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Effect of flumazenil-augmentation on microsleep and mood in depressed patients during partial sleep deprivation

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Abstract

The antidepressive effect of sleep deprivation (SD) in depressed patients disappears after sleep of the recovery night and after early morning naps. Both can provoke a rapid relapse into depression in SD-responders. In addition, the occurrence of short episodes of sleep (termed microsleep, MS) during partial SD (PSD) is associated with SD-nonresponse, suggesting that MS during the time awake may be related to relapse or PSD-nonresponse.

The GABA-benzodiazepine receptor antagonist flumazenil augments vigilance and reduces NonREM-sleep pressure in early morning recovery sleep in volunteers after SD.

Therefore, in this study 27 patients with major depression were subjected to a PSD. In a double blind randomized design either flumazenil or placebo was orally applied during PSD in order to examine whether the application of flumazenil reduces sleep propensity and thus, increases antidepressant efficacy of PSD.

EEG was registered continuously for 60 h by a portable device for the assessment of microsleep episodes at baseline and during PSD. Flumazenil application significantly suppressed frequency and total amount of MS. While the antidepressant efficacy of PSD was not different between flumazenil and placebo during PSD, the subjective mood improved after the recovery night in patients treated with flumazenil.

It is concluded that GABAergic mechanisms are involved in the regulation of MS during PSD, which may be related to a mood stabilizing effect after the recovery night. However, the mechanisms underlying the association between the occurrence of MS during PSD and mood variation have to be further clarified.

Keywords: Sleep deprivation; Major depression; Polysomnography; Microsleep; Flumazenil; GABA

1. Introduction

Sleep deprivation (SD) which acts rapidly within hours without serious side effects is an efficient antidepressant therapy in about 60% of depressed patients (Wu and Bunney, 1990). Beside SD of the total night, partial sleep deprivation (PSD) in the second half of the night has also shown substantial antidepressant effects in patients with major depression (Giedke and Schwarzler, 2002; Wirz-Justice et al., 2005).

A major shortcoming of sleep deprivation therapy (total and partial SD), however, is that the antidepressant effect in SD-responders usually lasts only until the recovery night, as almost all SD-responders relapse into depression after the first night of sleep following SD. Repeated application of SD (up to three times a week) and concomitant antidepressant medication, may stabilize the antidepressant effect of SD (Holsboer-Trachsler and Ernst, 1986).

In addition, short naps during SD are able to provoke a rapid relapse into depression in patients responding to SD

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(Knowles et al., 1979). A systematic series of studies on the effect of naps on SD response revealed that naps which occurred in the early morning hours were significantly related to a worsening of mood in contrast to afternoon or evening hours regardless of sleep-EEG characteristics (REM or NonREM sleep) during the naps (Wiegand et al., 1987; Wiegand and Berger, 1989; Wiegand et al., 1993).

These findings suggest that sleep per se (sleep of the recovery night and sleep of naps during daytime) may itself counteract the antidepressant efficacy of SD and/or exert depressogenic effects.

To further examine the relationship between sleep and mood response we performed a first study which included a continuous EEG-registration during partial sleep deprivation (PSD) in order to assess short phases of daytime sleepiness by EEG registration (termed microsleep (MS)), which may not be recognized by the patient or by the nursing staff in relation to mood response (Hemmeter et al., 1998). The data of this first pilot study showed, that PSD increases daytime EEG sleepiness and that the accumulated amount of short sleep phases during sleep deprivation (MS) evaluated by EEG, predominantly until noon, were closely related to PSD nonresponse and impaired cognitive performance (Hemmeter et al., 1998). In addition, the short episodes of sleep consisted almost exclusively of NonREM-sleep and the cumulated amount of daytime sleep was inversely related to the amount of slow wave sleep in the recovery night. Predominantly patients who already presented with daytime sleep episodes before PSD displayed increased daytime sleep during PSD (Hemmeter et al., 1998). Therefore, a disposition for the expression of increased daytime sleepiness was hypothesized, which seemed to be related to PSD response.

Based on these findings we concluded: (1) that EEG assessed daytime sleep during PSD may affect mood response, (2) that the occurrence of daytime MS is predominantly involved in NonREM-sleep regulation due to an increased NonREM sleep pressure, and (3) that the reduction of daytime sleep by a reduction of NonREM sleep pressure at times which are critical for SD-response may increase the antidepressant efficacy of PSD. In addition, we suggested that the occurrence of daytime sleep episodes during PSD might be related to the occurrence of daytime sleep already presented at baseline.

Studies with GABAergic substances have shown that GABAergic neurotransmission is deeply involved in the regulation of sleep and wakefulness affecting predominantly NonREM sleep (Lancel, 1999).

The benzodiazepine receptor antagonist flumazenil is able to reverse sleepiness (Ziegler et al., 1986; Lavie, 1987; Steiger et al., 1994) at regular sleep times and during the day (Freye and Fournell, 1988). Furthermore, the application of flumazenil has been shown to antagonize memory impairment induced by benzodiazepine receptor agonists in healthy subjects (Pregler et al., 1994; Wesensten et al., 1995). This is accompanied by alerting effects on EEG frequency bands, indicating increased arousal and vigilance (Freye and Fournell, 1988).

Finally, we showed, that in healthy sleep deprived subjects, flumazenil is able to reduce daytime sleepiness and NonREM-sleep pressure in the early morning which may be the crucial time for mood response in depressed patients during SD (Seifritz et al., 1995).

Based on these findings flumazenil is a promising candidate to maintain wakefulness during PSD via a reduction of NonREM sleep pressure.

Flumazenil may therefore counteract sleep pressure during PSD and thus prevent the occurrence of sleep at times when sleep seems to be most detrimental. Thus, flumazenil should augment the antidepressant efficacy of PSD by reducing the expression of MS during sleep deprivation.

Therefore, in this study we examined the effect of flumazenil on the occurrence of MS and mood response during PSD. Based on the findings of our previous study, we were additionally interested in the modulating effect of MS at baseline on the occurrence of MS during PSD and mood response.

2. Methods

In order to test the hypothesis that reduction of daytime sleepiness expressed as microsleep is related to an augmentation of the antidepressant efficacy of PSD we designed the following placebo controlled study.

In a combined within/between subjects design which included a PSD in depressed patients (within subjects factor), half of the patients received flumazenil by oral application, half of the patients placebo (between subjects factor) under double-blind conditions. During the whole study EEG was registered continuously for 60 h by an ambulatory device allowing the evaluation of sleep EEG (before and after PSD) and MS episodes (at baseline and during PSD).

2.1. Study population

Twenty-seven inpatients (15 men, 12 women) age 44.1 ± 9.1 years (mean $\pm$ SD), range 22–59 years with the diagnosis of major depression according to the SCID (Wittchen et al., 1987) participated in the study, who all were naïve to SD-therapy. The flumazenil group consisted of 8 men and 6 women, age 42.0 ± 10.1 years, in the placebo group were 7 men and 6 women, age 46.1 ± 8.0 years.

Severity of depression was evaluated by the 21-Item version of Hamilton depression rating scale (HDRS; Hamilton, 1967). In addition, self-rating of mood was assessed with a visual analogue scale (VAS). For the inclusion into the study a HDRS-score of $\geqslant 15$ was required. In all patients clinical and laboratory examinations including clinical chemistry, blood count, electroencephalogram (EEG) and electrocardiogram (ECG) were performed to rule out any other somatic disease.

For comparison with our previous study (Hemmeter et al., 1998) all patients were treated with a constant dosage of a trimipramine monotherapy (200 mg/d at 7.15 p.m.) for at least one week before entering the study without any other medication. The reason for the selection of trimipramine is that it does not disturb sleep architecture and especially does not suppress REM sleep (Ware et al., 1989; Wiegand and Berger, 1989; Sonntag et al., 1996), which allows an evaluation of the effects of flumazenil and sleep deprivation on sleep architecture.

All patients who participated in this study were not allowed to go to bed or to sleep at any time during baseline and PSD. They participated in the regular activities on the special ward for affective disorders, such as ergotherapy and sports. All subjects were completely monitored by the nursing staff, no periods of obvious sleep have been detected.

The study was approved by the local ethics committee, written informed consent was obtained from all patients before entering the study.

One patient of the placebo group dropped out, because he refused to continue the EEG registration during PSD. Therefore, only 27 of the initially planned 28 patients could be included into data analyses.

2.2. Study design

The study was designed as a placebo controlled double blind combined within/between subjects design. All patients underwent a continuous 60-h EEG recording for the assessment of baseline sleep EEG, a sleep EEG immediately before PSD and a sleep EEG of the recovery night. In addition, the EEG during the entire waketime before and during PSD was recorded in order to assess the amount of MS during wakefulness (see Fig 1).

Beside the continuous EEG registration at day 1 (baseline before PSD), day 2 (during PSD) and day 3 (day after the first sleep night after PSD) a Hamilton depression rating scale (Hamilton, 1967) was applied. Subjective psychopathology was scored using a visual analogue scale of mood.

During PSD half of the patients were additionally treated with 30 mg of flumazenil (oral application) from 1.30 a.m. in 2 h intervals until 10.30 a.m. according to the results of the nap studies (Wiegand et al., 1993; Riemann et al., 1993) and the previous microsleep study in depressed patients which demonstrated that MS within this time interval may be decisive for antidepressant response (Hemmeter et al., 1998). The second half of the patients received placebo at the same times.

2.3. Outcome measures

PSD-response was assessed by 6-Items of the Hamilton Depression Rating Scale covering the Items depressive mood, feelings of guilt, working and leisure activities, depressive inhibition, psychological symptoms of anxiety, somatic symptoms (Bech et al., 1975). An improvement of at least 40% from baseline was required to determine response.

In addition, a visual analogue scale (VAS) of mood and tiredness (total length = 10 cm, poles extremely bad mood vs. extremely good mood, extremely tired vs. extremely awake) was completed by patients in 2-h intervals during PSD in order to detect changes in subjective mood during PSD. In addition, the VAS was administered the day

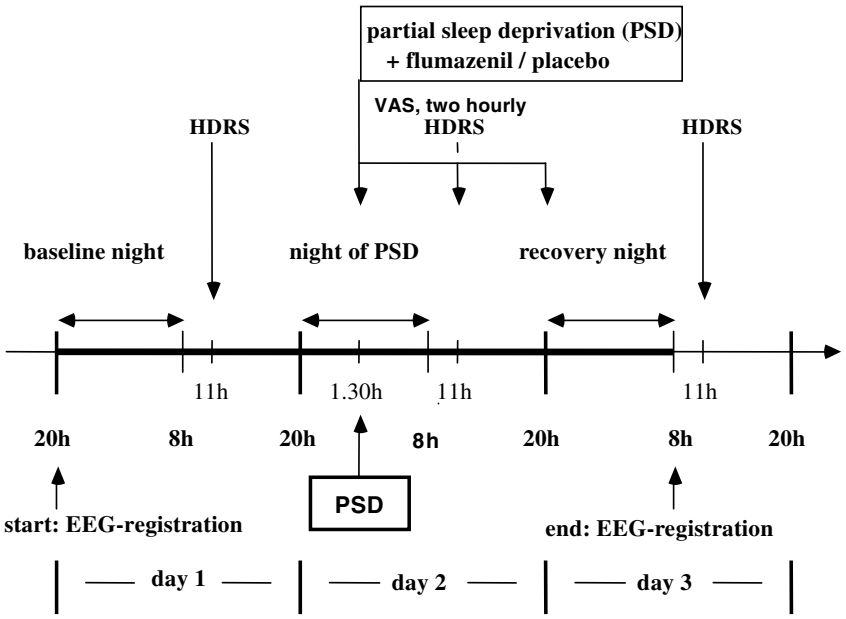

Fig. 1. Study design. VAS, visual analogue scale, HDRS, Hamilton depression rating scale, PSD, partial sleep deprivation.

before PSD both in the morning (8 a.m.) and in the evening (8 p.m.) and after the recovery night (8 a.m.).

2.4. Quantification of microsleep (MS)

Sleep was registered using a portable ambulatory device (EEG: C3-A2, left and right EOG, submental EMG). To evaluate sleep episodes during the day, MS was scored according to the criteria of Rechtschaffen and Kales (1968), with the exception in the length of the sleep sequences: for the purpose of the previous and this study, all sequences lasting 15 or more seconds (as opposed to 30 or more seconds) and fulfilling the criteria of sleepiness (=stage 1), sleep (=stages 2, 3, 4 and REM-sleep) were defined as MS. The total amount of MS and the amount of the different sleep entities were quantified in minutes and number of MS episodes. To exclude biases resulting from a single rater, all sequences were evaluated by two experienced raters and ambiguous sequences have been printed on EEG paper and additionally evaluated by an independent third rater.

2.5. Statistics

Due to the design of the combined within/between subjects design of the study analyses of variance with the between subjects factor (flumazenil vs. placebo) and the within subjects factor (before and during/after PSD) were performed on the dependent variables of mood and MS.

In case of significant effects of group, time or interaction effects post hoc analyses by *t*-tests were performed.

Correlative associations were assessed by Pearson product moment correlation coefficient.

Nonparametric statistics were used when appropriate.

An alpha level of $\alpha = .05$ was regarded as significant, an $\alpha \leqslant .10$ as trend.

3. Results

3.1. The effects of flumazenil on microsleep during partial sleep deprivation

The ANOVA with treatment (flumazenil vs. placebo) as between subjects factor and time (baseline vs. PSD) as within subjects factor revealed significant effects of time $F = 4.49$, $p < .05$ and interaction ($F = 4.17$, $p < .05$). In patients treated with placebo a significant increase of frequency and cumulative amount of MS compared to baseline was observed. This increase was not present in patients treated with flumazenil (*t*-tests, $p < .05$, Fig. 2, Table 1).

3.2. Antidepressive response in the total group

In the total group of patients severity of depression assessed by HDRS 21-scale was 25.59 ± 7.81 at baseline and was significantly reduced after the recovery night (18.37 ± 7.65).

The 6-Item Hamilton score, which has been applied to assess the change of depression also during PSD, resulted in a significant improvement during PSD (*t*-test vs. baseline, $t = 4.20$, $p < .001$) and after the recovery night (*t*-test vs baseline, $t = 3.87$, $p < .001$).

Based on the HDRS-6-change the response criteria of 40% improvement vs. baseline revealed a response rate of 14 responders (51.9%) for all 27 patients.

After the recovery night 10 patients still presented with an at least 40% improvement vs. baseline (before PSD) reflecting a response rate of 37%.

3.3. The effects of flumazenil vs. placebo on depressive mood

At baseline HDRS-6 values were increased by trend in the placebo group. Therefore, an ANCOVA with HDRS-6

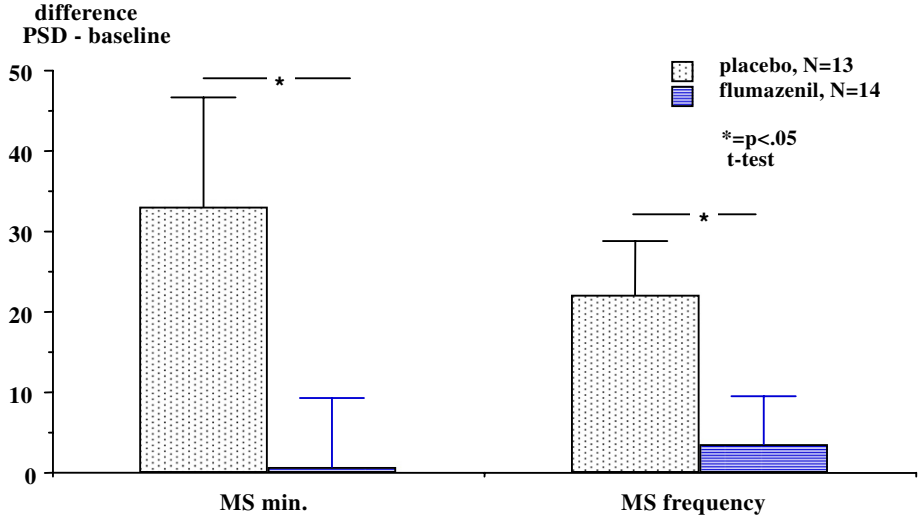

Fig. 2. Frequency and cumulative amount of microsleep (MS) before and during partial sleep deprivation (PSD) in patients with additional flumazenil- or placebo treatment – differences between PSD and baseline.

Table 1
Microsleep (MS) at baseline (cumulative amount, min) before and during partial sleep deprivation in patients with additional flumazenil- or placebo treatment

Microsleep variables (min)	Before PSD (baseline)	During PSD	ANOVA reap. measurement			
				df	*F*	*p*
Total sample (N = 27)						
Microsleep (MS) total			Group	1	0.08	n.s.
Placebo, $N = 13$	4.51 ± 7.09	37.51 ± 51.76[a]	Time	1	4.49	.05
Flumazenil, $N = 14$	17.80 ± 33.99	18.42 ± 27.97	Int. act.	1	4.17	.05
MS 1.30 – 6.00 h			*t*-Test			
Placebo, $N = 13$		15.50 ± 27.14	$t = 1.61$, $p =$ n.s.			
Flumazenil, $N = 14$		3.46 ± 6.95				
MS 6.00 – 12.00 h			Group	1	0.32	n.s.
Placebo, $N = 13$	0.35 ± 1.04	11.46 ± 20.59	Time	1	2.96	.10
Flumazenil, $N = 14$	3.39 ± 12.52	4.51 ± 9.40	Int. act.	1	1.97	n.s.
MS 12.00 – sleep onset			Group	1	0.46	n.s.
Placebo, $N = 13$	4.16 ± 6.25	10.54 ± 14.43	Time	1	0.15	n.s.
Flumazenil, $N = 14$	14.41 ± 27.68	10.45 ± 26.51	Int. act.	1	2.74	n.s.
Patients with MS at baseline ($N = 11$)						
Placebo $N = 6$	9.78 ± 7.68	69.34 ± 63.30[c]				
Flumazenil, $N = 5$	49.84 ± 41.94[b]	23.28 ± 45.86				

[a] $p < .05$, *t*-test vs. baseline.
[b] $p < .05$, *U*-test between groups.
[c] $p < .05$, Wilcoxon-test vs. baseline.

Table 2
Ratings of depression before, during and after partial sleep deprivation in patients with flumazenil vs. placebo treatment

	Before PSD (baseline)	During PSD (day 1)	After PSD (day 2)	ANOVA reap. measurement			
					df	F	p
Ratings of depression							
HDRS 21-Scale				Group	1	1.93	n.s.
Placebo, $N = 13$	27.46 ± 6.81		19.92 ± 8.17	Time	1	27.38	.01
Flumazenil, $N = 14$	23.86 ± 6.40		16.93 ± 7.12	Int.act.	1	0.05	n.s.
HDRS 6-Scale[d]				Group	1	0.39	n.s.
Placebo, $N = 13$	11.54 ± 2.70	6.77 ± 4.38	8.46 ± 3.95	Time	1	0.03	n.s.
Flumazenil, $N = 14$	9.07 ± 3.79[a]	7.00 ± 4.11	6.93 ± 3.79	Int. act.	1	1.16	n.s.
VAS mood (morning)[d]				Group	1	0.12	n.s.
Placebo, $N = 13$	4.50 ± 1.26	4.00 ± 1.37	4.27 ± 1.35	Time	1	5.85	.02
Flumazenil, $N = 14$	3.50 ± 1.33[a]	3.86 ± 1.67	4.96 ± 2.15[b,c]	Int. act.	1	6.87	.03
VAS mood (evening)				Group	1	0.82	n.s.
Placebo, $N = 13$	4.30 ± 1.56	4.65 ± 2.02	No rating	Time	1	0.05	n.s.
Flumazenil, $N = 14$	5.21 ± 1.60	4.75 ± 1.50	Available	Int. act.	1	1.49	n.s.

[a] $p < .10$, *t*-test between groups.
[b] $p < .01$, *t*-test vs. baseline.
[c] $p < .05$, *t*-test vs. day 1.
[d] ANCOVA with baseline as covariate.

values at baseline as a covariate was performed in order to detect significant differences between flumazenil and placebo during PSD and after the recovery night. No significant effects of treatment (flumazenil vs. placebo) or interaction (treatment × time) were observed for this variable (Table 2).

3.4. Response rate in both groups

Under flumazenil administration six patients responded to PSD (according to an improvement of at least 40% in the HDRS-6 Item scale), in the placebo group a response was observed in eight patients (61.5%). At day 2 after the recovery night in the placebo group only four patients showed a 40%-improvement compared to baseline (30.8%), while in the flumazenil group 6 patients (42.9%) were still improved by at least 40%.

3.5. Subjective mood under placebo and flumazenil

For the assessment of the effects of flumazenil on subjective mood (VAS mood ratings) during PSD an ANOVA

with treatment (flumazenil vs. placebo) as between subjects factor and time (2 hourly assessed ratings of subjective mood) has been performed, which did not reveal any significant treatment- or interaction-effects (treatment: df = 1, $F = 0.16$, p = n.s., time df = 10, $F = 1.58$ p = n.s., interaction treatment × time: df = 10, $F = 0.71$, p = n.s.)

In addition, the inclusion of subjective mood ratings at baseline (evening values), during PSD (morning and evening) and after the recovery night (morning) did not reveal significant differences between groups during PSD (Table 2).

However, there was a significant interaction effect of treatment and time (PSD vs. recovery night) for the mood rating in the morning, which is due to a significant increase of mood in the morning after the recovery night in patients treated with flumazenil compared to the corresponding mood ratings (at 8 a.m.) of the placebo group (ANCOVA, interaction flumazenil vs. placebo × $F = 6.87$, $p = .03$, t-test, $p < .05$, Table 2).

3.6. Correlational analyses

In the placebo group MS during PSD, predominantly the cumulative amount of MS expressed in the first half of PSD until noon was closely correlated with subjective mood at the end of PSD (4 p.m. $r = -.59$, $p < .05$), 8 p.m. ($r = -.57$, $p < .05$), showing that patients with a low amount of MS during PSD were in a better mood at the end of PSD compared to patients showing a high amount of MS.

No correlations between the cumulative amount of MS during PSD and subjective mood were detected in patients with flumazenil application during PSD.

3.7. Additional effects of microsleep at baseline on daytime sleep (MS) during PSD

In this study only 11 of the 27 patients presented with short daytime sleep episodes (MS) at baseline (6 men, 5 women, age 41.82 ± 9.90 years). In 16 patients (9 men, 7 women; age 45.50 ± 8.77 years) at baseline no MS could be detected.

First of all, patients without MS at baseline (7 patients (46.2%) in the placebo group, 9 patients (64.3%) in the flumazenil group) did not increase significantly with MS during PSD without differences between both groups (10.22 ± 11.70 min (placebo) vs. 15.72 ± 14.05 min (flumazenil), U-test = n.s.).

Differences however, were evident in patients who already presented with MS at baseline.

These patients already differed significantly at baseline (Pl, $n = 6$: 9.78 ± 7.68 min vs. Flu, $n = 5$: 49.84 ± 41.94 min, $n = 5$, U-test, $p < .05$, Table 1).

During PSD the placebo group significantly increased with MS (predominantly in the first half of the day) ($p < .05$ Wilcoxon test), while in flumazenil treated patients MS decreased to from 49.84 ± 41.94 min to 23.28 ± 45.86 min (Fig. 3, Table 1).

Due to the small sample size no parametric testing was performed.

During the time of the flumazenil application MS could be almost completely suppressed in patients who presented with MS already at baseline In contrast, patients without MS at baseline showed some MS under flumazenil application during this time span which was descriptively larger than that of patients of the placebo group (patients without MS at baseline and placebo treatment) (Fig. 3).

3.8. Response rate in the subgroups

Response (according to the HDRS-6 improvement of at least 40% at the day of PSD) was best in patients with no MS at baseline treated with placebo (71.4%) followed by placebo treated patients with MS at baseline (50%) and by patients treated with flumazenil without MS at baseline (44%) and with MS at baseline (40%).

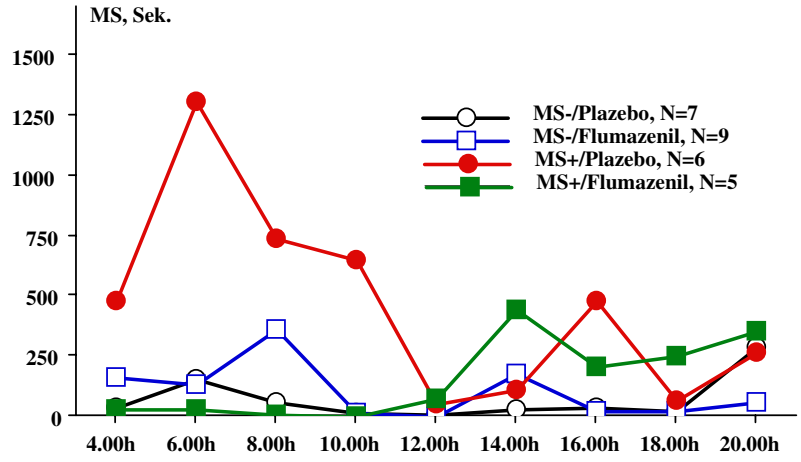

Fig. 3. Microsleep during partial sleep deprivation under flumazenil or placebo application in patients with (MS+) and without microsleep (MS−) at baseline.

4. Discussion

The main finding of this study is that the application of flumazenil during PSD significantly reduced MS in depressed patients, predominantly in the first part of PSD, the time interval which corresponds closely with the time when flumazenil was applied.

The application of flumazenil did not have an effect on antidepressant response during PSD, but it improved subjective mood in the morning after the recovery night.

The effects of flumazenil and PSD were substantially influenced by the occurrence of MS at baseline. In particular, MS at baseline was closely related to the development of MS in the placebo group during PSD, as patients who already presented with MS at baseline exhibited a significant increase in MS. This was not the case in the flumazenil group, in which MS during PSD could be almost completely suppressed.

The substantial suppression of MS by flumazenil confirms the results of previous studies on the effects of flumazenil on sleep and vigilance showing that flumazenil is able to reduce daytime sleepiness (Lavie, 1989; Patat et al., 1994) and homeostatic NonREM sleep pressure reflected by a more shallow NonREM sleep (Gaillard and Blois, 1989; Steiger et al., 1994; Mendelson, 2000; Ozone et al., 2001). In addition, the conclusions drawn from the findings of a previous study of our group (Seifritz et al., 1995) are confirmed. They showed that sleep pressure of early morning recovery sleep after sleep deprivation can be reduced and thus also MS, predominantly in the early morning hours, may be suppressed by the application of flumazenil.

The second hypothesis of this study was not confirmed. The suppression of MS by flumazenil did not lead to an augmentation of the response to PSD.

While flumazenil was able to substantially reduce MS compared to placebo, subjective mood and severity of depression assessed by the 6 Item Hamilton score during PSD, were not different under placebo.

An explanation for this result may be provided by the fact that the relationship between the occurrence of MS and mood, which has been detected in our first study (Hemmeter et al., 1998) and confirmed recently by another group (Wiegand et al., 2002), was only present in the placebo group of this study but not in patients treated with flumazenil. This finding, that in placebo treated patients mood and cumulative amount of microsleep were correlated, while under flumazenil treatment no correlation was observed, suggests that flumazenil may induce a dissociation between mood and MS during PSD.

A further explanation for the lack of an efficacy of flumazenil may be due to the heterogenity of the occurrence of MS under baseline conditions. Nine of the 14 patients who were treated with flumazenil did not present with MS at baseline, while the five patients with MS at baseline presented with much more MS than the corresponding placebo group. The effect on MS seen in flumazenil treated patients, therefore, is due to the substantial suppression of MS in five patients.

According to the results of our first study, patients with a low amount of MS before PSD were more likely to respond to PSD than patients with a high amount of MS at baseline. This result is also observed in the present data. 71.4% of patients without MS at baseline vs. only 50% of patients with MS in the placebo group responded to PSD.

Therefore, in the five patients with MS at baseline of the flumazenil group we expected an increase with MS during PSD without a mood response, while for the nine patients without MS a mood response should have been more likely.

Interestingly, in both groups treated with flumazenil (no MS and MS before PSD) the response rate was comparable and lower than in the placebo group.

In contrast to the substantial suppression of MS by flumazenil in patients who presented with MS at baseline, patients without MS at baseline showed MS episodes under the application of flumazenil, which were descriptively longer and more frequent than in patients of the corresponding placebo group (without MS at baseline). After cessation of flumazenil only a very small amount of MS was observed in this group.

This surprising effect may be explained by a possible partial agonistic effect, which has been previously described for flumazenil (Gaillard and Blois, 1989; Steiger et al., 1994).

Another explanation for this finding is provided by the observation of different effects of flumazenil on sleepiness dependent in the dosage and vigilance state of the subjects (Lavie, 1989). In this study flumazenil in a higher dosage was able to provoke arousal reactions and increased vigilance in sleepy subjects and flumazenil in a lower dosage led to sleepiness and tiredness in vigilant subjects. Therefore, patients with MS and without MS at baseline who may differ in the vigilance state, may respond differently to flumazenil application due to the expression of MS comparable to the different response reported in the study of Lavie (1989).

The different effects of flumazenil on MS in patients with a low and a high amount of MS at baseline, thus, may be explained by the antagonistic and the partial agonistic effects of flumazenil dependent on vigilance state.

A further explanation for the dissociation of MS and mood under flumazenil treatment may be provided by the well known antidepressant effects of GABA-agonistic substances such as alprazolam (Flugy et al., 1992), and fengabine (Lloyd et al., 1989). In addition, there are further data on the relation between the GABAergic system and depression, such as low GABA-levels in plasma and liquor of depressed patients (Petty et al., 1995) which are associated with an upregulation of GABA-A receptors in the frontal cortex (Cheetham et al., 1988). These findings provide the basis of the GABAergic theory of depression (see Lloyd et al., 1989).

The GABA-antagonistic effect of flumazenil, which is clearly demonstrated by the suppression of MS, could, therefore, in parallel induce a worsening of mood, which counteracts the positive effects on mood exerted by the suppression of MS. This consideration is supported by the effects of flumazenil contrasting to those of benzodiazepines on subjective mood in humans (Duka et al., 1986; Higgitt et al., 1986) and depression like behaviour in animals (Miyata et al., 2005) and may explain the observed discoupling of MS and mood during PSD by flumazenil in this study.

After the recovery night, however, subjective mood increased significantly only in the flumazenil group. In addition, this response was descriptively more stable in PSD-responders under flumazenil.

These findings suggest that flumazenil may not augment, but rather contribute to the stabilization of the effect of PSD on mood response. The study sample, however, was too small to definitely corroborate this conclusion, because the detection of a statistical significance based on the observed differences would require a much larger study sample.

Concerning this explorative hypothesis, however, it is very unlikely that direct GABAergic effects of flumazenil are responsible for this observation, as the half life of flumazenil is very short. Therefore, an indirect effect of flumazenil has to be assumed. The better mood in the morning after PSD may be explained as a consequence of the suppression of MS during daytime, which may consecutively affect homeostatic sleep pressure (= Process S) within the two process model of sleep regulation (Borbèly, 1982). The suppression of MS by flumazenil counteracts the increased sleep pressure of patients during PSD, allowing a less disturbed and thus better accumulation of process S during waketime. The accumulation of Process S is accompanied by an intensification of GABAergic neurotransmission. This has been shown by Seifritz et al. (1995), who were able to suppress the slow wave activity increase induced by SD by the administration of flumazenil. Therefore, the stronger accumulation of process S, which is also reflected by an intensification of NonREM sleep (Hemmeter et al., 2003) after flumazenil application in our patients, may be accompanied by an increase in GABAergic neurotransmission, which is still present after the recovery night. The improvement of mood, may thus, be explained in terms of the GABAergic model of depression due to a secondary increase of GABAergic neurotransmission.

In summary, the results of this study suggest a close contribution of GABAergic neurotransmission to mechanisms of sleep–wake regulation, while a contribution to modulating effects on mood can only be hypothesized.

It is worth noting to say that in this study all patients were treated with a constant dosage of trimipramine for at least one week before they entered the study. The duration of the trimipramine treatment, the duration of the depressive illness and number of previous episodes had no effect on the antidepressant efficacy of PSD and the expression of MS in this study. Moreover, no effects of these variables on MS had been detected in the first MS study (Hemmeter et al., 1998). In addition, the results of a meta-analysis (Leibenluft and Wehr, 1992) did not show any effects of these variables on PSD response. Furthermore, in this study no significant effects of age and gender on the cumulative amount of MS were found.

However, it is still unclear how MS is affected by an antidepressant treatment with a different pharmacological and clinical profile. Furthermore, it is unknown whether the application of flumazenil additional to any other antidepressant therapy will lead to comparable effects as observed in this study.

In addition, it must also be stressed that due to the double blind character, the patients in this study could not be randomized according to the amount of MS presented at baseline.

Therefore, only five patients who presented with MS at baseline were treated with flumazenil. Due to the strong impact of baseline MS on subsequent MS expression during PSD and mood response in the placebo group the inclusion of this variable was essential for the explanation of the effects observed.

However, including MS as an additional independent variable caused the groups to become very small and confirmative statistics were not possible. Therefore, this study has to be regarded as an exploratory study performed for the development of new hypotheses on the relationship between MS, mood response and GABAergic neurotransmission. Further research on this topic with larger samples and the control of the expression of MS is necessary.

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