pharmacol 171:207–218

Dijk DJ, Hayes B, Czeisler CA (1993): Dynamics of electroencephalographic sleep spindles and slow wave activity in men: Effect of sleep deprivation. Brain Res 626:190–199

Franken P, Dijk DJ, Tobler I, Borbély AA (1991): Sleep deprivation in rats: Effects on EEG power spectra, vigilance states, and cortical temperature. Am J Physiol 261:R198–R208

Gaillard JM, Schulz P, Tissot R (1973): Effect of three benzodiazepines (nitrazepam, flunitrazepam and bromazepam) on sleep of normal subjects, studied with an automatic sleep scoring system. Pharmacopsychiatry 6:207–217

Jahnsen H, Llinás R (1984): Electrophysiological properties of guinea-pig thalamic neurones: An in vitro study. J Physiol 349:205–226

Johnson LC, Seales DM, Naitoh P, Church MW, Sinclair M (1979): The effects of flurazepam hydrochloride on brain electrical activity during sleep. Electroenceph Clin Neurophysiol 47:309–321

Juhász G, Emri Z, Kékesi KA, Salfay O, Crunelli V (1994): Blockade of thalamic $GABA_B$ receptors decreases EEG synchronization. Neurosci Lett 172:155–158

Kim Y, Zhuang H, Tsutsumi M, Okabe A, Kurachi M, Kamikawa Y (1993): Comparison of the effect of zopiclone and brotizolam on sleep EEG by quantitative evaluation in healthy women. Sleep 16:655–661

Kuriyama K, Hirouchi M, Nakayasu H (1993): Structure and function of cerebral $GABA_A$ and $GABA_B$ receptors. Neurosci Res 17:91–99

Lancel M, Kerkhof GA (1989): Effects of repeated sleep deprivation in the dark- or light-period on sleep in rats. Physiol Behav 45:289–297

Lancel M, van Riezen H, Glatt A (1992): The time course of σ activity and slow-wave activity during NREMS in cortical and thalamic EEG of the cat during baseline and after 12 hours of wakefulness. Brain Res 596:285–295

Lancel M, Crönlein TAM, Müller-Preuß P, Holsboer F (1994): Pregnenolone enhances EEG delta activity during non-rapid eye movement sleep in the rat, in contrast to midazolam. Brain Res 646:85–94

Macdonald RL, Olsen RW (1994): $GABA_A$ receptor channels. Ann Rev Neurosci 17:569–602

McCormick DA, Pape HC (1990): Properties of a hyperpolarization-activated cation current and its role in rhythmic oscillation in thalamic relay neurons. J Physiol 431:291–318

Mendelson WB, Martin JV (1990): Effects of muscimol and flurazepam on the sleep EEG in the rat. Life Sci 47:99–101

Mendelson WB, Monti D (1993): Do benzodiazepines induce sleep by a GABAergic mechanism? Life Sci 53:81–87

Mendelson WB, Martin JV, Perlis M, Wagner R, Majewska MD, Paul SM (1987): Sleep induction by an adrenal steroid in the rat. Psychopharmacology 93:226–229

Neckelmann D, Ursin R (1993): Sleep stages and EEG power spectrum in relation to acoustical stimulus arousal threshold in the rat. Sleep 16:464–477

Nuñez A, Curró Dossi R, Contreras D, Steriade M (1992): Intracellular evidence for incompatibility between spindle and delta oscillations in thalamocortical neurons of cat. Neuroscience 48:75–85

Paré D, Curró Dossi R, Steriade M (1991): Three types of inhibitory postsynaptic potentials generated by inter-

neurons in the anterior thalamic complex of cat. J Neurobiol 66:1190–1204

SAS/STAT (1987): SAS/STAT TM Guide for Personal Computers, Cary NC, SAS/STAT.

Steriade M, Deschênes M (1988): Intrathalamic and brainstem-thalamic networks involved in resting and alert states. In Bentivoglio M, Spreafico R (eds), Cellular Thalamic Mechanisms. Amsterdam, Elsevier, pp 37–62

Steriade M, Deschênes M, Domich L, Mulle C (1985): Abolition of spindle oscillations in thalamic neurons disconnected from nucleus reticulars thalami. J Neurophysiol 54:1472–1497

Steriade M, Domich L, Oakson G, Deschênes M (1987): The deafferented reticularis thalami nucleus generates spindle rhythmicity. J Neurophysiol 57:260–273

Steriade M, Curró Dossi R, Nuñez A (1991): Network modulation of a slow intrinsic oscillation of cat thalamocortical neurons implicated in sleep delta waves: Cortically induced synchronization and brainstem cholinergic suppression. J Neurosci 11:3200–3217

Steriade M, McCormick DA, Sejnowski TJ (1993): Thalamocortical oscillations in the sleeping and aroused brain. Science 262:679–685

Tobler I, Borbély AA (1990): The effect of 3-h and 6-h sleep deprivation on sleep and EEG spectra of the rat. Behav Brain Res 36:73–78

Trachsel L, Dijk DJ, Brunner D, Klene C, Borbély AA (1990): Effect of zopiclone and midazolam on sleep and EEG spectra in a phase-advanced sleep schedule. Neuropharmacology 3:11–18