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Sleep in depression: the influence of age, gender and diagnostic subtype on baseline sleep and the cholinergic REM induction test with RS 86

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Summary. One hundred and eight healthy controls and 178 patients with a major depressive disorder according to DSM-III were investigated in the sleep laboratory after a 7-day drug wash-out period. Subsamples of 36 healthy controls and 56 patients additionally took part in the cholinergic rapid eye movement (REM) sleep induction test with RS 86. Data analysis revealed that age exerted powerful influences on sleep in control subjects and depressed patients. Sleep efficiency and amount of slow wave sleep (SWS) decreased with age, whereas the number of awakenings, early morning awakening, and amounts of wake time and stage 1 increased with age. REM latency was negatively correlated with age only in the group of patients with a major depression. Statistical analysis revealed group differences for almost all parameters of sleep continuity with disturbed indices in the depressed group. Differences in SWS were not detected. REM latency and REM density were altered in depression compared to healthy subjects. Sex differences existed for the amounts of stage 1 and SWS. The cholinergic REM induction test resulted in a significantly more pronounced induction of REM sleep in depressed patients compared with healthy controls, provoking sleep onset REM periods as well in those depressed patients showing baseline REM latencies in the normal range. Depressed patients with or without melancholia (according to DSM-III) did not differ from each other, either concerning baseline sleep or with respect to the results of the cholinergic REM induction test. The results stress the importance of age when comparing sleep patterns of healthy controls with those of depressed patients. Furthermore they underline the usefulness of the cholinergic REM induction test for differentiating depressed patients from healthy controls and support the reciprocal interaction model of nonREM-REM regulation and the cholinergic-aminergic imbalance hypothesis of affective disorders.

Key words: Major depression – Slow wave sleep – REM sleep – Cholinergic REM induction test

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Introduction

Since the initial reports by Kupfer (Kupfer and Foster 1972; Kupfer 1976), it is widely accepted that sleep in depression is characterized by disturbances of sleep continuity and more specific alterations of the sleep pattern like a reduction of slow wave sleep (SWS) and a desinhibition of rapid eye movement (REM) sleep including a shortening of REM latency, prolongation of the first REM period and heightening of REM density. Initial claims that these abnormalities constitute markers for the primary/endogenous or melancholic subtype of the disorder are debated (Berger et al. 1982; 1983b; Feinberg et al. 1982; Feinberg and Carroll 1984; Thase et al. 1984).

The reduction of SWS and REM sleep abnormalities is presently a major focus of psychiatric sleep research and has raised much controversy concerning the meaning and relevance for the pathophysiology of depression.

The two-process model of sleep and slepp regulation formulated by Borbély (Borbély 1982; Borbély and Wirz-Justice 1982) makes the assumption (based on frequency analysis of the sleep EEG (Borbély et al. 1984) that the generation of SWS is impaired in depression. The reduction of SWS, especially in the first third of the night, is thought to be responsible for the advance of REM sleep. Borbély and Wirz-Justice speculated that nocturnal amounts of SWS are the expression of a hypothesized process "S", which builds up during wakefulness. Reduced amounts of SWS are ascribed to a disturbed buildup of "S" during the day, which is assumed to be implicated in the pathogenesis of depression. Total sleep deprivation, i.e. prolonged wakefulness, according to Borbély and Wirz-Justice, enhances SWS and acts antidepressively via an increased buildup of process "S".

The reciprocal interaction model of nonREM and REM sleep regulation and its implications for depression (McCarley 1982) postulates a primary role for the advance of REM sleep with the assumption of an overactive cholinergic/muscarinic system in depression. Hobson and coworkers have demonstrated (Hobson et al. 1975, 1986) with experiments in cats that the regular alternating pat-

tern of nonREM and REM sleep is governed by cell groups in the brain system. In short, noradrenergic/serotonergic neurons in the locus coeruleus and the dorsal raphe are active during nonREM sleep, whereas cholinergic cell groups in the pontine reticular formation become active immediately prior to and during REM sleep. Evidence for a strong cholinergic involvement in the triggering and maintenance of REM sleep comes from stimulation studies, for example with carbachol, which induced long-lasting REM periods in cats (for overview, see Steriade and McCarley 1990). Studies in healthy humans with the cholinomimetics arecoline (Sitaram et al. 1978a, b), physostigmine (Berger et al. 1983a; Sitaram et al. 1976, 1977), galanthamine (Riemann et al., in press), RS 86 (Spiegel 1984; Riemann et al. 1988), pilocarpine (Berkowitz et al. 1989) and SDZ 210–086 (Hohagen et al. 1993) also demonstrated an unequivocal shortening of REM latency following cholinergic challenge.

Given the strong interrelatedness and interconnection between cholinergic networks in the brain stem and higher brain areas, especially in the limbic system (Hobson et al. 1986), REM sleep disinhibition in depression may be cited as further evidence for the cholinergic-aminergic neurotransmitter imbalance model (Janowsky et al. 1972; Janowsky and Risch 1986) which initially was based on pharmacological evidence concerning the behaviour of humans and animals following manipulations of cholinergic/noradrenergic neurotransmission.

Attempts to evaluate empirically both theories in humans have included numerous sleep studies, among them frequency analysis of the sleep EEG, sleep deprivation studies and challenge studies with cholinergic drugs (for an overview, see Reynolds and Kupfer 1987).

The influence of age on human sleep has been well documented in healthy subjects (Williams et al. 1974; Feinberg et al. 1967; Miles and Dement 1980; Spiegel 1981). Over the life span amounts of "light" sleep (i.e. stage 1) and nocturnal awakenings increase, whereas slow wave sleep decreases. Total sleep time, when including naps during the day, probably does not decrease. In some older healthy subjects REM sleep abnormalities otherwise usually observed only in depressed patients may occur.

For depressives a significant influence of age has been documented as well. Slow wave sleep declines with age (Gillin et al. 1981; Kerkhofs et al. 1988; Lauer et al. 1991; Reynolds et al. 1990; Thase et al. 1986; Ulrich et al. 1980). REM latency is negatively age-related in depressives (Gillin et al. 1981; Kumar et al. 1987; Kupfer et al. 1986a, b; Lauer et al. 1991; Thase et al. 1986; Ulrich et al 1980).

Concerning sex differences, decreased amounts of slow wave sleep have been demonstrated for males compared with females both in healthy and depressed subjects (Reynolds et al. 1990).

These data stress the fact that, when comparing depressed patients and healthy subjects, age and gender have to be controlled.

1. The first aim of the present study is to provide a comparative analysis of the sleep patterns of a large sample of 108 healthy subjects and 178 patients with a major depressive disorder (MDD), analysing the impact of age, gender and diagnostic subtype (melancholic vs. nonmelancholic depression according to DSM-III). This part of the study is an extension of results published earlier by Lauer et al. (1991), where we compared the sleep data of 50 healthy controls and 74 patients with MDD, who are also included in the present evaluation.

2. Subgroups of the sample participated in the cholinergic REM induction test (CRIT) with RS 86. Cholinergic stimulation studies (for an overview, see Berger et al. 1989; Gillin et al. 1991) showed that in depression the reaction to cholinergic stimulation prior to or during sleep is significantly more pronounced compared to healthy subjects and patients with other psychiatric diagnoses, with the exception of patients with schizophrenia (Riemann et al. 1991). Whereas in healthy subjects cholinergic stimulation with RS 86 led to a mean reduction of REM latency by approximately 20 min (from 70 to 50 min), in depressed patients almost exclusively sleep onset REM periods (SOREMP = REM latency $\leq$ 25 min) occurred following cholinergic stimulation. These results were interpreted as evidence for a muscarinic supersensitivity or cholinergic overactivity in depression. Furthermore, these data can be regarded as supportive of the reciprocal interaction model of nonREM-REM regulation as formulated by Hobson et al. (1975, 1986).

The present analysis will focus on the questions whether the REM sleep response to cholinergic challenge in healthy subjects and depressed patients is age-dependent and if patients with or without melancholia differ from each other.

Methods

Samples

Study I: comparison of baseline sleep. Healthy controls: 108 healthy control subjects (69 males and 39 females) were studied. Mean age (SD) was 34.5 (12.5) years. Prior to inclusion in the study all subjects were carefully screened for physical and mental health. To rule out organic diseases, routine blood tests, ECG and a physical examination were performed. Only subjects with values within normal limits were included. To rule out psychiatric disorders a thorough psychiatric examination was performed. Only subjects with no personal or family history of affective or psychiatric disorders were included in the study. All of the control subjects were required to be free of any kind of medication for at least four weeks prior to study. All subjects were paid for participating.

Depressed patients: 178 inpatients with a major depressive disorder (MDD according to DSM-III, APA 1980) were studied. 59 of the patients were male and 119 were female. Mean age (SD) was 41.2 (12.7) years. According to DSM-III, 113 patients fulfilled the criteria for melancholia, 65 patients were nonmelancholic. Diagnoses were made by experienced psychiatrists familiar with DSM-III criteria. No structured interview was used, but psychiatrists were required to fill out checklists for each patient encompassing DSM-III criteria for MDD and subtype melancholia.

Prior to the study patients underwent routine blood tests, ECG, EEG and CCT. Only subjects with values within normal limits and an unsuspicious physical examination were included. The severity of depression was measured by the 21-item Hamilton scale. Patients were required to score $\geq$ 18 points on the Hamilton scale to be included. All patients were free of any kind of medication for at least seven days prior to the study.

Table 1. Demographic and clinical data

	Healthy controls (n = 108)	MDD (n = 178)	MDD Melancholic (n = 113)	MDD Nonmelancholic (n = 65)
Age ($x \pm$ SD)	34.5 ± 12.5 years	41.2 ± 12.7 years***	43.6 ± 13.2 years	37.1 ± 10.8 years***
Male : female	69 : 39	59 : 119***	37 : 76	22 : 43
DSM-III Melancholia (yes/no)	–	113 : 65	–	–
DSM-III Subtypes (Mono-/bipolar I/bipolar II)	–	151 : 18 : 9	91 : 14 : 8	60 : 4 : 1
21-item HAMD	–	26.4 ± 5.7	27.3 ± 5.5	24.3 ± 5.7**
Age at onset of depression	–	34.8 ± 12.8 years	36.3 ± 13.2 years	32.3 ± 10.6 years*
No. depressive episodes	–	2.9 ± 3.9	3.4 ± 4.7	2.0 ± 1.2**
Duration current episode	–	51.1 ± 109.0 weeks	33.8 ± 63.2 weeks	81.1 ± 156.4 weeks*
Drug wash-out period:				
7– 14 days n =	–	103	76	27
15– 28 days n =	–	26	13	13
29– 90 days n =	–	7	6	1
91–180 days n =	–	1	1	0
180 days < n =	–	32	12	20
never medication n =	–	9	5	4

Results of two-tailed paired t-tests or a Chi-square test (for sex distribution) comparing either MDD with healthy controls or nonmelancholic with melancholic patients

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

Table 1 summarizes demographic and clinical data for healthy subjects and depressed patients.

It can be seen that depressed patients and healthy controls differed significantly concerning age- and sex-distribution. Furthermore melancholic and nonmelancholic patients differed significantly with respect to age and sex distribution, severity of depression (21-HAMD), age at onset of depression, the number of depressive episodes and the duration of the current episode.

Study II: cholinergic REM induction test (CRIT). Healthy subjects: 36 healthy subjects (15 males and 21 females) with a mean age of 41.8 ± 15.6 years participated in the study. Data of this study have already been published (Riemann et al. 1988). These subjects constitute a subsample of the 108 healthy controls.

Depressed patients: 56 patients (20 males, 36 females) with a major depressive disorder according to DSM-III (APA, 1980) with a mean age of 40.6 ± 13.6 years were investigated. The data of 16 of these patients have already been published (Berger et al. 1989). These patients are a subsample of the 178 patients from study I. Depressed patients in this sample did not differ from healthy controls with respect to age and gender. 33 of the depressed patients fulfilled criteria for melancholia [11 males, 22 females; mean age (SD): 42.4 (12.5) years] and 23 patients [9 males, 14 females; mean age (SD): 40.4 (12.7) years] were nonmelancholic. Melancholic and nonmelancholic patients in this group did not differ from each other with respect to age- and sex-distribution or any other of the demographic or psychopathological variables listed in table 1.

All studies had been approved by the local ethics committee. Control subjects and patients were informed in detail about the experimental procedures and gave their informed written consent.

Sleep

Sleep recordings were performed and scored according to standard procedures (Rechtschaffen and Kales 1968) from "lights out" (22.45–23.15 h) to "lights on" (6.30–7.15 h). EEG was recorded from positions C3–A2, C4–A1, EOG was recorded horizontally and EMG was recorded submentally.

For study I the second laboratory night of the healthy volunteers was included in the analysis. For depressed patients the second or third laboratory night was included in this analysis. For study II subjects took part in two additional sleep recordings with administration of placebo/1.5 mg RS 86 prior to sleep at 22.00 h. Placebo/RS 86 were administered in a randomized double-blind crossover design.

Sleep recordings were scored visually blind to experimental condition and diagnosis and evaluated for parameters of sleep continuity, sleep architecture and REM sleep. The following sleep parameters were evaluated: (1) Time in bed (time from lights out till lights on in the morning) and sleep period time: time from sleep onset till final awakening (last stage 2, 3, 4 or REM) during the recording. (2) Total sleep time: time from sleep onset till final awakening in minutes minus intermittent awakenings. (3) Sleep efficiency: ratio of total sleep time (TST) to time in bed (TIB) × 100%. (4) Stages Wake, 1, 2, SWS (Stages 3 and 4 combined and separately), and REM (expressed in % of sleep period time = SPT). (5) Latencies, i.e., time from beginning of the record to the first occurrence of Stage 2 (= sleep onset latency) and from sleep onset to the first occurrence of stage REM (= REM latency) (in minutes). (6) Number of awakenings during SPT. (7) Early morning awakening (EMA) (in minutes), i.e., time span between the last epoch of Stages 2, 3, 4, REM occurring during the record and lights on in the morning. (8) Number of REM periods. (9) Duration of the first REM period (in minutes). (10) REM density for the first REM period and for the whole night (REM density = the ratio of 3-s mini-epochs per REM period including rapid eye movements to the total number of all 3-s mini-epochs per REM period × 100%).

RS 86

RS 86 is a spiropiperidyl derivative which passes the blood-brain barrier and has a half-life of approximately 6–8 h (for pharmacol-

ogy of RS 86, see Palacios et al. 1986). RS 86 has only minor side effects, therefore the administration of a peripheral antidote like methylscopolamine is not necessary. Initially it was assumed that RS 86 primarily acts on the M1 receptor. In the meantime data have been published (Velasquez-Moctezuma et al. 1989) indicating that primarly M2 agonists increase REM sleep whereas pure M1 agonists do not seem to influence REM sleep. As our studies with RS 86 (Berger et al. 1989; Lauer et al. 1988; Riemann et al. 1988; Riemann and Berger 1989; Riemann et al. 1991) demonstrated a significant influence on REM latency and REM density, it seems likely that RS 86 acts on both M1 and M2 receptors. Furthermore, recent pharmacological data (Stoll and Müller 1991) indicate that RS 86 is a mixed M1/M2 receptor agonist with only a slight preference for the M1 receptor.

Statistics

For descriptive purposes mean (SD) were calculated. To compare sleep patterns of depressed patients and healthy subjects and melancholic/nonmelancholic patients, a two-way ANCOVA (with age as covariate and the factors group and sex or diagnostic subtype) was applied to correct for the statistically significant age difference between controls and depressed patients and between the diagnostic subtypes of depression. A prerequisite for ANCOVA is a uniform relationship between the covariate and the dependent variables in the different groups investigated. Therefore product-moment correlations of age with sleep variables were computed prior to the ANCOVA to ascertain that this was the case. For single comparisons between groups, *t*-tests were used. The level of significance was set at $P < 0.05$ (two-tailed).

All statistical analyses were adjusted to the number of comparisons performed to control for type-I error according to the method of Bonferroni. For the set of sleep variables ($n = 17$), an obtained p value of $P = 0.0029$ then corresponds to a statistical significance at the 5% level. An obtained p value of $P = 0.0005$ corresponds to a significance level of 1% and an obtained *P*-value of $P = 0.00005$ to a significance of 0.1%. Only adjusted significance levels will be given in tables and figures. The SAS statistic programme (1985) was used to compute statistics.

Results

1. Baseline sleep

When comparing the samples of healthy subjects and depressed patients concerning age, a statistically significant difference emerged ($P < 0.001$, T-test). As well, both groups differed concerning sex distribution ($P < 0.001$; chi-square test).

1.1. The impact of age. In a first step the influence of age on sleep patterns was investigated by computing separately for both groups correlation coefficients of sleep variables with age (see Table 2).

Data analysis revealed a highly significant influence of age on many of the sleep variables in both groups. Sleep period time, total sleep time and sleep efficiency decreased with age. The number of awakenings, early morning awakening, wake time (%SPT) and stage 1 (%SPT) increased with age. Slow wave sleep (%SPT) decreased with age. In depressed subjects REM latency negatively correlated with age.

1.2. Comparison of healthy subjects and depressed patients. Owing to the considerable age and sex differences between healthy controls and depressed subjects and the described age – sleep relationships, an analysis of variance with age as covariate was performed when comparing both groups. This seemed reasonable, as the relationship between age and sleep variables was similar in both groups investigated. The two-way ANCOVA included the factors diagnosis (healthy controls versus MDD), gender (males versus females) and interaction (diagnosis x gender). The mean (SD) of sleep variables for both groups and both sexes separately and the results of the statistical analysis are depicted in Table 3 a and b.

The values for the covariate age confirmed the results of the correlation analysis.

A number of significant differences between the two groups were found: In depressives total sleep time and sleep efficiency were reduced whereas the number of awakenings, early morning awakening and wake time (%SPT) were enhanced. Stage 2 (%SPT) was reduced and REM sleep (%SPT) heightened. REM latency was significantly reduced, whereas REM density of the first REM period and total REM density were significantly heightened in depression.

Statistically significant sex differences occurred for Stage 1 (%SPT) with males showing higher values, whereas SWS (%SPT) was reduced in males compared to females.

Table 2. Correlations of age and sleep variables (product-moment correlation)[a]

	Healthy Controls ($n = 108$)	MDD ($n = 178$)
Sleep continuity		
Time in bed min.	0.09	0.11
Sleep period time min.	–0.33*	–0.31*
Total sleep time min.	–0.48**	–0.39**
Sleep efficiency %	–0.47**	–0.40**
Sleep latency min.	0.14	0.06
No. awakenings	0.46**	0.27*
Early morning awakening min.	0.25	0.41**
Sleep architecture		
Stage wake % SPT[b]	0.42**	0.28*
Stage 1% SPT	0.42**	0.26
Stage 2% SPT	0.13	0.04
Stage 3% SPT	–0.50**	–0.40**
Stage 4% SPT	–0.44**	–0.53**
SWS % SPT	–0.55**	–0.53**
REM % SPT	–0.15	–0.08
REM-sleep		
No. REM periods	–0.18	–0.05
REM latency min.	–0.09	–0.27*
1. REMP duration min.	0.20	0.04
1. REMP density %	0.01	0.10
Total REM density %	–0.07	–0.07

[a] *P*-values adjusted to the number of coefficients (Bonferroni method)
[b] SPT = Sleep period time, time from sleep onset till final awakening
* $P < 0.05$; ** $P < 0.01$

Table 3a. Sleep variables of healthy controls and patients with MDD (mean ± SD)

	Healthy controls		MDD	
	Males (n = 69)	Females (n = 39)	Males (n = 59)	Females (n = 119)
Sleep continuity				
Time in bed min.	452.8 (27.0)	439.3 (28.3)	451.3 (31.1)	449.6 (36.7)
Sleep period time min.	429.7 (29.0)	412.5 (34.0)	411.6 (33.0)	400.4 (52.0)
Total sleep time min.	416.1 (36.4)	384.8 (51.9)	371.0 (67.1)	361.9 (72.7)
Sleep efficiency %	91.9 (6.0)	87.6 (10.4)	82.2 (14.9)	80.3 (15.1)
Sleep latency min.	20.1 (14.5)	22.6 (20.5)	24.7 (16.5)	29.0 (28.4)
No. awakenings	8.3 (6.1)	7.9 (5.4)	15.3 (13.3)	13.5 (12.2)
Early morning awakening min.	2.7 (7.8)	4.2 (11.7)	16.3 (26.4)	20.8 (34.6)
Sleep architecture				
Stage Wake % SPT[a]	3.3 (4.0)	6.7 (9.6)	10.1 (13.6)	10.1 (12.5)
Stage 1% SPT	7.7 (4.3)	6.2 (4.3)	10.1 (6.1)	8.2 (4.5)
Stage 2% SPT	56.4 (7.1)	54.6 (7.4)	47.2 (11.6)	48.5 (9.8)
Stage 3% SPT	7.3 (5.8)	8.8 (4.9)	6.4 (5.4)	7.3 (5.3)
Stage 4% SPT	2.7 (4.6)	3.4 (4.4)	2.2 (3.7)	2.7 (4.7)
SWS % SPT	9.9 (9.0)	12.2 (7.9)	8.6 (8.0)	10.1 (8.5)
REM % SPT	21.6 (5.6)	19.5 (5.7)	23.1 (7.9)	22.8 (6.7)
REM sleep				
No. REM periods	4.0 (0.8)	3.8 (0.9)	4.2 (1.0)	3.9 (1.1)
REM latency min.	83.0 (38.2)	91.8 (59.8)	49.0 (32.6)	56.5 (36.2)
1. REMP duration min.	18.3 (11.3)	15.8 (12.0)	24.8 (22.3)	23.0 (15.5)
1. REMP density %	16.9 (11.2)	20.0 (10.5)	32.0 (16.7)	31.9 (16.2)
Total REM density %	23.7 (9.6)	23.7 (10.0)	35.1 (14.4)	32.4 (12.4)

[a] SPT = Sleep period time, time from sleep onset till final awakening

Table 3b. Results of statistical analysis: ANCOVA[a]

	Diagnosis df = 1,285		Gender df = 1,285		Diagnosis × Gender df = 1,285		Covariate Age df = 1,285	
	F =	P <	F =	P <	F =	P <	F =	P <
Sleep continuity								
Time in bed min.	0.81	n.s.	1.82	n.s.	0.50	n.s.	1.70	n.s.
Sleep period time min.[b]	3.24	n.s.	4.75	n.s.	0.02	n.s.	27.81	**
Total sleep time min.	9.31	*	3.69	n.s.	0.79	n.s.	51.36	**
Sleep efficiency %	15.99	**	1.49	n.s.	0.02	n.s.	52.39	**
Sleep latency min.	2.57	n.s.[c]	1.03	n.s.	0.19	n.s.	1.78	n.s.
No. awakenings	13.74	**	2.17	n.s.	0.00	n.s.	27.99	**
Early morning awakening min.	11.59	**	0.06	n.s.	1.06	n.s.	38.70	**
Sleep architecture								
Stage wake % SPT[b]	7.04	*	0.42	n.s.	0.71	n.s.	26.73	**
Stage 1 % SPT	7.24	n.s.	12.53	**	0.01	n.s.	28.78	**
Stage 2 % SPT	43.04	**	0.12	n.s.	1.95	n.s.	1.53	n.s.
Stage 3 % SPT	0.00	n.s.	9.11	*	1.52	n.s.	74.03	**
Stage 4 % SPT	0.90	n.s.	5.76	n.s.	1.24	n.s.	95.77	**
SWS % SPT	0.22	n.s.	11.48	**	2.11	n.s.	126.49	**
REM % SPT	9.66	*	1.68	n.s.	0.89	n.s.	2.35	n.s.
REM sleep								
No. REM periods	2.32	n.s.	3.63	n.s.	0.05	n.s.	1.69	n.s.
REM latency min.	36.31	**	3.98	n.s.	0.18	n.s.	11.23	*
1. REMP duration min.	8.92	n.s.	1.53	n.s.	0.11	n.s.	2.38	n.s.
1. REMP density %	47.1	**	0.47	n.s.	0.58	n.s.	1.19	n.s.
Total REM density %	44.8	**	0.57	n.s.	0.97	n.s.	1.31	n.s.

[a] P-value adjusted to the number of comparisons (Bonferroni method); * $P < 0.05$; ** $P < 0.01$

[b] SPT = Sleep period time, time from sleep onset till final awakening

[c] n.s. = nonsignificant

No significant interaction between diagnosis and gender was encountered.

In order to get a more detailed impression of differences between depressed and healthy subjects concerning age, the samples were split up into five age groups (18–25 years; 26–35 years; 36–45 years; 46–55 years; 56–65 years).

Figures 1 and 2 depict the data for SWS, %SPT and REM sleep variables for the five age groups when contrasting depressed patients with healthy controls.

As can be seen, SWS (%SPT) did not distinguish at any age between healthy controls and MDD. For REM sleep variables, REM latency differed between both groups apart from the 26–35 year-olds, whereas parameters of REM density distinguished between both groups at any age.

1.3. The influence of diagnostic subtype: melancholic vs. nonmelancholic depression. As a number of significant differences between melancholic and nonmelancholic patients concerning demographic and clinical variables were noted (see Table 1), in a first step the impact of these variables on sleep was analysed. When analysing the influence of severity of depression, age at first onset of depression, number of depressive episodes and duration of the current episode, age was used as a covariate, as age may for example simultaneously influence sleep and clinical characteristics. None of the computed correlations even approached statistical significance (when correcting for the number of coefficients calculated according to the Bonferroni method; data not shown).

Table 4 displays mean values (SD) of sleep variables of melancholic and nonmelancholic patients. For data analysis, an analysis of covariance with the factor diagnosis and the covariate age was performed.

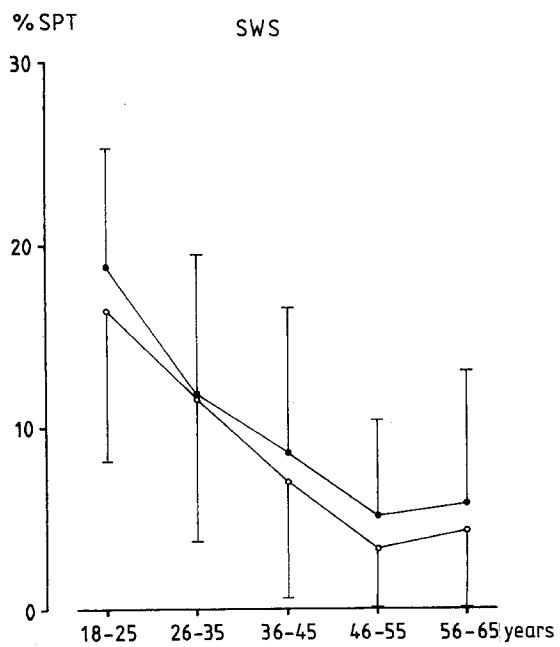

Fig. 1. SWS %SPT for healthy controls (*open circles*) and depressed patients (*closed circles*) separated for five age groups

The covariate age confirmed the correlational analysis performed in the preceding section of this paper. None of the sleep variables differentiated significantly between both groups.

2. The cholinergic REM induction test with RS 86

2.1. Comparison of healthy controls and depressed patients. Figure 3 displays the results of the cholinergic REM induction test for the central variable, REM latency, for healthy controls and patients with MDD. For this analysis, both samples were split up into three age groups (18–35 years; 36–50 years; 51–65 years). A two-factorial analysis of variance (factor 1: age group; factor 2: diagnosis) with repeated measurement (placebo vs. RS 86) revealed a highly significant impact of RS 86 on REM latency ($df = 1,88$; $f = 58.2$; $P = 0.0001$), as well as a highly significant difference between depressed patients and

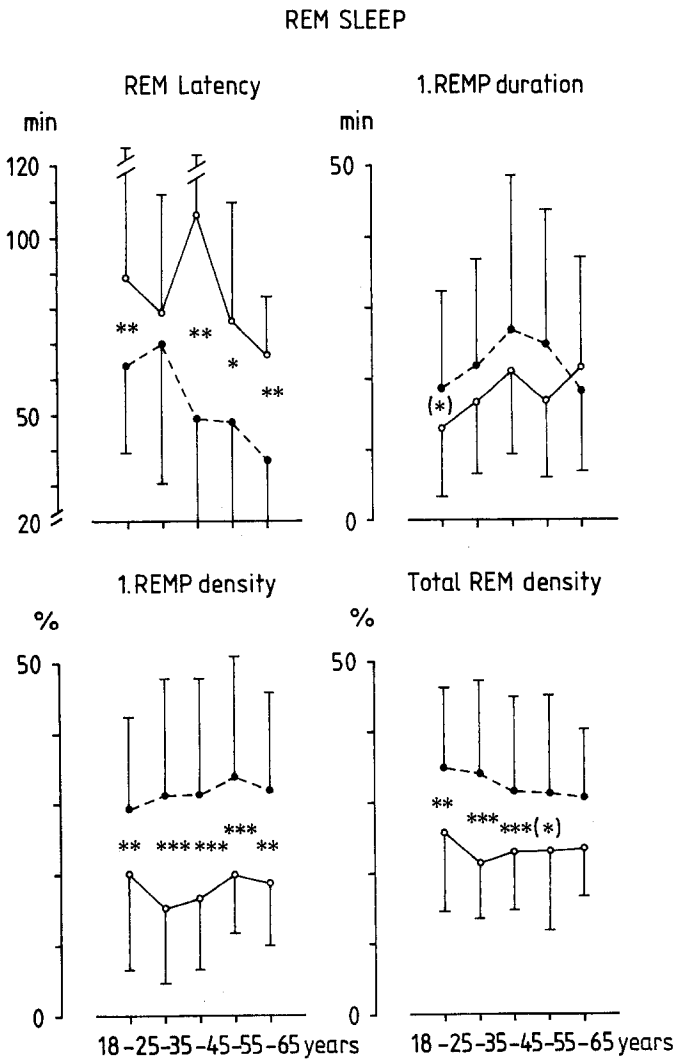

Fig. 2. Variables of REM sleep for healthy controls (*open circles*) and depressed patients (*closed circles*) separated for five age groups. *Stars* indicate statistical significance (two-tailed *t*-test) for between-group comparisons. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

Table 4. Sleep varibales ($x \pm$ SD) for melancholic and nonmelancholic patients and statistical analysis (ANCOVA)[a]

	Melancholic patients (n = 113)	Nonmelancholic patients (n = 65)	Diagnosis df = 1,177		Covariate Age df = 1,177	
			F =	P <	F =	P <
Sleep continuity						
Time in bed min.	454.8 (40.7)	445.6 (38.9)	0.10	n.s.[c]	0.90	n.s.
Sleep period time min.	402.0 (46.9)	407.9 (46.5)	0.06	n.s.	18.25	**
Total sleep time min.	355.2 (72.4)	381.9 (65.2)	1.62	n.s.	25.96	**
Sleep efficiency %	78.1 (15.9)	85.7 (12.8)	4.73	n.s.	26.78	**
Sleep latency min.	28.1 (21.5)	26.8 (30.6)	0.02	n.s.	0.61	n.s.
No awakenings	16.3 (12.7)	10.5 (11.5)	5.13	n.s.	9.19	*
Early morning awakening min.	24.5 (35.9)	10.2 (21.6)	2.94	n.s.	28.29	**
Sleep architecture						
Stage wake % SPT[b]	12.1 (13.7)	6.6 (10.3)	3.89	n.s.	10.80	*
Stage 1 % SPT	9.5 (5.7)	7.9 (3.8)	1.83	n.s.	6.30	n.s.
Stage 2 % SPT	47.4 (11.2)	49.2 (8.7)	1.71	n.s.	0.76	n.s.
Stage 3 % SPT	6.1 (5.2)	8.6 (5.2)	3.54	n.s.	26.42	**
Stage 4 % SPT	1.9 (3.8)	3.7 (5.2)	1.40	n.s.	58.49	**
Stage SWS % SPT	8.0 (7.7)	12.3 (8.8)	3.73	n.s.	58.30	**
Stage REM % SPT	22.5 (6.9)	23.4 (7.4)	0.33	n.s.	0.82	n.s.
REM sleep						
No. REM periods	4.0 (1.1)	4.0 (1.0)	0.01	n.s.	0.34	n.s.
REM latency min.	52.3 (37.2)	57.1 (31.3)	0.00	n.s.	12.44	**
1. REMP duration min.	22.4 (17.7)	25.8 (18.4)	1.95	n.s.	0.75	n.s.
1. REMP density %	31.5 (16.8)	32.8 (15.5)	0.72	n.s.	2.21	n.s.
Total REM density %	33.3 (13.4)	33.4 (12.7)	0.04	n.s.	0.94	n.s.

[a] P-values adjusted to the number of variables * $P < 0.05$; ** $P < 0.01$

[b] SPT = Sleep period time, time from sleep onset till final awakening

[c] n.s. = nonsignificant

control subjects (df = 1,88; f = 38.5; P = 0.0001). There was also a statistical trend for an effect of age group (df = 2,88; f = 2.7; P = 0.0760). The interaction between diagnostic group × treatment was also significant (df = 1,88; F = 5.3, P = 0.0233), indicating a more pronounced effect of RS 86 on REM latency in depressives compared to healthy subjects.

When looking at the frequency of SOREMPs (sleep onset REM periods, REM latency ≤ 25 min) following cholinergic stimulation, 13 out of 18 young depressed, 18 out of 25 middle-aged depressives and 8 out of 13 "older" depressed patients displayed a SOREMP after cholinergic stimulation. The respective figures for healthy controls were none out of 13 younger subjects, two out of 10 middle-aged subjects and four out of 13 "older" subjects. When correlating delta-REM latency (placebo – RS 86) with age, in healthy controls a nonsignificant coefficient (r = 0.19) was obtained. In depressed patients, the correlation coefficient was r = -0.344 (P = 0.0095), thus indicating that with increasing age, the REM sleep response to RS 86 decreased.

Gender differences were not detected (data not shown).

2.2. Melancholic vs. nonmelancholic patients. Figure 4 depicts the results of the cholinergic REM induction test for REM latency when comparing patients with or without melancholia.

It is obvious that both subgroups of patients did not differ from each other concerning REM latency. Neither for placebo, RS 86 nor for delta-values (placebo – RS 86) was any significant difference concerning REM latency between both groups found.

Discussion

In the first part of this study the influence of age and gender on baseline sleep of healthy controls and depressed patients was analysed. It was confirmed that age has a powerful influence on sleep in both groups. Indices of sleep continuity like total sleep time, sleep efficiency, number of awakenings, early morning awakening and wake time deteriorated with increasing age in both groups. On the other hand, slow wave sleep decreased with age. For REM sleep parameters only one significant correlation was found, i.e. in depressed patients REM latency decreased with age. These data are in accord with already published data on the relationship of age and sleep in healthy subjects and depressed patients (Williams et al. 1974; Feinberg et al. 1967; Miles and Dement 1980; Spiegel 1981; Gillin et al. 1981; Kerkhoffs et al. 1988; Reynolds et al. 1990; Thase et al. 1986; Ulrich 1980).

Concerning sex differences stage 1 (%SPT) was heightened in males, whereas slow wave sleep (%SPT) was reduced in males. The data concerning slow wave sleep are in agreement with those recently published by Reynolds et al. (1990). Summarizing, the data concerning sex dif-

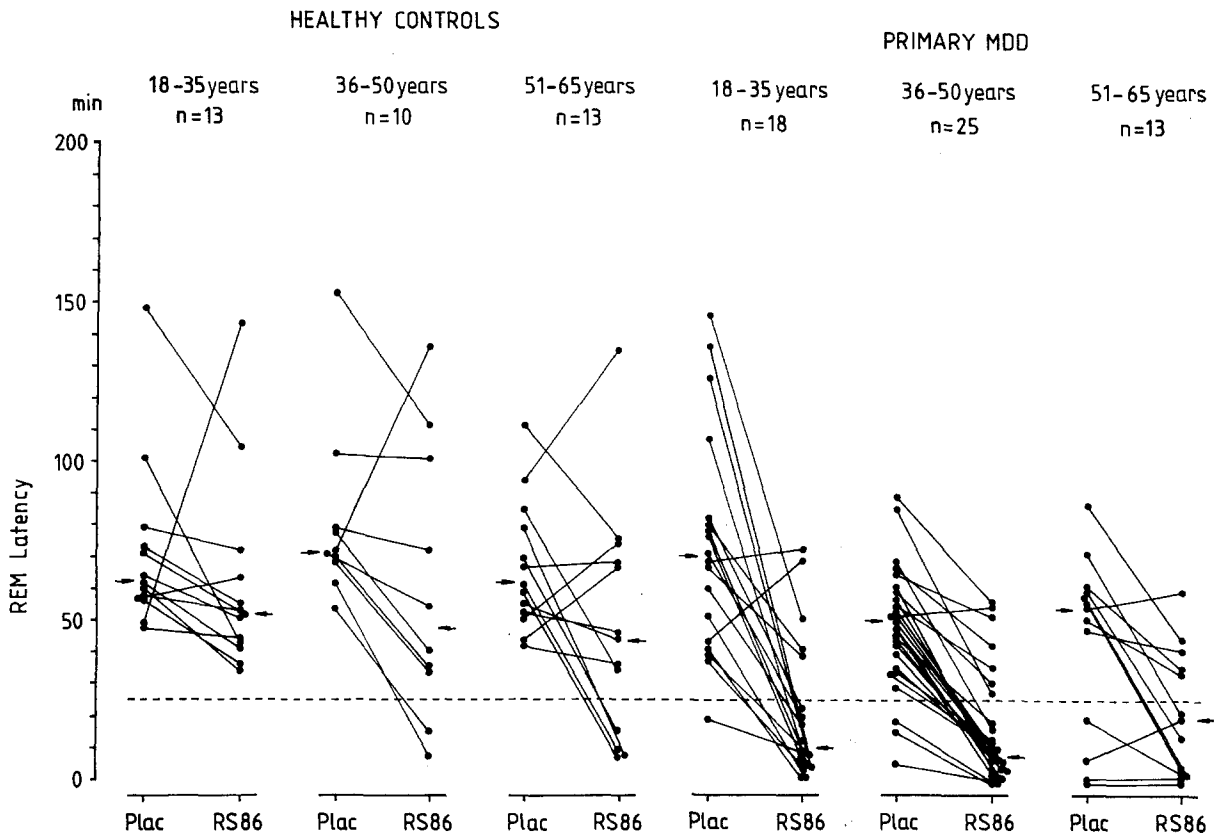

Fig. 3. Influence of 1.5 mg RS 86 on REM latency compared to placebo in healthy subjects and depressed patients for three different age groups. *Dashed line:* REM latency threshold of 25 min. *Arrows* mark medians

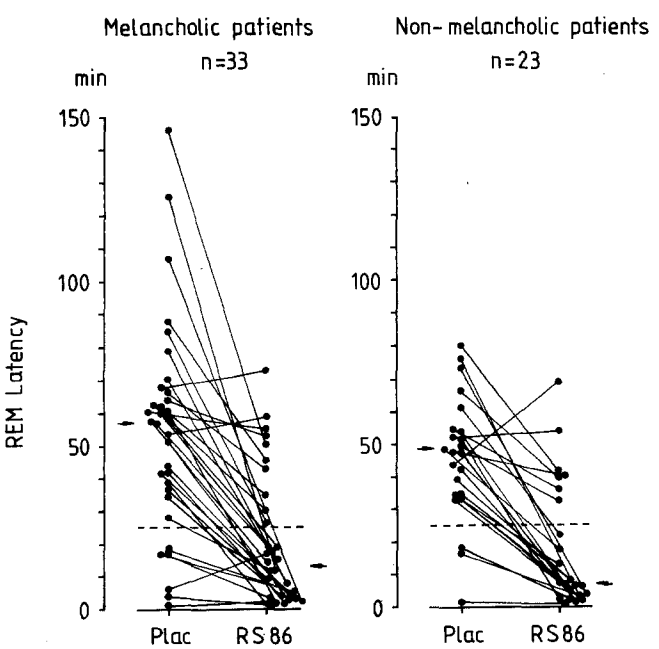

Fig. 4. Influence of 1.5 mg RS 86 in comparison to placebo on REM latency in 33 melancholic and 23 nonmelancholic patients. *Dashed line:* REM latency threshold of 25 min. *Arrows* mark medians

ferences in both groups confirm earlier results (see, for example, Williams et al. 1974) that the sleep of males is more shallow than that of females. The meaning of sex differences in SWS is far from clear. Reynolds et al. (1990), for example, speculated that greater lifetime alcohol consumption may explain the reduction of SWS in males. Dijk et al. (1989) on the other hand, suggested that extracerebral factors like skull thickness (which differs between males and females) may generate artifactual differences with respect to SWS between males and females.

Comparison of healthy subjects and depressed patients when controlling for age revealed significant differences in the expected direction for sleep continuity with impaired indices in the depressed group. Contrary to our expectation no difference concerning SWS was noted, which is in agreement with results of a recent meta-analysis of sleep studies in depression (Knowles and MacLean 1990). The fact that no differences between the slow wave sleep of depressed patients and healthy controls were noted is of high theoretical importance. These data contradict the assumptions of the two-process model of sleep and sleep regulation for depression (Borbély and Wirz-Justice 1982) which postulates reduced slow wave sleep in depressives compared with healthy subjects. Although there was a parallel decline in slow wave sleep, REM latency decreased more strongly in depression compared with healthy controls, thus refuting the assumption that the advance of REM sleep is an epiphenomenon of decreased slow wave sleep. However, it has to be mentioned that the sleep analysis in the present study was performed on a vi-

sual basis and not by frequency analysis, on which the original assumptions of the two-process model are based. As Brunet et al. (1988) have demonstrated that visually scored amounts of SWS and delta power as evaluated by frequency analysis are highly correlated, our results can at least be taken as indirect hints against the basic assumption of the two-process model of sleep and its implications for depression. Two studies (Mendelson et al. 1987; Van den Hoofdakker and Beersma 1988) which applied frequency analysis to sleep data of depressed patients did not detect differences in the amount of delta power between depressed patients and healthy controls, thus contradicting directly the assumption that generation of delta waves is impaired in depression compared to healthy subjects.

Almost all REM sleep parameters in the depressed patients were altered as expected. Differences in REM latency became more obvious with increasing age. On the other hand, first REM density and total REM density were highly significantly altered throughout the whole age range, suggesting that REM density may be even better suited as a biological marker for depression. Data from animal experiments (for example Velazquez-Moctezuma et al. 1990) indicated that the components of the complex phenomenon of REM sleep, i.e. EEG desynchronisation, rapid eye movements, muscle atonia, may be under the differential control of nicotinic and muscarinic (M1, M2) receptors. Studies with direct muscarinic agonists and cholinesterase inhibitors in humans have also shown a differential profile of action on the components on REM sleep (Riemann et al., in press). Maybe future research will allow combination of selective alterations of the components of REM sleep with specific neurobiological mechanisms.

In a next step of the data analysis sleep data of patients with or without melancholia were compared. As both groups differed on a variety of demographic and clinical variables, first of all the impact of these variables (severity of depression, number of depressive episodes, duration of the current episode and age at first onset of depression) were analysed. When controlling for age, none of these variables exerted significant influences on sleep data. Therefore sleep data of melancholic and nonmelancholic patients were compared using only age as a covariate. With this analysis, no differences were found between the groups. Our data contradict the assumed differential-diagnostic nature of sleep variables for the endogenous/melancholic subtype of depression (Feinberg et al. 1982; Feinberg and Carroll 1984; Giles et al. 1987; Kerkhofs et al. 1988; Rush et al. 1982) but are in line with previous reports from our group in smaller samples (Berger et al. 1982, 1983b). The discrepancy between our data and those from other studies may be due to the control of the confounding influence of age and severity of depression. In the studies by Feinberg, patients with endogenous depression were on average 15 years older than the nonendogenous group and no statistical age correction was applied. In the study by Giles et al. (1987), inpatients with endogenous depression were compared with nonendogenous outpatients, with the latter group being probably less depressed. When including a broad range of depressive patients whose depression ranges from minor to severe forms, severity of depression also influences sleep (Akiskal et al. 1982; Cartwright 1983; Spiker et al. 1978; Thase et al. 1986). A comparison of more severely depressed endogenous inpatients with less depressed nonendogenous outpatients may then lead to artifactual differences between the groups.

Summarizing, the data of the present study clearly contradict the assumption that sleep variables, especially REM sleep parameters, may help to differentiate melancholic from nonmelancholic depression. In contrast to earlier studies, differences in REM sleep regulation between both groups were far from being significant. Insofar, the results support the assumption of a continuous (Kendell 1978) rather than a strict dichotomous distribution (Carney et al. 1965) of depressive disorders.

In the second part of the study the impact of the cholinergic agonist RS 86 on REM latency was analysed. It was demonstrated that cholinergic stimulation provoked a highly significant shortening of REM latency in depression compared to the healthy state. As depicted in Fig. 3, younger depressed patients (18–25 years) who showed placebo REM latencies similar to those of healthy controls, following cholinergic stimulation displayed a clear-cut difference from the respective values of control subjects. Extrapolating from these data is seems plausible to assume in younger depressives too an increased – but still subthreshold – functional activity of the REM sleep triggering cholinergic transmitter system (see also our previous results, Lauer et al. 1990). Pharmacological stimulation with a cholinergic agonist like RS 86 seems to be able to perturb the threshold and demask a latent neurotransmitter imbalance.

The REM sleep response to cholinergic stimulation decreased with age in depression. A parsimonious explanation for this phenomenon may be a "ceiling" effect, as baseline REM latency in depressives decreased with age, perhaps thus leading to a decrease in reactivity to cholinergic challenge. There may be, however, also alternative approaches to explain the decrease in reactivity of the REM sleep system with increasing age in depression. Assuming that decreased placebo REM latencies in older depressed patients reflect higher central nervous cholinergic activity, additional cholinergic stimulation instead of leading to a still earlier onset of REM sleep may provoke arousal and thereby prevent REM sleep. In earlier studies with physostigmine for example, the onset of REM sleep was advanced in healthy subjects, but not in depressed patients, who awoke more frequently in the non-REM period subsequent to cholinergic challenge (Berger et al. 1983a). That increased cholinergic tone beyond a certain level does not provoke REM sleep but leads to arousal is also supported by animal data (Prospero-Garcia et al. 1993).

The data for RS 86 support the reciprocal interaction model of non-REM-REM regulation, which suggests a primary role for cholinergic neurons in the brain stem in the triggering and maintenance of REM sleep (McCarley 1982). Furthermore the data of heightened reactivity in the depressed patients are in favour of the cholinergic-aminergic imbalance model of depression (Janowsky et

al. 1972). The age dependency of REM latency during baseline sleep and following cholinergic stimulation may argue for an interaction of age with the assumed central nervous transmitter imbalance between cholinergic and aminergic systems in depression. A disturbance of REM sleep may become manifest during baseline sleep as a consequence of a neurotransmitter dysfunction and increasing age, additional cholinergic stimulation may help to demask an imbalance of neurotransmission on the level of REM sleep regulation in younger patients. In earlier studies we have been able to show that the REM sleep response to a cholinergic agonist is much more pronounced in depression than in healthy controls or in patients with eating and anxiety disorders as well as personality disorders (Berger et al. 1989; Gann et al. 1992; Lauer et al. 1988, 1990). In a recent study in patients with schizophrenia an almost equally strong REM sleep response was, however, observed (Riemann et al. 1991). In schizophrenia, however, these results may be interpreted as the consequence of a disturbance of the balance between dopaminergic and muscarinic systems (Tandon et al. 1988; Tandon and Greden 1989).

Summarizing, the results stress the important impact of age on sleep in healthy subjects and depressed patients and warrant a thorough control of this variable in psychiatric sleep research. REM sleep variables like REM latency and REM density seem to stigmatize the sleep of depressives as opposed to healthy subjects. Cholinergic stimulation accentuates the differences between depressives and healthy volunteers. From that point of view, baseline and challenge studies of the sleep EEG seem to provide a reliable and valid method of measuring and/or provoking biological abnormalities which are well understood from the underlying neurobiological processes. In comparison with the states of wakefulness and NonREM sleep, the neurobiological mechanisms involved in the generation and maintenance of REM sleep are by far better understood (Hobson and Steriade 1986; Steriade and McCarley 1990). Assuming that the cholinergic cell groups in the brain stem that govern REM sleep are interrelated and interconnected with cholinergic neuronal networks in higher brain areas, for example in the limbic system (Hobson et al. 1986), studies on REM sleep regulation during baseline and after cholinergic stimulation may serve as indicators of central nervous processes involved in the regulation of affective states.

Further studies will be necessary to clarify the state/trait question of REM sleep abnormalities. Data on this issue unfortunately do not allow decisive conclusions. Some studies found a persistence of REM sleep abnormalities beyond the acute state of depression, whereas others did not (for overview, see Riemann and Berger 1989). Concerning the question of vulnerability for affective disorders and REM sleep abnormalities, a first study by Schreiber et al. (1992) indicated that high-risk probands for depression (i.e. first-degree relatives of depressed patients) also displayed REM sleep abnormalities to a certain degree following cholinergic challenge with RS 86. Future studies will also have to clarify the question whether REM sleep abnormalities during baseline and after cholinergic stimulation are of clinical value concerning, for example, differential-therapeutic strategies for patients with affective disorders.

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