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Sleep disturbances are correlated with decreased morning awakening salivary cortisol

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Summary Morning and evening salivary cortisol levels were correlated with sleep parameters in 14 patients with primary insomnia and 15 healthy controls. Salivary cortisol was sampled immediately after awakening (T1), 15 min later (T2), and immediately before going to bed (T3) for 1 week at home. In parallel with this, subjects estimated parameters of sleep in a daily sleep log. Patients and controls were all non-smokers who did not differ regarding morning awakening time or bedtime.

Cortisol after awakening was significantly decreased in primary insomnia. Salivary cortisol at the time of awakening correlated negatively with the subjective estimation of sleep quality, i.e. a low salivary cortisol level directly after awakening correlated with a higher frequency of nightly awakenings ($r = -0.50$), a diminished sleep quality ($r = -0.34$) and a decreased feeling of recovery after awakening ($r = -0.35$; all $p < 0.05$). Furthermore, awakening cortisol was negatively correlated with the Pittsburgh Sleep Quality Index ($r = -0.43$) and with a questionnaire on sleep-related cognitions with the subscales rumination in bed ($r = -0.56$) and focusing on sleep-related thoughts ($r = -0.46$; all $p < 0.05$).

1. Introduction

Chronic insomnia is a widespread disorder (Hohagen et al., 1993; Leger et al., 2000; Backhaus et al., 2002b) that increases with age, especially beyond the age of 40 years. Around this age the amount of slow wave sleep naturally decreases and wakefulness during bedtime increases, both of which might contribute to insomnia (e.g. Van Cauter et al., 2000). According to DSM-IV criteria (Association AP, 2000), patients with primary insomnia suffer from problems of sleep onset, sleep maintenance, and/or non-refreshing sleep. They experience daytime impairments, such as tiredness, disturbed concentration, or reduced efficiency, as a consequence of the disturbed night sleep. For diagnosis, an insomnia duration of at least 4 weeks is necessary.

Primary insomnia is associated with a disturbance of cortisol secretion, whereby an increase in 24-h urinary cortisol levels has been shown (Vgontzas et al., 1998; Shaver et al., 2002). Vgontzas et al. (2001) found an increase in serum cortisol in the evening and in the first hours of nocturnal sleep in a sample of younger patients with primary insomnia. This result was confirmed

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by Rodenbeck et al. (2002) on a small sample of patients, while Riemann et al. (2002) could not replicate these findings. The latter study, however, exclusively used the DSM-IV criteria for sample selection, while Vgontzas et al. (2001) only included subjects with a disturbed sleep EEG in addition to the DSM-IV criteria, thus probably sampling patients with a more severe disturbance of sleep.

Studies on serum or plasma cortisol allow for assessments during sleep, but they require use of an intravenous cannula that in itself can be a stressor influencing cortisol secretion and sleep parameters (Jarrett et al., 1984; Vitiello et al., 1996; Prinz et al., 2001). Furthermore, the sampling of blood at short time intervals usually restricts assessment to unnatural laboratory conditions and to a relatively small number of nights that might not reflect the natural variation in nightly sleep. While the sampling of urinary cortisol would allow for assessment across more nights, this method only allows for a more general assessment of cortisol secretion over a longer time period. Salivary cortisol, by contrast, can easily and without stress be collected at home and across a longer time period. Furthermore, it can be collected at specific time points close to the sleep period, thus allowing for some assessment of the consequences of disturbed sleep on the cortisol activity. Like urinary cortisol it is unbound, and thus a measure of the biochemically active component of cortisol (Kirschbaum and Hellhammer, 1989). It has a circadian rhythm and a reactivity to stressors that correlates with serum and plasma cortisol (Kirschbaum and Hellhammer, 1994). The morning awakening cortisol response (over the first 30–45 min) is an especially reliable marker of hypothalamus–pituitary–adrenal axis (HPA) activity (Pruessner et al., 1997) that in turn is dependent on a moderate genetic influence and on stressful events preceding the sample collection (Pruessner et al., 1999; Wust et al., 2000).

Rounding up, the collection of salivary cortisol allows for an assessment of HPA activity while at the same time circumventing some of the methodological problems associated with cortisol assessment in the urine and the blood. It allows cortisol activity to be assessed immediately before and after sleep in the natural home environment and for a longer time period. We therefore used this technique in a controlled design in order to study the cortisol activity immediately before and after sleep in patients meeting the DSM-IV criteria of primary insomnia. Furthermore, we wanted to explore whether the changes in cortisol secretion are related to the typical complaints associated with sleep impairment.

2. Methods

2.1. Subjects

Fourteen patients with primary insomnia and 15 healthy controls between 32 and 62 years of age participated (see Table 1). Patients were recruited from the outpatient sleep disorders clinic of the Department of Psychiatry and Psychotherapy of the University of Luebeck. Controls were recruited using advertisements. All subjects were non-smokers and had a body mass index in the normal range between 19 and 25. They went to bed at a certain time regularly and were not shift workers. None of the subjects took any psychoactive drugs or medications that could affect sleep. Participants had a thorough physical and psychiatric assessment including the structured clinical interview for the diagnosis of disorders according to DSM-IV (SCID, extended German version by Wittchen et al., 1997). Moreover, a battery of clinical tests including electrocardiography, electroencephalography, thyroid hormones, blood count, and urine analysis was evaluated.

For assessment of the sleep disorder, a structured sleep interview was applied and a medical history taken. The participating insomniac patients all met the criteria for primary insomnia according to DSM-IV. In order to lower the risk

Table 1 Descriptive statistics of the sample (means ± SD)

	Controls	Patients	*t*-test *p*
Mean age (years)	47.1 ± 8.8	46.8 ± 7.9	0.929
Age range (years)	32–61	33–62	
Sex: females (*n*)/males (*n*)	10/5	11/3	0.474 (Chi^2)
Body mass index	23.8 ± 3.7	22.9 ± 3.0	0.497
Time of awakening (h ± min)	6 : 59 ± 35.6	7 : 08 ± 52.3	0.585
Time of going to bed (h ± min)	23 : 15 ± 43.2	23 : 19 ± 71.3	0.850
Duration of insomnia (years)	–	11.6 ± 11.2	–

that the sleep disturbances could be the precursor of a depressive disorder, all patients in this study had to fulfill the criteria of primary insomnia for at least 2 years without fulfilling the criteria for depression in between.

Patients who, in addition to primary insomnia, met the criteria for any other concurrent psychiatric disorder, for another sleep, or for a somatic disorder possibly affecting sleep were excluded.

The study had been conducted in accordance with the declaration of Helsinki and had been approved by the local ethics committee. All subjects gave their written informed consent after complete description and adequate understanding of the study prior to participation.

2.2. Procedure

The participants collected 21 saliva samples on seven consecutive days (3 per day) with Salivette sampling devices (Sarstedt, Rommelsdorf, Germany). They were instructed to collect them immediately after awakening (T1), 15 min later (T2), and just before bedtime (T3). The time of awakening and of going to bed and the exact time of every saliva sampling had to be recorded. The participating subjects were explicitly advised not to collect the sample outside the mentioned time points. If they missed a time point, they were to omit this sample. Participants were informed that the exact time point was very important for the study protocol and the following analyses, and that there would be no consequences for the participants in case of missing data. To increase compliance further we restricted the samples per day to three and used only time points that seemed unproblematic for sampling, as they would not interfere with other daily obligations including work schedule.

Participants were free to use an alarm clock or wake up spontaneously at home. They were instructed to refrain from brushing teeth, eating food, drinking alcoholic beverages, caffeine, or fruit juices 1 h before saliva sampling. They stored their samples in a refrigerator at home for the 1 week of sampling before bringing them to the sleep laboratory. There the samples were centrifuged and the supernatant frozen at $-20\ ^{\circ}C$ until assayed in the laboratory. After the 1-week saliva collection at home, subjects slept one night in the sleep laboratory to rule out sleep-related breathing disorders and periodic leg movements.

2.2.1. Cortisol analysis

For assessment of the cortisol values a radioimmunoassay was used (Diagnostic Products Corp. Herrman Biermann, Bad Nauheim, Germany; sensitivity 0.2 μg/dl, intra-assay coefficient of variation $\leq$5%, inter-assay coefficient of variation $<$6.5%). The assessing staff were blind with respect to the participating subjects and their diagnosis.

2.2.2. Sleep logs

All participants filled out daily sleep logs in the morning, in which they documented the time of going to bed and switching the light off as well as the time of awakening. Furthermore, subjects were asked to estimate the sleep onset latency, the frequency of nightly awakenings, wake time after sleep onset, and total sleep time. Additionally, participants rated their sleep quality and feeling of recovery after awakening using a rating scale ranging from 1 (very good) to 6 (very bad).

2.2.3. Questionnaires

As a general measure of sleep the Pittsburgh Sleep Quality Index (PSQI, Buysse et al., 1989) was used. It has a good test–retest reliability and validity especially for patients with primary insomnia and healthy controls (Backhaus et al., 2002a). The PSQI score can range from 0 to 21, with higher values showing a lower sleep quality and a higher level of sleep disturbances. Five points or more indicate decreased sleep quality. For assessment of rumination in bed and focusing on sleep-related thoughts, a standardized questionnaire (FEPS, Hoffmann et al., 1996) was used. On these two subscores a higher score signifies increased ruminations about the sleep disturbance and a stronger focus on sleep-disturbance-related consequences, respectively.

2.2.4. EEG-recording

To rule out sleep apnea or periodic leg movements all subjects spent one night in our sleep laboratory where their sleep was monitored polysomnographically using (1) electrodes positioned at C3 referenced against A2, and at C4 referenced against A1 as defined by the international 10–20 system, (2) an electromyographic recording of the submental region, and (3) electrooculographic recordings assessing eye movements with the help of two horizontal and one vertical recording. Furthermore, electromyographic recordings of the legs and three recordings of breathing (chest and abdominal excursions, nose–mouth airflow) together with oxygen monitoring were applied. No subject enrolled in the study showed clinically relevant sleep apneas (>5/h) or nocturnal periodic leg movements with EEG-arousal (>5/h).

As only one night was spent in the sleep laboratory, data regarding sleep stages were not taken into consideration for the study.

3. Statistical analysis

The means of the cortisol values of each measurement point were calculated for each participant over the whole week of measurement. The inter-day variations of salivary cortisol were evaluated by correlation analyses. Group differences for the salivary cortisol values were analyzed using ANOVAs with repeated measurements and *t*-tests wherever the ANOVAs revealed a significant effect. For calculating the average bedtime and time of awakening, time points were converted into decimal figures, averaged, and then retransformed into the ordinary representation of time. Sociodemographic, questionnaire, and sleep log data were analyzed using two-tailed *t*-tests for independent samples wherever appropriate. Correlation analyses were performed with Pearson correlations. Tests were calculated with an alpha of 0.05. Data were analyzed with SPSS for Windows, Version 11. Variability values were expressed as standard deviations (SD) unless otherwise specified.

4. Results

4.1. Sample statistics

Patients and controls did not differ with regard to age, sex, body mass index, time of awakening or bedtime (see Table 1).

4.2. Salivary cortisol profiles of insomnia patients and controls

The ANOVA for the 1-week salivary cortisol measurement showed a significant sampling time as well as a significant sampling time × group interaction effect (time effect: $F = 133.1$, df = 2, $p = 0.000$; interaction effect: $F = 3.45$, df = 3, $p = 0.047$). Post-hoc *t*-tests revealed a significantly decreased cortisol level after awakening (T1) for the primary insomnia patients in comparison with the healthy controls ($p = 0.001$), while there were no significant differences between the groups for the other time points (T2, T3) (see Fig. 1).

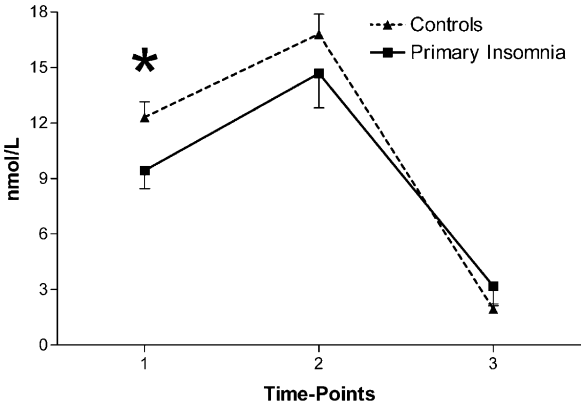

Fig. 1. Salivary cortisol at awakening (T1), 15 min later (T2), and at bedtime (T3): average values over 1 week at home (±standard error of the mean) $^{*}p < 0.05$.

The fall of salivary cortisol from awakening (T1) to evening (T3) was significantly smaller for the insomnia patients than for the controls (patients: 6.4 ± 3.5; controls: 10.8 ± 3.4; $p = 0.013$). A smaller decrease between the cortisol values 15 min after awakening (T2) and the evening cortisol (T3) just failed to reach significance (patients: 12.2 ± 5.5; controls: 15.3 ± 4.3; $p = 0.054$). The morning increase in cortisol from awakening (T1) to 15 min after awakening (T2) was not significantly different between the groups (patients: 4.4 ± 0.6; controls 5.2 ± 1.5; $p = 0.311$).

The inter-day variations of the salivary cortisol samples for T1, T2 and T3 over 1 week are shown in Table 2.

4.3. Sleep logs

Patients and controls differed significantly in their subjective estimation of all sleep parameters. Insomnia patients recorded higher sleep onset

Table 2 Inter-day correlation matrix for cortisol at T1, T2 and T3

Correlation of cortisol values on Sunday with	T1	T2	T3
Monday	0.655*	0.242	0.106
Tuesday	0.732*	0.347*	0.541*
Wednesday	0.437*	0.069	0.468*
Thursday	0.629*	0.181	0.595*
Friday	0.314#	0.055	0.294
Saturday	0.703*	0.591*	0.716*

*$p < 0.05$.
#$p < 0.1$.

Table 3 Sleep logs and questionnaires (means ± SD)

	Controls	Patients	*t*-test *p*
Sleep log			
Sleep onset latency (min)	9.9 ± 5.94	67.16 ± 62.08	0.004
Frequency of nightly awakening	0.54 ± 0.6	2.06 ± 1.99	0.019
Wake time after sleep onset (min)	6.50 ± 6.80	82.88 ± 79.45	0.003
Total sleep time (min)	442.65 ± 39.66	312.55 ± 62.14	0.000
Rating sleep quality (1 = very good until 6 = very bad)	1.63 ± 0.53	3.56 ± 0.69	0.000
Rating recovery after awakening (1 = very good until 6 = very bad)	1.90 ± 0.57	3.47 ± 0.75	0.000
PSQI (≥5 indicates decreased sleep quality)	2.40 ± 1.64	12.50 ± 3.61	0.000
FEPS: ruminating	13.33 ± 16.83	47.21 ± 23.29	0.000
FEPS: focusing on sleep	10.13 ± 11.96	53.21 ± 22.61	0.000

latency, a higher frequency of nightly awakenings, a longer duration of time awake after sleep onset, and a shorter total sleep time. Ratings of sleep quality and feeling of recovery after awakening were decreased in insomnia patients. See Table 3 for means and statistics.

4.4. Questionnaires

Insomnia patients had a significantly lower sleep quality as shown by the PSQI sum score and higher values for sleep-related rumination and focusing on sleep-related thoughts (FEPS-II) in comparison with the controls. See Table 3 for exact values and statistics on the questionnaire items.

4.5. Correlations between cortisol and sleep logs/questionnaires

Salivary cortisol at awakening showed significant negative correlations with the sleep log parameters frequency of nightly awakenings, sleep quality, and feeling of recovery after sleep (see Fig. 2).

Negative correlations with the awakening cortisol values could also be demonstrated for the applied questionnaires: the cognitive questionnaire (FEPS-II) with its subscales focusing on sleep and rumination in bed and the PSQI (see Fig. 3). There was a trend for the correlation between cortisol at T1 and the sleep log parameter total sleep time ($r = 0.28$, $p = 0.07$), but no significant correlation between cortisol at T1 and wake time after sleep onset ($r = -2.1$, $p = 0.135$).

With the exception of rumination and cortisol at T2, no further significant correlations between T2 or T3 and the sleep log or the questionnaire parameters ($p > 0.05$) were found.

5. Discussion

The data from this study show that subjects with a higher frequency of nightly awakenings, a low rating of sleep quality, and with the impression of a lack of recovery after awakening have lower awakening cortisol values. Furthermore, similar correlations of the awakening cortisol value with the PSQI, focusing on sleep-related thoughts, and rumination in bed were found. All these results therefore show that a higher disturbance of sleep is associated with a lower cortisol level directly after awakening. The similar correlation results for focusing, ruminations, and PSQI score on sleep quality are in accordance with the hypothesis that all three measures represent typical aspects of the cognitive evaluation of sleep disturbances and thus should correlate with morning cortisol in the same way.

Patients with primary insomnia had a significantly lower awakening salivary cortisol in comparison with healthy subjects. The decrease in cortisol from awakening to going to bed in the evening was significantly lower in the patients. This was due mainly to the lower awakening cortisol level in the patients, since the evening values yielded no significant differences between groups.

Edwards et al. (2001) as well as Kudielka and Kirschbaum (2003) demonstrated the dependence of salivary awakening cortisol on the time of awakening. In the present study, the bedtime and the time of awakening did not differ significantly between patients and controls, so that the results cannot be explained by differences in the awakening times. The stability of bedtime and time of awakening across the whole sample was probably due to the fact that the participants had regular work schedules.

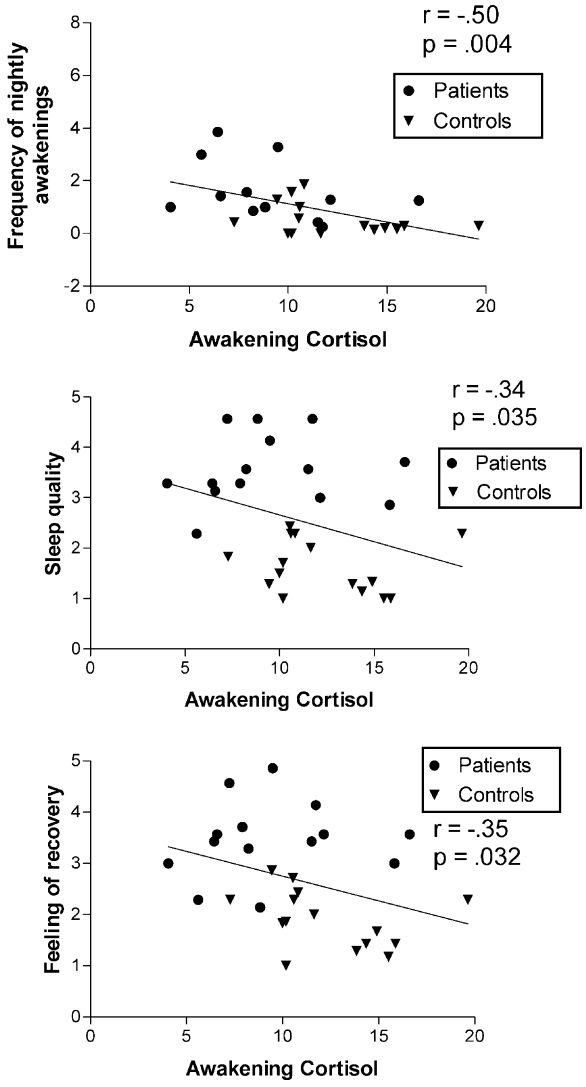

Fig. 2. Correlations of awakening salivary cortisol with the sleep log parameters frequency of nightly awakenings, sleep quality (1, good; 5, bad sleep quality), and feeling of recovery (1, high; 5, low feeling of recovery).

In a recent study Kudielka et al. (2003) found that 26% of their study population did not completely adhere to their sampling protocol despite the fact that the importance of exact timing of the samples was stressed. This non-compliance was very low for the salivary cortisol at the time of awakening, but highest at measurement 30 min after awakening. We also found significant inter-day correlations for the cortisol samples which were sampled immediately after awakening as well as for those which were sampled before going to bed (T1 and T3). The inter-day correlations for T2 (15 min after awakening), however, were much lower. We suppose that this is due to a lower compliance for sampling at this time point.

With more than 30 years, the age range of the subjects in our study was wide but representative of patients with primary insomnia and thus provides clinical relevance to these data. A more homogeneous, younger sample with a smaller age range would probably have had the advantage of a smaller variability of cortisol values thus increasing the chances of obtaining (more) significant results, but it would not have been as representative for typical patients with primary insomnia.

As the awakening cortisol correlated negatively with the frequency of nightly awakenings, the lower morning values might be the result of more nightly cortisol activation due to more waketimes after sleep onset with a consequently decreased HPA-axis activation after awakening. This hypothesis is supported by results from other studies. Thus Vgontzas et al. (2001) found a higher HPA-axis activation in insomniac patients in the evening and the first half of the night, Spath-Schwalbe et al. (1991) demonstrated an increase in HPA-axis activation following experimentally induced nightly awakenings in healthy subjects, and Waye et al. (2003) in a study on young healthy subjects showed that experimental nightly exposure to low frequency noise is associated with lower subjective sleep quality and with attenuated salivary morning cortisol values 30 min after awakening. A direct demonstration of an increase in cortisol in the night and a consequent decrease at awakening has to meet with the confounding technical problem that an intravenous collection of cortisol in itself is a stress factor increasing cortisol excretion (Prinz et al., 2001) that will also change the relevant sleep parameters (Jarrett et al., 1984; Vitiello et al., 1996). This issue cannot therefore be easily settled.

Several epidemiological studies on patients with insomnia found a higher risk to develop a major depression in the following years (e.g. Ford and Kamerow, 1989; Livingston et al., 1993; Breslau et al., 1996; Chang et al., 1997). Harris et al. (2000) found that women with increased morning cortisol levels (at 08:00 h) were at a higher risk for a major depressive episode within a 12–13 months' follow-up. Depressed patients and the elderly have a diminished variability in circadian cortisol levels and a raised morning cortisol level in common (Kern et al., 1996; Van Cauter et al., 1998; Deuschle et al., 1998). Our group of patients with primary insomnia had a mean insomnia duration of 11 years without development of a concurrent depression. Instead of higher cortisol values they had a lowered cortisol level in the morning. Further prospective studies are necessary to investigate whether patients with shorter primary insomnia duration and a higher risk of

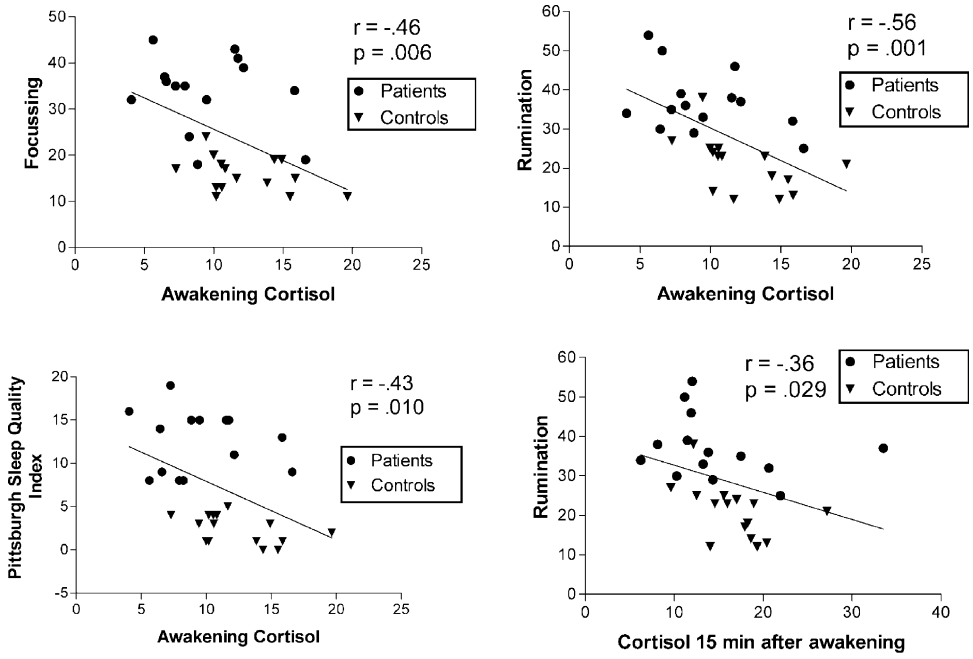

Fig. 3. Pearson correlations of awakening cortisol with the sleep parameters focusing on sleep, rumination, and the PSQI scores $\geq$5 indicate decreased sleep quality.

depression show a differing deviation in morning cortisol.

In addition, it seems a worthwhile task to investigate whether a low awakening cortisol in primary insomnia might be a state marker that normalizes after a successful therapy for primary insomnia. Since short-term cognitive-behavioral therapies showed stable positive therapy effects at follow-ups ranging from several months up to 3 years after therapy (Morin et al., 1994; Murtagh and Greenwood, 1995; Backhaus et al., 2001), an intervention is available to allow this issue to be investigated further.

In conclusion, our data show that the method of repetitive awakening salivary cortisol collection at home allows a successful investigation of sleep disturbances in the home environment. Awakening salivary cortisol might be a biological marker for several subjective parameters of sleep disturbance. It will have to be shown in further studies whether this marker is predictive for an increased risk for somatic or psychiatric disorders and whether it can be influenced by successful treatment.

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