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Brain-derived neurotrophic factor is a biomarker for subjective insomnia but not objectively assessable poor sleep continuity

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ABSTRACT

Objectives: Brain-derived neurotrophic factor (BDNF) is a central mediator of the effects of stress on neuronal plasticity. Patients with subjective insomnia have significantly lower serum BDNF (sBDNF) levels. The aims of the present study were to investigate the associations of sBDNF with, 1) subjective and 2) objective sleep; 3) to investigate the associations between dimensions of psychopathology, subjective sleep and sBDNF, and 4) to investigate the associations between insomnia, sBDNF and cortisol.

Methods: 60 patients with insomnia (IG; mean age: 40.4 years; 48.3% females) and 30 healthy, age and gender-matched controls (CG) took part in the study. Subjective sleep was assessed using the Insomnia Severity Index (ISI), objective sleep was assessed once via sleep-EEG recordings. Both sBDNF and salivary cortisol were sampled once the following morning. Last, experts rated participants' symptoms of depression and anxiety.

Results: sBDNF was significantly lower in the IG than in the CG (large effect size; Hedge's g = 1.75), while higher insomnia scores, but not depression or anxiety ratings, predicted lower sBDNF levels. Concerning objective sleep, low sBDNF did not correlate with sleep continuity measures, but with decreased REM-sleep; the latter was also characteristic of the IG. sBDNF and salivary morning cortisol were unrelated.

Conclusions: Independently of symptoms of depression or anxiety, sBDNF appears to be a biomarker for the clinical diagnosis of insomnia, but not for objectively assessed poor sleep continuity. A possible link between sBDNF and insomnia seems to be via regulation of REM-sleep, but not salivary morning cortisol.

1. Introduction

Brain-derived neurotrophic factor (BDNF) belongs to a family of growth factors located in the brain and peripheral tissues and playing an essential role in neuronal cell differentiation, growth, and survival (Skaper, 2018). Neurotrophic functions of BDNF have implications for cognitive functions, appetite, body weight, and sleep (Duman et al., 2000; Faraguna et al., 2008; Garner et al., 2017; Lu et al., 2014; Schmitt et al., 2016; Xu and Xie, 2016). In the last two decades, BDNF has become increasingly accepted as a central mediator of the effects of stress on neuronal plasticity. Stress-induced decreased BDNF expression has also been related to the psychopathology of stress-related mental disorders (Duman et al., 1997, 2006; Krishnan and Nestler, 2008). Following the so-called "neurotrophin hypothesis of stress" BDNF seems to be involved in the pathophysiology of major depression (Molendijk et al., 2011; Duman et al., 2016).

While previous studies have investigated the role of BDNF in depression, we focus on the relation between BDNF and insomnia as a common feature in many stress-related mental disorders, and an independent risk factor for higher stress vulnerability (Morin et al., 2003). Thus, insomnia is often observed among patients with major depressive disorders (Steiger and Kimura, 2010). In a previous pilot study (Giese et al., 2013, 2014a), we investigated the serum BDNF (sBDNF) levels of adults with current symptoms of insomnia, and of

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non-sleep disturbed controls. These symptoms were associated with significantly lower sBDNF, while severity of insomnia and sBDNF were negatively correlated. Overall, the results supported the view that lower sBDNF levels are linked to insomnia. Moreover, in a mediation model of the interplay of perceived stress, subjective sleep quality and BDNF, insomnia was the mediator between stress and changes in BDNF. While there was a direct association between perceived stress and low BDNF levels, perceived stress was only linked to lower BDNF levels when associated with insomnia (Giese et al., 2013). These preliminary results suggest that insomnia is the key-mediator for low levels of BDNF.

To summarize, lower BDNF levels appear to be involved in poor subjective sleep regulation and psychopathologies such as major depressive disorders. In several studies patients with major depressive disorders have also suffered from insomnia (Geoffroy et al., 2018) and displayed dysregulated hypothalamic-pituitary-adrenal axis activity (HPA-AA), with higher cortisol levels as stable and robust markers of increased HPA-AA (Ising et al., 2007; Holsboer and Ising, 2010). The present study aimed at further investigating sBDNF as a candidate biomarker for both subjective insomnia (Giese et al., 2013, 2014a) and objective poor sleep, along with traits of psychopathology and cortisol secretion as a marker of the HPA-AA. To this end, we employed established measures of stress-related disorders, namely sleep-EEG (Steiger and Kimura, 2010) and saliva morning cortisol (Wüst et al., 2000).

One hypothesis and two research questions were formulated.

Following Giese et al. (2013, 2014a) and Schmitt et al. (2016) we anticipated that more severe subjective insomnia would be related to lower sBDNF levels in patients with insomnia. As insomnia is highly comorbid with depression and anxiety, and sBDNF is also lower in such psychopathological states, we examined whether the relationship between sBDNF and insomnia was independent of depression and anxiety.

Next, we explored whether sBDNF was associated with objective sleep EEG parameters of sleep continuity (sleep onset latency, sleep duration, number of awakenings, wakefulness after sleep onset, and sleep efficiency) and sleep architecture (stage 1 and 2, slow wave sleep and REM sleep).

We also sought to determine whether increased HPA-AA reflected in saliva morning cortisol was a link between insomnia and decreased sBDNF; as insomnia is acknowledged as a state of hyperarousal (Riemann et al., 2015) we anticipated elevated morning cortisol levels. And based on the neurotrophin-hypothesis of depression and on the assumption that chronic (Duman et al., 1997) and perceived stress (Giese et al., 2013) lead to lower sBDNF levels, we expected a negative correlation between sBDNF and cortisol levels.

2. Methods

2.1. Study participants

Sixty male and female patients aged between 18 and 65 and suffering from insomnia according to DMS-IV criteria were enrolled in this study (insomnia group = IG). The diagnostic criteria were a predominant complaint of difficulties initiating or maintaining sleep, or nonrestorative sleep, for at least one month and that the sleep disturbance (or associated daytime fatigue) caused clinically significant distress or impairment in social, occupational, or other important areas of functioning. An additional inclusion criterion was an Insomnia Severity Index (ISI, Bastien et al., 2001) score above 8, the cut-off sum score for subthreshold insomnia. Exclusion criteria were severe medical or neurological conditions (e.g., intoxication, drug abuse, stroke), a lifetime diagnosis of alcohol dependence, along with severe psychiatric issues such as acute and chronic psychosis, eating disorders, suicidal behavior, or cognitive issues, such to impair an individual to comply with the study conditions. As regards medications and substances, the intake of stimulants and illicit drugs, including amphetamines and methamphetamines, were explicit exclusion criteria. A control group (CG) of thirty healthy subjects, matched for age, gender, and body mass index, was recruited. A brief psychiatric interview ensured that only healthy individuals without any clinical sleep complaints or other mental or physical health problems were included.

2.2. Procedure

Participants were informed about the aims of the study and the confidential and anonymous data handling and signed a written informed consent. Next, they completed questionnaires covering sociodemographics, subjective sleep quality (Insomnia Severity Index; Bastien et al., 2001; Gerber et al., 2016) and overall symptom load. Experts rated participants' degree of anxiety and depression. Overnight sleep-EEG recordings were made once and the following morning saliva and blood samples were taken once. The local ethics committee (Ethikkommission Nordwest-und Zentralschweiz; EKNZ Nr. 146/13) approved the study, which was performed in accordance with the rules laid down in the Declaration of Helsinki and its later amendments.

2.3. Assessments

2.3.1. Baseline examination

After enrolment, all patients underwent a baseline examination to assess insomnia severity, psychopathology, somatic symptoms and general health condition including social functioning and stressful life events. Assessments were made by experienced raters using standardized rating scales. Insomnia was assessed with the self-rating Insomnia Severity Index (ISI, Bastien et al., 2001) based on the DSM-IV diagnostic criteria for insomnia. Additional data collected included: self-rating on the Symptoms Check-List (SCL-90-R, Derogatis et al., 1976); previous treatments with pharmacotherapy; smoking status and current cigarette consumption per day. Trained psychiatrists rated participants' symptoms of depression (Hamilton Depression Rating Scale; Hamilton, 1960) and symptoms of anxiety (Hamilton Anxiety Rating Scale; Hamilton, 1959).

2.3.2. Sleep EEG

Following the sleep units schedule, sleep could take place between 10 p.m. and 7 a.m. Within this interval, patients could sleep according to their individual needs. EEG records were visually scored blind by two experienced raters applying standardized criteria (Rechtschaffen and Kales, 1968) to derive the following parameters: total sleep time (TST), sleep efficiency (SEI; ratio of TST to time in bed), sleep onset latency (SOL), number of awakenings, wake-time after sleep onset (WASO), sleep architecture measures (Sleep stages 1, 2, slow wave sleep (stage 3 and 4), and REM sleep). For sleep medicine assessment, periodic limb movements and breathing were recorded.

2.3.3. Laboratory assessments

2.3.3.1. Serum samples; BDNF (brain-derived neurotrophic factor). For the assessment of BDNF levels, a blood sample was drawn between 7 and 8 a.m. after overnight fasting according to a standardized protocol, using serum vacutainer tubes (Becton Dickinson). The serum tube was centrifuged at $1300 \times g$ for 10 min. Aliquoted samples were stored at −80 °C before assaying BDNF content. Serum BDNF levels were assessed with an enzyme-linked immunosorbent assay (ELISA) kit (Biosensis® Mature BDNF RapidTM ELISA Kit: Human, Mouse, Rat; Thebarton, SA, Australia). Serum samples were appropriately diluted (1:100) and detection of BDNF was via a pre-coated mouse monoclonal anti-mature BDNF 96-well plate as described in the manufacturer's protocol. The absorbance was measured within 5 min after addition of the stop solution in a microplate reader set at 450 nm and a correction wavelength set to 690 nm, to determine BDNF concentrations according to the standard curve. All assays were carried out in duplicate and means were calculated.

Table 1
Sample characteristics. Comparison between Insomnia group (IG) and Control group (CG).

	IG (N = 60)	CG (N = 30)	tests, *p*	effect sizes, g
age, *years, mean (SD)*	40.4 (12.9)	37.9 (11.9)	$t_{1,88} = 0.9$, p = 0.39	0.20
gender, females, N (%)	29 (48.3)	17 (56.7)	χ^2(N = 90, df = 1) = 0.56; p = 0.45	–
males, N (%)	31 (51.7)	13 (43.3%)		
BMI, kgm^{-2}, *mean (SD)*	25.1 (4.1)	23.4 (3.6)	$t_{1,79} = 1.9$, p = 0.065	0.43 [S]
WHR, *ratio, mean (SD)*	0.87 (0.08)	0.85 (0.07)	$t_{1,85} = 1.2$, p = 0.240	0.26 [S]
educational level			F = 4.65, p = 0.103	–
high school, *N (%)*	32 (54.2)	22 (75.9)		
middle, *N (%)*	14 (23.7)	2 (6.9)		
low, *N (%)*	13 (22.0)	5 (17.2)		
smoking status, *yes, N (%)*	16 (26.7)	10 (34.5)	χ^2(N = 89, df = 1) =0.58; p = 0.45	–
psychotropic medication, *N (%)*	49 (89.1)	0 (0)	–	–
antidepressants, *N (%)*	37 (67.3)	0 (0)	–	–
augmentational therapy, *N(%)*	17 (30.6)	0 (0)	–	–
sedative/sleep medication, *N (%)*	19 (34.5)	0 (0)	–	–
ISI, *total score, mean (SD)*	17.6 (4.0)	2.4 (1.9)	$w_{1,85} = 24.1$, p < 0.001	4.40 [L]
HAMD, *total score, mean (SD)*	12.6 (7.1)	2.4 (2.6)	$w_{1,81} = 9.8$, p < 0.001	1.70 [L]
HAMA, *total score, mean (SD)*	11.5 (7.4)	2.4 (2.2)	$w_{1,75} = 8.7$, p < 0.001	1.47 [L]
SCL 90R, *GSI, mean (SD)*	0.74 (0.49)	0.13 (0.10)	$w_{1,69} = 9.2$, p < 0.001	1.51 [L]

BMI: body mass index, WHR: waist-hip-ratio, ISI: Insomnia Severity Index, HAMD: Hamilton Depression Rating Scale, HAMA: Hamilton Anxiety Rating Scale, SCL 90R: Symptom Checklist, GSI: global severity index.

2.3.3.2. Saliva sampling: cortisol awakening response (CAR). The CAR has been shown to be a reliable index of basal HPA axis activity (Pruessner et al., 1997). On two days of the week (on the morning immediately after sleep EEG recording and one day later), four saliva cortisol samples were taken [0, 10, 20, and 30 min immediately after awakening; for more details see Hatzinger et al. (2013)]. Participants were instructed to start saliva sampling immediately after awakening without first rinsing their mouth with water. They were also instructed not to eat breakfast or to brush their teeth before sampling was completed. To facilitate verifying the exact timing of the saliva sampling, we instructed participants immediately to record each of the four time points of saliva collection in a log provided with the collection tubes/swabs.

Saliva samples were obtained using the "Salivette" device for quick and hygienic sampling (Sarstedt, Nümbrecht, Germany). This device includes a small cotton swab on which the subject gently chews for 0.5–1 min. Thereafter, the swab is transferred into a small plastic tube, the Salivette container, and stored in a freezer. Saliva samples were returned to the laboratory where they were centrifuged at 4 °C (2000 rpm, 10 min) and stored at -20 °C until assay. Free salivary cortisol concentrations were analysed using a time-resolved immunoassay with fluorometric detection "Coat-Account" Cortisol RIA from DPC (Diagnostics Products Corporation; Biermann GmbH, Bad Nauheim, Germany) as detailed elsewhere (Dressendorfer et al., 1992). Intra- and inter assay variability of this assay was less than 2.00% and 2.04%. The hormone concentration of the morning cortisol samples was computed as the area-under-the-concentration-time-curve (AUC) using trapezoidal integration (Forsythe et al., 1969). AUCtotal refers to the entire amount of cortisol concentration under the time curve, whereas AUCbasal describes the initial and averaged amount of cortisol secretion over time, as if the HPA-axis had not been stimulated. Accordingly, AUCnetto reflects the difference between AUCtotal and AUCbasal.

2.4. Statistical analysis

A series of t-, w-, and X^2-tests was performed to detect possible differences in sample characteristics between IG and CG. For preliminary calculations, a series of Pearson's correlations and t-tests were computed to determine whether traits of depression and anxiety or sBDNF were systematically associated with sample characteristics and thus requiring inclusion as covariates in subsequent analyses.

Comparisons of group differences between IG and CG were calculated with t-tests. Additionally, a series of Pearson's correlations was calculated between sBDNF, traits of depression and anxiety, objective sleep parameters, and CAR measures.

Next, a multiple regression analysis was performed with sBDNF as dependent variable and ISI, HAMD, HAMA and psychotropic medication status as independent variables. To test the statistical independence of the residuals, Durbin-Watson coefficient was reported.

Test results with an alpha level below 0.05 are reported as significant. The following abbreviations were used to denote significance levels: ° = p < 0.10, * = p < 0.05; ** = p < 0.01; *** = p < 0.001. Effect sizes for t- and w-tests were calculated with Hedge's g (with adaptation to different sample sizes) with, following Cohen (1988), 0.49 ≥ g ≥ 0.20 indicating small [S] (i.e., negligible practical importance), 0.79 ≥ g ≥ 0.50 indicating medium [M] (i.e., moderate practical importance), and g ≥ 0.80 indicating large [L] (i.e., crucial practical importance) effect sizes.

Analyses were conducted using SPSS® 25.0 (IBM Company, Armonk, NY, USA) for Windows®.

3. Results

3.1. Sample characteristics

Characteristics of the insomnia patients (IG) and control group (CG) are presented in Table 1. The groups did not differ significantly in age, gender distribution, education, smoking status, or waist-hip-ratio, though the IG had a marginally higher BMI (g = 0.43 [S]). In the IG, sleep medication was common (89.1%). The main drugs were antidepressants (67.3%) and hypnotics (34.5%). None of the CG was treated with psychotropic drugs.

As expected, insomnia scores were higher in the IG, while all CG participants were below the ISI cut-off for insomnia (< 8). The IG had higher scores for depression, anxiety, and symptom load.

3.2. Preliminary calculations

Age was correlated weakly with insomnia scores (r = 0.19, p = 0.08). There were no significant correlations between age and depression or anxiety scores (r's < 0.05; p's > 0.70).

Insomnia, depression, and anxiety scores did not differ significantly as a function of gender or smoking status. Intake of psychotropic medications was associated with higher scores for insomnia, depression, and anxiety (p < 0.05).

Levels of sBDNF were unrelated to age or BMI (r = 0.11, p = 0.31;

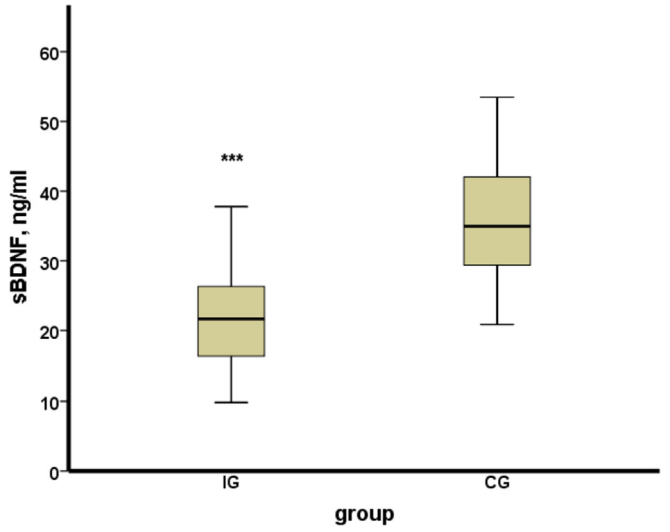

Fig. 1. Comparison of sBDNF between insomnia patients (IG) and healthy controls (CG); *t*-test for independent samples; ***: $p < 0.001$.

r = 0.01, p = 0.99, resp.) and did not differ between genders (t(81) = 0.05, p = 0.96) or smoking statuses (t(80) = 1.41, p = 0.16) but were lower in participants with psychotropic medication (22.7 ± 7.0 vs. 33.0 ± 10.6 ng/ml, w(76) = 4.95, $p < 0.001$).

3.3. sBDNF and insomnia scores

sBDNF serum levels were significantly lower in the IG (21.9 ± 7.0 vs. 35.6 ± 9.3 ng/ml; t(81) = 7.57, $p < 0.001$, g = 1.75 [L], Fig. 1). There were also significant negative correlations between sBDNF levels and insomnia severity, depression and anxiety in the total sample (see Table 2). The correlation between ISI and BDNF (Fig. 2) remained robust even after controlling for depression (partial correlation: r = −0.41, $p < 0.01$), anxiety (partial correlation: r = −0.49, $p < 0.01$), or both (partial correlation: r = −0.43, $p < 0.01$). In other words the negative relation between sBDNF levels and insomnia severity was unrelated to comorbid symptoms of depression or anxiety.

A stepwise multiple regression with sBDNF as dependent variable and insomnia, depression, anxiety and psychotropic medication status as independent variables confirmed that ISI scores predicted sBDNF levels, while the other independent variables did not reach statistical significance and were excluded from the equation (Table 3).

3.4. sBDNF and objective sleep measures

Objective sleep measures derived by sleep-EEG are reported in Table 4. Compared to healthy controls, IG had a longer sleep onset latency (SOL, g = 0.49 [S]), less REM-sleep (duration: g = 0.45 [S]; percentage: g = 0.52 [M]) and a longer REM-latency (g = 0.39 [S]). The IG group had marginally longer WASO and a higher number of awakenings (g = 0.40 [S]/g = 0.38 [S], respectively). The groups did not differ on either respiratory distress or period limb movement indices.

Table 5 reports relationships between objective sleep measures, sBDNF and insomnia. As objective sleep depends on age, partial correlations were adjusted for age. With one exception, there were no significant associations between sBDNF and objective sleep continuity measures; lower sBDNF levels were weakly associated with increased SOL. Concerning measures of sleep architecture, sBDNF positively and insomnia negatively correlated with percentage of REM-sleep.

Table 2
Correlations between sBDNF and psychometrics in the total sample, insomnia group and control group, respectively.

	total sample	IG	CG
ISI, score	r = −0.544	r = 0.099	r = −0.202
	p < 0.001	p = 0.475	p = 0.324
HAMD, score	r = −0.404	r = 0.044	r = −0.145
	p < 0.001	p = 0.753	p = 0.462
HAMA, score	r = −0.292	r = 0.152	r = 0.108
	p = 0.003	p = 0.284	p = 0.586

pearson's correlations r (two-tailed).

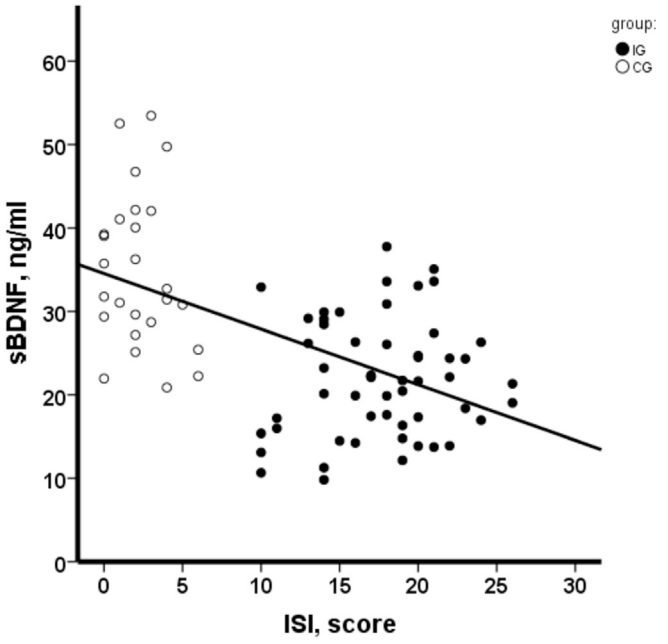

Fig. 2. Correlation between sBDNF levels and insomnia severity index (ISI). (Pearson's r = −0.54, $p < 0.001$).

3.5. sBDNF, insomnia and morning cortisol secretion (HPA-AA)

The IG had significantly lower AUCtotal and AUCbaseline values than the CG (respectively, 244 ± 101 vs. 301 ± 88, p = 0.035, g = 0.59 [M] and 185 ± 99 vs. 245 ± 127, p = 0.041, g = 0.53 [M]). AUCnetto values differed slightly but not significantly (IG: 69 ± 66 vs. CG: 89 ± 82, p = 0.33, g = 0.28 [S]).

ISI scores were significantly and negatively correlated with AUCtotal and AUCbasal scores (r = −0.294, p = 0.033 and r = −0.292, p = 0.025, respectively).

AUCtotal correlated positively with amount of REM-sleep (percentage: r = 0.301, p = 0.027) and negatively with amount of NREM S2 sleep (percentage: r = −0.369, p = 0.006).

sBDNF did not correlate with CAR measures (|r|'s < 0.1, p's > 0.4).

4. Discussion

In the present study sBDNF appeared to be a biomarker for subjective insomnia, but not objectively assessed poor sleep. The association between sBDNF and insomnia was also independent of any comorbid depression, anxiety or psychotropic medication, suggesting that sBDNF serves as a biomarker for insomnia distinct from psychopathologies, psychopharmacotherapy, or objective sleep activity and does so dimensionally rather than categorically. Furthermore, sBDNF was unrelated to morning cortisol, indicating that the relationship between sBDNF and insomnia is not mediated by HPA-AA.

We believe that the present results add to the current literature in showing that sBDNF is related to subjective sleep perception but not to objective sleep continuity. This suggests that subjective and objective

Table 3
Multiple linear regression with sBDNF as dependent variable, and ISI, HAMD, HAMA and psychotropic medication status as predictors.

Dimension	Variables	Coefficient	Standard error	Coefficient β	t	p	R	R^2	Durbin-Watson coefficient
sBDNF	Intercept	34.92	1.78	-	19.67	0.000	0.543	0.295	1.84
	ISI	-0.66	0.12	-0.543	5.45	0.000			
Excluded variables	HAMD	-0.01	-	-	-0.08	0.939			
	HAMA	0.11	-	-	0.82	0.417			
	Medication	0.10	-	-	0.51	0.610			

Note: ISI: insomnia severity index, HAMD: Hamilton Depression Rating Scale, HAMA: Hamilton Anxiety Rating Scale.

Table 4
Comparison of polysomnography results between IG and CG.

	IG	CG	Tests *p*	Effect sizes *g*
	Mean (SD)	*Mean (SD)*		
Sleep continuity variables				
SPT, min	388 (60)	371 (45)	0.156	0.31 [S]
TST, *h:min*	6:28 (1:27)	6:50 (1:01)	0.231	0.19 [S]
SEI, *%*	78.8 (14.6)	84.8 (13.4)	0.063	0.42 [S]
SOL, *min*	25.9 (28.4)	14.0 (13.2)	**0.009**	**0.49 [S]**
WASO, *min*	74.2 (64.5)	50.7 (46.8)	0.080	0.40 [S]
Waketime, *min*	66.4 (58.5)	47.2 (46.1)	0.122	0.35 [S]
awakenings, *N*	19.2 (9.0)	16.0 (7.2)	0.099	0.38 [S]
awakening index, Nh^{-1}	2.95 (1.30)	2.63 (1.25)	0.278	0.25 [S]
Sleep architecture				
NREM Stage 1, *min*	38.8 (23.5)	36.2 (17.7)	0.594	0.12 [S]
Stage 2, *min*	241.1 (57.6)	247 (52.7)	0.624	0.11 [S]
Stage 3, *min*	21.3 (15.4)	19.6 (10.1)	0.515	0.12 [S]
Stage 4, *min*	13.1 (16.5)	17.0 (20.3)	0.331	0.22 [S]
REM sleep, *min*	73.1 (38.4)	88.7 (25.6)	**0.048**	**0.45 [S]**
NREM Stage 1, *%*	10.2 (6.8)	8.7 (5.0)	0.291	0.24 [S]
Stage 2, *%*	62.2 (8.5)	59.4 (8.1)	0.140	0.34 [S]
Stage 3, *%*	5.0 (4.2)	4.2 (2.7)	0.325	0.21 [S]
Stage 4, *%*	3.0 (4.2)	3.8 (4.6)	0.435	0.19 [S]
REM sleep, *%*	17.4 (7.7)	21.0 (5.1)	**0.021**	**0.52 [M]**
REM latency, *min*	143 (105.8)	107.8 (44.7)	**0.034**	**0.39 [S]**
RDI, h^{-1}	1.9 (5.0)	0.5 (0.8)	0.332	0.31 [S]
PLM-index, total, h^{-1}	18.8 (29.3)	15.2 (25.7)	0.664	0.13
PLM-index, w awakenings, h^{-1}	5.0 (5.9)	3.1 (6.6)	0.273	0.31 [S]

Note: REM = rapid eye movement, NREM = non-rapid eye movement, TST = total sleep time, SEI = sleep efficiency index, SOL = sleep onset latency, WASO = waketime after sleep onset, Awakening index = number of awakenings per hours of sleep period time, which is TST plus WASO, RDI: respiratory distress index, PLM-index: periodic limb movement index.

Table 5
Partial correlations adjusted to age between sleep-EEG measures, ISI and sBDNF in the total sample.

	ISI *r*	sBDNF *r*
Sleep continuity variables		
TST, *h:min*	-.129	.155
SEI, *%*	-.184°	.181
SOL, *min*	.176	-.204°
WASO, *min*	.202°	-.104
awakenings, *N*	.194°	.056
awakening index, Nh^{-1}	.150	.080
Sleep architecture		
NREM Stage 1, *min*	.046	.171
Stage 2, *min*	-.042	.076
Stage 3, *min*	.043	-.183
Stage 4, *min*	-.128	-.015
REM sleep, *min*	-.214*	.199°
NREM Stage 1, *%*	.127	.074
Stage 2, *%*	.157	-.139
Stage 3, *%*	.089	-.238*
Stage 4, *%*	-.101	-.025
REM sleep, *%*	-.255*	.234*
REM latency, *min*	.263*	-.185

Note: Pearson's correlations r (two-sided); °: $p < 0.10$, *: $p < 0.05$, **: $p < 0.01$.

sleep reflect two distinct neurophysiological processes.

Our first hypothesis was that sBDNF levels and insomnia severity would be inversely related and this was confirmed. Compared to healthy controls, insomnia patients had higher insomnia scores and lower sBDNF levels. This replicates previous findings (Giese et al., 2013, 2014a; Schmitt et al., 2016) but expands upon these in confirming the relationship in a larger sample of both diagnosed insomniacs and healthy controls. The association between sBDNF and insomnia was robust, even after controlling for depression, anxiety, and psychotropic medication. This finding is particularly impressive, as 1) sBDNF is lower in depression (Bocchio-Chiavetto et al., 2010) and 2) insomnia is a key symptom of major depression (Steiger and Kimura, 2010). Our results therefore suggest that sBDNF as biomarker is independently linked to the pathophysiological backgrounds of both insomnia and depression. As these disorders share activation of the stress response system, our results are consistent with the notion of stress-induced suppression of BDNF (cf. Duman et al., 1997). In other words, stress may induce different clinical syndromes such as insomnia, anxiety and depression, which is consistent with our finding that BDNF correlated with all three dimensions.

We treated as exploratory the question as to whether sBDNF indices are associated with objective sleep EEG parameters of sleep continuity and sleep architecture. We found that 1) sBDNF and objective sleep continuity dimensions were unrelated, while 2) there was a positive correlation with REM sleep percentage. This pattern is in line with findings reported by Deuschle et al. (2018) in a similar study of insomnia patients and healthy controls. As regards the mismatch of relationships between BDNF and subjective versus objective sleep, previous research has suggested that subjective insomnia and objective sleep are distinct clinical entities which are only weakly associated. In fact, objective sleep EEG measures of polysomnography are insufficient to discriminate between subjective insomnia and healthy sleep (Baglioni et al., 2014; Riemann et al., 2015). To conclude, as there is no close correlation between subjective and objective sleep, their associations with sBDNF levels should also be different.

As regards the positive correlation between BDNF and REM-sleep percentage, this is consistent with the relation observed between BDNF and insomnia, less REM-sleep in the IG and the negative correlation between insomnia severity and REM-sleep (%); the latter accord with results of a meta-analysis of polysomnography studies on insomnia disorders (Baglioni et al., 2014). There is evidence from numerous studies that insomnia is associated with reduced REM-sleep and increased REM-latency. And this consistent and replicable finding of a REM-sleep deficit has been identified as a key issue in insomnia (Riemann et al., 2012); REM sleep appears to play a key role in emotional processing with relevance to mood and anxiety disorders

(Goldstein and Walker, 2014), while insomnia is related to alterations of the emotional system resulting in emotional and physiological hyperarousal and hence sleep disturbances (Baglioni et al., 2010; Fernandez-Mendoza et al., 2015).

Concerning the positive correlation between BDNF and REM-sleep, preclinical studies support this relationship: 1) experimental REM-sleep deprivation reduced BDNF in brain stem regions but not in the hippocampus (Sei et al., 2000), indicating that lack of REM-sleep is at least partially related to a deficit in BDNF. 2) BDNF has been linked to REM sleep homeostasis: selective REM sleep deprivation (RSD) increases local BDNF expression in cholinergic cells in pedunculopontine tegmentum (PTT) and glutamatergic cells of the subcoeruleus nucleus (SubCD), both of which are involved in REM sleep generation. Moreover, BDNF/TrkB signalling in the PTT with induction of BDNF expression correlates with increased REM sleep homeostatic drive (RSHD) (Barnes et al., 2017; Datta et al., 2015). Finally, heterozygous BDNF (±) knockdown rats failed to exhibit an RSHD and no REM rebound after RSD (Garner et al., 2017). These findings suggest that acute RSD increases BDNF while a lack of BDNF expression correlates with weak REM homeostasis and a deficit in REM sleep, as we found for insomnia.

Interestingly, in our sample, we found a positive correlation between sBDNF and REM-sleep (%). This suggest that insomnia and decreased levels of BDNF may be functionally linked via REM-sleep regulation and brain stem circuits. Remarkably, in a study of depressed patients before and after partial sleep deprivation (i.e., deprivation of the second REM sleep-rich half of the night) deprivation induced both increased BDNF serum levels and a REM-sleep rebound during the recovery night (Beck et al., 2010; Giese et al., 2014b). Moreover, a flexible and normalized circadian BDNF rhythm seemed to be a precondition of these homeostatic interactions (Giese et al., 2014b).

The overall pattern of results suggests that sBDNF is a more precise biomarker for subjectively assessed insomnia than for objective sleep-EEG dimensions. However, impairment of REM sleep regulation may link insomnia and BDNF.

Following the “neurotrophin hypothesis of insomnia”, our second exploratory question was whether consistent low levels of sBDNF in insomnia were related to hyperactivity of the stress response system, specifically elevated HPA-AA reflected in raised saliva morning cortisol. However, this hypothesis received no support; insomnia was characterized by lower saliva morning cortisol while sBDNF did not correlate with CAR measures at all.

With respect to research on HPA-activity in insomnia, we note that though insomnia is acknowledged as state of hyperarousal (Bonnet and Arand, 2010; Riemann et al., 2010, 2015) and hyperactivity of HPA axis is expected, findings regarding cortisol levels are more complex. In longitudinal studies with nocturnal trajectories of plasma cortisol, cortisol levels most consistently increased in the evening and at midnight (Rodenbeck et al., 2001, 2002; Vgontzas et al., 2001; Seelig et al., 2013), but not in the morning (Rodebeck et al., 2002; Vgontzas et al., 2001; Seelig et al., 2013; Riemann et al., 2002). More specifically, studies of morning saliva cortisol and CAR in insomnia found a lower AUCtotal but also a steeper rise in CAR (i.e., AUCnetto) (Backhaus et al., 2004; Hansen et al., 2012; Zhang et al., 2014; Abell et al., 2016). As in our study, Backhaus et al. (2004) also found a negative correlation between severity of insomnia and decrease in morning salivary cortisol.

Following to Duman et al. (1997), our third exploratory question concerned a possible association between an increased stress response system indicated by CAR and lower levels of sBDNF; no such association was found. As proposed above, CAR may not reflect HPA-activity in general over the entire day, and if evening and nocturnal cortisol levels do increase this may amount to a netto hypercortisolism despite decreased CAR. However, we did not have the data able to test this possibility. It is also conceivable that suppression of sBDNF in insomnia depends on mechanisms other than increased levels of glucocorticoids. Consequently, further research is needed to explain the association between sBDNF levels and insomnia severity.

4.1. Limitations

The following considerations warn against overgeneralization of the present results. First, we assessed patients’ objective sleep only once. While there are conflicting results as regards the so-called first-night effect in patients with chronic insomnia, a second assessment might have provided more insight into the association between objective sleep and sBDNF. Second, while the standard procedures to assess sleep and gather blood and saliva samples were rigorously supervised, it is conceivable that small differences in the timing of sampling might have produced different blood and saliva levels; circadian changes in cortisol and sBDNF secretions could therefore have blurred the relationships. However, as regards the saliva/plasma ratio (SPR) of cortisol, Bosch (2014) reported in the review that reliable measurements of blood borne constituents implies a constant, such that the cortisol concentration in saliva reliably follows intra- and interindividual variations in plasma. Finally, most of our insomnia patients were taking hypnotics or antidepressants, which could have an impact on sleep architecture such as suppression of REM sleep. The sleep EEG related results should therefore be interpreted carefully. In this view, participants with insomnia and reporting medication intake were also those participants with lower sBDNF levels, along with higher symptoms of depression and anxiety. While medication intake remained unaltered during the entire study assessment, and while the quality of the data does not allow the causal and temporal relationship between sBDNF levels, symptoms of insomnia, depression and anxiety, and medication intake, future studies might apply longitudinal designs to deal with such issues.

5. Conclusion

In line with our previous research, we found low levels of sBDNF are strongly associated with subjective insomnia both in a categorial and a dimensional fashion. We found no association between objective poor sleep and sBDNF, consistent with the loose association between insomnia and objective measures of sleep disturbances. The pathophysiological background of insomnia and its link to sBDNF requires further research.

Conflicts of interest

All authors declare no conflicts of interest.

Contributors

JB, AE and EHT designed the study and wrote the protocol, and all were involved in the literature searches and analyses. JB and TM were highly engaged with data collection and data entry. AE supervised the laboratory analysis of BDNF. SB and TM undertook the statistical analyses. TM drafted the manuscript and coordinated the integration of authors' comments and corrections. EHT and JB are TM's senior researchers. All authors contributed to and have approved the final manuscript.

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