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Pineal gland volume in primary insomnia and healthy controls: a magnetic resonance imaging study

JAN M. BUMB[1], CLAUDIA SCHILLING[1], FRANK ENNING[1], LEILA HADDAD[1], FRANC PAUL[1], FLORIAN LEDERBOGEN[1], MICHAEL DEUSCHLE[1], MICHAEL SCHREDL[1] and INGO NOLTE[2]

[1]Department of Psychiatry and Psychotherapy, Central Institute of Mental Health, Medical Faculty Mannheim, Heidelberg University, Mannheim, Germany and [2]Department of Neuroradiology, University Hospital Mannheim, Mannheim, Germany

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Correspondence
Jan Malte Bumb, Central Institute of Mental Health, J5, 68159 Mannheim, Germany.
Tel.: +49 621 1703 6161;
fax: +49 621 1703 2325;
e-mail: bumb@cimh.de

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SUMMARY

Little is known about the relation between pineal volume and insomnia. Melatonin promotes sleep processes and, administered as a drug, it is suitable to improve primary and secondary sleep disorders in humans. Recent magnetic resonance imaging studies suggest that human plasma and saliva melatonin levels are partially determined by the pineal gland volume. This study compares the pineal volume in a group of patients with primary insomnia to a group of healthy people without sleep disturbance. Pineal gland volume (PGV) was measured on the basis of high-resolution 3 Tesla MRI (T1-magnetization prepared rapid gradient echo) in 23 patients and 27 controls, matched for age, gender and educational status. Volume measurements were performed conventionally by manual delineation of the pineal borders in multi-planar reconstructed images. Pineal gland volume was significantly smaller ($P < 0.001$) in patients (48.9 ± 26.6 mm^3) than in controls (79 ± 30.2 mm^3). In patients PGV correlated negatively with age ($r = -0.532$; $P = 0.026$). Adjusting for the effect of age, PGV and rapid eye movement (REM) latency showed a significant positive correlation ($r_S = 0.711$, $P < 0.001$) in patients. Pineal volume appears to be reduced in patients with primary insomnia compared to healthy controls. Further studies are needed to clarify whether low pineal volume is the basis or the consequence of functional sleep changes to elucidate the molecular pathology for the pineal volume loss in primary insomnia.

INTRODUCTION

Insomnia is characterized by difficulties in falling asleep, maintaining sleep or non-restorative sleep accompanied by significantly impaired daytime functioning in the absence of a specific physical, mental or substance-related cause (Riemann *et al.*, 2010). Moreover, insomnia patients suffer from 'hyperarousal' on an autonomous and central nervous level (Feige *et al.*, 2013), indicated by highly significantly elevated numbers of micro-arousals and awakenings during rapid eye movement (REM) sleep in comparison with healthy good sleepers.

Recently, several magnetic resonance imaging (MRI) and computerized tomography (CT) studies assessed the prevalence of pineal calcification (Mahlberg *et al.*, 2009; Nolte *et al.*, 2009) and pineal cysts (Bumb *et al.*, 2012), speculating about their potential role in the aetiopathology of various disorders (e.g. sleep disorders) by disturbing melatonin release. Morphological changes, such as cysts or calcifications of the pineal gland, might cause altered melatonin secretion due to reduced functional parenchyma. Melatonin levels in human plasma have been shown to correlate linearly with solid pineal gland volume (PGV) (Nolte *et al.*, 2009). A pilot CT study has demonstrated that the degree of pineal calcification might cause alterations in the sleep–wake cycle (e.g. chronic daytime tiredness and sleep disturbances) (Kunz *et al.*, 1998).

For a long time, volumetric analyses of PGV have been carried out by post-mortem measurements (Golan *et al.*, 2002; Hasegawa *et al.*, 1987). In healthy volunteers, MRI evaluations on PGV are rare, and only few studies provide reference data. In a Chinese population, the mean PGV of healthy

volunteers was 94.20 ± 40.65 mm^3 (Sun *et al.*, 2009). In clinical samples (inpatients suffering from various medical diagnoses), comprising infants and adults, PGV ranged between 94.3 ± 159.1 mm^3 in infants (Bumb *et al.*, 2012) and 78.33 ± 89.0 mm^3 in adults (Bumb *et al.*, 2013). Intriguingly, interrelations of PGV and insomnia have not yet been addressed. In schizophrenia, PGV was found to be reduced in male patients compared to healthy controls (Bersani *et al.*, 2002), whereas PGV did not differ significantly between bipolar patients and healthy controls (Sarrazin *et al.*, 2011).

To address these issues, in this study we provide comparative volumetric analyses of the pineal gland in patients suffering from primary insomnia and age- and sex-matched healthy volunteers and a volumetric data link with both polysomnographic parameters and sleep quality scores. We hypothesize that primary insomnia may be related to reduced pineal gland volume and that the latter may be correlated with parameters of disturbed sleep.

MATERIALS AND METHODS

Subjects

The present sample comprised 23 untreated patients suffering from primary insomnia and 27 healthy controls matched groupwise according to age, gender and educational status. The control group comprised 16 females and 11 males (age range 21–67 years; mean age 39 ± 13.1 years). The patient group comprised 11 females and 12 males (age range 25–55 years; mean age 43 ± 7.4 years). Mean duration of illness was 8.6 ± 7.3 years (range 0.5–30 years).

Insomnia patients fulfilled the pertinent diagnostic criteria as defined by the *Diagnostic and Statistical Manual IV*–text revision (DSM-IV-TR) (American Psychiatric Association, 2000) and the *International Classification of Sleep Disorders*, 2nd edn (ICSD-2) (Edinger *et al.*, 2004).

All patients were recruited from our outpatient sleep disorders clinic. Healthy control subjects were recruited from the community via a newspaper advertisement. Exclusion criteria for both groups comprised present or past psychiatric or central nervous system disorders, pathological MRI findings (not including pineal structural abnormalities), severe other somatic diseases, substance dependency or substance abuse (except nicotine), as well as intake of any psychopharmacological drugs within the last 2 weeks. In both groups, sleep disorders not detected previously by sleep history, such as sleep-related breathing disorders and periodic limb movements during sleep, were ruled out by polysomnography.

In addition, the 'Schlaffragebogen B' sleep questionnaire (SF-B) (Goertelmeyer, 1986) was administered in 19 patients and 11 healthy controls. The SF-B comprises 28 items measuring composite scores such as sleep quality (11 items). The estimates refer to the previous 2 weeks. The interitem consistency for the composite scores range from $r = 0.77$ to $r = 0.87$ and the retest reliability (4 weeks) was approximately $r = 0.70$ (Goertelmeyer, 1986). Construct validity was shown in several factor analyses and comparisons with expert ratings were satisfactory; for example, $r = -0.67$ between sleep quality and the degree of insomnia (Goertelmeyer, 1986).

The local ethics committee approved the study and written informed consent was obtained from all patients and controls prior to enrolment in the study.

MRI protocol and estimation of PGV

MRI scans were performed using one 3-T MR scanner (Magnetom Trio, Siemens, Erlangen, Germany). MRI sequences for all subjects comprised EPI (echo planar imaging) (TR/TE 2000 ms per 30 ms, matrix 64 × 64, axial slices with 1-mm gap), and magnetization prepared rapid gradient echo (MPRAGE) (TR/TE 1570 ms per 28 ms, field of view 256 × 256, matrix 256 × 256, slice thickness 1 mm).

All MR images were checked for artefacts affecting the image interpretation (movement artefacts, flow artefacts, etc.) by an experienced neuroradiologist (IN) and all images were evaluated digitally using OsiriX software (www.osirix-viewer.com). PGV was measured blinded to diagnosis by defining the pineal borders manually on transversal reconstructed MPRAGE sequences. For identification of the pineal gland, the adjacent quadrigeminal plate, the medial borders of the thalami and the habenulae were identified. The pineal gland can be identified readily as it is surrounded by cerebrospinal fluid, except for the attachment to the habenulae. Special care was taken to discriminate between the pineal gland and the adjacent vessels (as the thalamostriate veins).

To determine the intrarater variability, the same evaluator assessed all data sets a second time with a time gap of 6 months. To determine the inter-rater variability, a second evaluator unaware of the results of the first evaluation assessed 10 randomly chosen data sets.

Polysomnography

All subjects underwent 2 consecutive nights of polysomnographic recording in our sleep laboratory. The first night was considered an adaptation night, and only the results of the second night were used for analysis. Polysomnographies were performed with a Schwarzer comlab 32 polysomnograph (Schwarzer GmbH, Munich, Germany) using a standard polysomnography montage according to the American Academy of Sleep Medicine (AASM) criteria, including electroencephalography in seven derivations (F3, F4, C3, C4, Cz, O1 and O2, linked to mastoids), left and right electrooculography, chin electromyography and surface electromyography of both tibialis anterior muscles, and recording electrocardiogram and respiratory variables (oronasal airflow, thoracal and abdominal respiratory effort and oxygen saturation). Sleep stages and periodic limb movements in sleep were recorded and scored according to standard procedures (Bonnet *et al.*, 1992, 1993; Iber *et al.*, 2007). Sleep stage scoring and counting of arousals and periodic limb

movements during sleep were performed visually by an experienced technician blind to the patient's condition and supervised by one of the authors (MS). The parameters evaluated involved sleep efficiency (%) (SE), sleep latency (min), total sleep time (TST) (min), wake time [% sleep period time (SPT)], sleep stage 1 (% SPT), sleep stage 2 (% SPT), sleep stage 3 (% SPT), rapid eye movement latency (REML) and REM density, as well as arousal index (per hour of SPT).

Statistics

Statistical analyses were performed using SPSS version 20 (IBM Corporation, Armonk, NY, USA) for Macintosh. Sociodemographic characteristics and differences in sleep quality were compared between both groups using the two-sample *t*-test. Regarding the MRI data, volume differences were assessed by Mann–Whitney *U*-test, because of non-normality of the data distribution (according to the Shapiro–Wilk test) and the relatively small sample size.

For the intra- and inter-rater variability of the volume measurements, Spearman's rank correlation test was determined. Further, in an exploratory analysis Spearman's rank correlation test was performed to detect correlations between PGV and both age and duration of illness, as well as associations between PGV and polysomnographic parameters. To explore the correlation between PGV and REM latency, adjustment for the effect of age first-order partial correlation (first-order Pearson's correlation) was performed. To determine the correlation between PGV and sleep quality, Pearson's correlation coefficient was conducted. Following this, if multiple correlations were used Bonferroni's correction was applied to significance values. *P*-values of less than 0.05 were considered significant. Descriptive values are given as mean ± standard deviation if not specified otherwise. Outliers were identified by *Z*-transformation (condition: significance level 0.0233, *Z*-values > ± 1.99) and were excluded from the analysis.

RESULTS

Reliability of volume measurements

All images were fully utilizable, and none of the images had to be excluded because of impaired image quality. Intrarater reliability was satisfactory (PGV: mean of first assessment 64.8 ± 32.3 mm^3, mean of second assessment 67.6 ± 31.3 mm^3). The Spearman's correlation coefficient was $r_S = 0.949$ ($P < 0.01$). There was no difference between the evaluations ($P < 0.01$). Analyses of inter-rater variability revealed that there was no difference between the two evaluators ($P < 0.01$). Spearman's correlation coefficient between both evaluators was $r_S = 0.833$ ($P < 0.01$).

Pineal volume and SF-B

Patients and healthy controls did not differ significantly concerning sociodemographic data, such as gender ($P = 0.430$), age ($P = 0.225$) and educational status ($P = 0.298$). PGV ranged from 21.1 to 138 mm^3 (48.9 ± 26.6 mm^3) in insomnia patients and from 33.1 to 128.5 mm^3 (mean 79 ± 30.2 mm^3) in healthy controls (one outlier was excluded by *Z*-transformation, PGV 192.5 mm^3). PGV in patients was significantly smaller than in controls ($P < 0.001$) (Fig. 1). PGV correlated negatively with age in insomnia patients ($r = -0.532$; $P = 0.026$, Bonferroni-adjusted) (Fig. 2), but not in healthy controls ($r_S = -0.058$; $P = 0.774$). PGV did not correlate with the duration of illness in insomnia patients ($r_S = -0.004$; $P = 0.986$).

The mean composite score of sleep quality was 2.7 ± 0.6 in insomnia patients and 4.4 ± 0.3 in healthy controls. The difference was statistically significant ($P < 0.001$, using the two-sample *t*-test, because data were distributed normally according to the Shapiro–Wilk test), confirming that patients had poorer sleep quality than healthy controls.

Sleep quality was associated significantly with PGV in the whole sample ($r = 0.632$, $P < 0.01$, Bonferroni-adjusted), showing an almost significant association in the insomnia subgroup ($r = 0.453$, $P = 0.06$, not corrected) but no significant correlation in healthy controls ($r = 0.037$, $P = 0.918$).

Polysomnography

Polysomnographic sleep data of the primary insomnia sample differed from healthy controls in the expected way: mean TST was 382.9 ± 39.3 min in healthy controls and 368.5 ± 53 min in insomnia patients. Mean SE was 85.5 ± 8.3% in healthy controls and 80.1 ± 11% in patients. Mean REML was 86.1 min in healthy controls (range 46.5–210 min) and 81.4 min (range 62–123 min) in patients (further details are given in Table 1).

Pineal volume and polysomnographic parameters

In insomnia patients, no significant correlation of PGV and polysomnographic parameters was revealed (PGV versus REM latency $r_S = 0.336$, $P = 0.136$; PGV versus TST $r_S = 0.135$, $P = 0.561$, PGV versus SE $r_S = 0.082$,

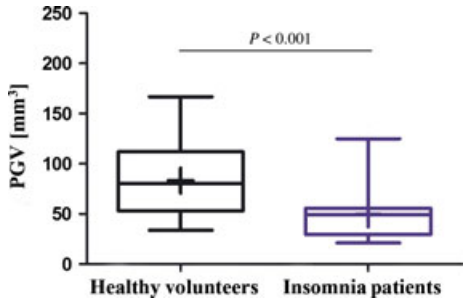

Figure 1. Box-and-whiskers plots (box shows 5 and 95 percentiles and mean value; one outlier has been excluded by *Z*-transformation: *Z*-score > ± 1.99) of pineal gland volume in healthy volunteers and insomnia patients. *P*-values were derived from the Mann–Whitney *U*-test.

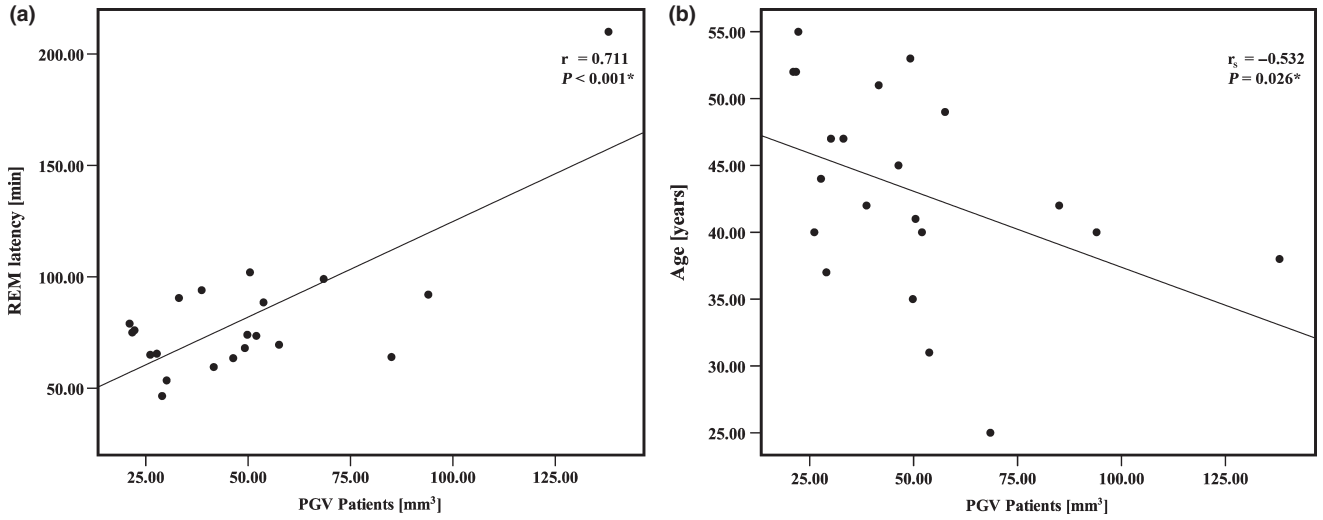

Figure 2. Spearman's correlation analyses of (a) pineal gland volume and rapid eye movement (REM) latency in insomnia patients adjusting for the effect of age and of (b) pineal gland volume and age in insomnia patients (*adjusted statistical significance).

Table 1 Polysomnographic parameters of healthy volunteers and insomnia patients

	Healthy volunteers	*Insomnia patients*
TST (min)	382.9 ± 39.3	368.5 ± 53
SE (TST/TIB)	85.5 ± 8.3	80.1 ± 11
Wake time	6.1 ± 4.6	12.5 ± 7.1
Sleep latency (min)	29.7 ± 16.8	20.1 ± 16.5
REM latency (min)	86.1 ± 40.9	81.4 ± 32.2
REM density	19.4 ± 6.8	19.2 ± 6.6
Arousal index	11.7 ± 6.2	14.4 ± 5.6
N1 (% SPT)	8.6 ± 4.8	13.5 ± 5.8
N2 (% SPT)	50.3 ± 7.8	50.3 ± 9.2
N3 (% SPT)	21 ± 12.1	11.4 ± 8.3
REM (% SPT)	13.1 ± 5.2	12.3 ± 4.4

Values are given as mean ± standard deviation.
TST, total sleep time; TIB, time in bed; SE, sleep efficiency; REML, rapid eye movement latency; N1, sleep stage 1 (% SPT); N2, sleep stage 2 (% SPT); N3, sleep stage 3 (% SPT); REM, REM sleep (% SPT); arousal index (per hour of SPT): wake time [% sleep period time (SPT)].

$P = 0.722$). Adjusting for the effect of age, PGV and REM latency showed a significant positive correlation ($r_S = 0.711$, $P < 0.001$, Bonferroni-adjusted), whereas results did not change concerning TST and SE (PGV versus TST $r_S = 0.04$, $P = 0.866$; PGV versus SE $r_S = 0.711$, $P = 0.993$).

In healthy controls, PGV was not correlated significantly with any polysomnographic parameter (PGV versus REM latency $r_S = 0.391$, $P = 0.235$; PGV versus TST $r_S = 0.327$, $P = 0.326$, PGV versus SE $r_S = 0.264$, $P = 0.433$). Adjusting for the effect of age, results did not change significantly (PGV versus REM latency $r_S = 0.417$, $P = 0.231$; PGV versus TST $r_S = 0.386$, $P = 0.270$, PGV versus SE $r_S = 0.135$, $P = 0.231$).

DISCUSSION

To our knowledge, this is the first comparison of MRI analyses of PGV in patients suffering from primary insomnia and healthy controls. In the present study, insomnia patients were characterized by significantly smaller PGV (Figs 1 and 3). Moreover, our results may suggest a correlation between PGV and sleep quality, which is in line with the findings from a very recent study showing a negative correlation trend ($r = –0.17$, $P = 0.089$) between solid pineal parenchyma and the sleep quality disturbance subscore of the Landecker Inventar für Schlafstörungen (LISST) in a homogeneous population without sleep impairments (Liebrich *et al.*, 2013).

Insomnia, pineal gland volume and melatonin

Small pineal glands might lead to low melatonin levels in both serum and cerebrospinal fluid, and thus poor sleep. Very recently, it has been demonstrated that 24-h melatonin serum levels in 15 healthy male volunteers correlated with pineal gland morphology. Solid PGV (cysts and calcifications excluded) correlated positively with hallmarks of the diurnal melatonin rhythm (Nolte *et al.*, 2009). This finding is in line with other reports (Kunz *et al.*, 1999; Mahlberg *et al.*, 2009) demonstrating that the degree of pineal calcification is correlated negatively with total sleep time and sleep efficiency, on one hand, and that uncalcified pineal tissue is correlated positively with 24-h excretion of 6-sulphatoxymelatonin on the other hand.

In young and elderly patients suffering from primary insomnia, nocturnal plasma melatonin levels were diminished compared to those in healthy controls (Riemann *et al.*, 2002). It seems conceivable that reduced melatonin secretion in insomnia patients may be related to smaller PGV. These results support the hypothesis that pineal gland abnormalities

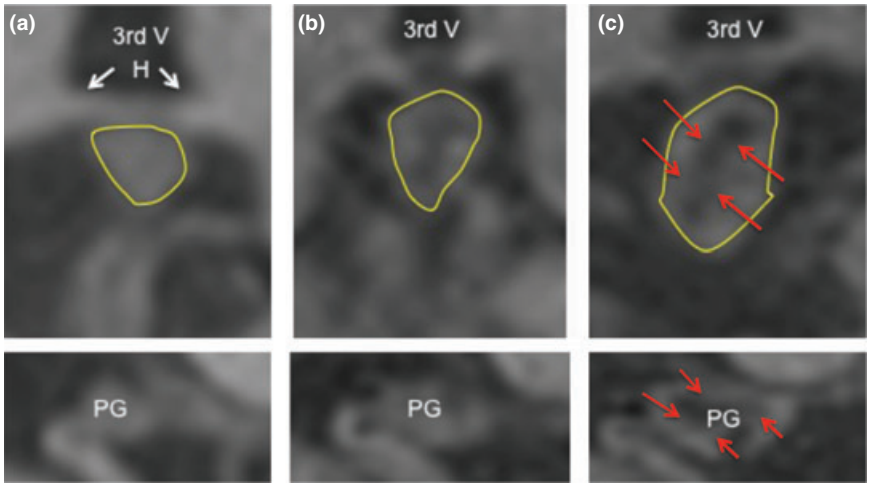

Figure 3. Magnetization prepared rapid gradient echo (MPRAGE) imaging of the pineal region illustrating the spectrum of pineal glands (PG) (upper row: axial reconstruction; lower row: sagittal reconstruction). (a) Smallest PG, 21.05 mm^3; (b) intermediate PG 90.85 mm^3; (c) largest PG 192.45 mm^3. The pineal gland was axially reformatted along its long axis. Contours of PG are shown in colour. Red arrows point to the habenulae. Arrows mark structural abnormalities (SA). H, habenula; third V, third ventricle; PG, pineal gland.

might cause disruptions in melatonin secretion, leading consequently to sleep problems or even insomnia.

At this point, however, it is not clear whether smaller glands reflect the cause or the consequence of insomnia. The observation that there was no correlation between PGV and the duration of illness, however, does not support the idea of small PGV being a consequence of insomnia.

In insomnia patients, age and PGV were correlated negatively. A very recent study (Bumb *et al.*, 2013) showed a negative correlation of PGV and age ($r = -0.130$, $P = 0.016$) in a large clinical sample, comprising different diagnostic groups. In healthy individuals the influence of age on PGV seems negligible (Golan *et al.*, 2002). Moreover, it is a well-known fact that sleep processes underlie changes linked to ageing. Older humans often complain about poorer sleep and shorter sleep duration accompanied by decreased sleep efficiency (Unruh *et al.*, 2008). Based on our results, we suggest that this effect is even more pronounced in insomnia patients, and prerequisites for insomnia might be prerequisites for an age-related degenerative process resulting in smaller PGV. Unfortunately, the present study did not record the use of neuropharmacological drugs dating back more than 2 weeks or during child- and adulthood. Therefore, a medication effect on the growth of PGV cannot be entirely excluded.

It has been hypothesized that PGV in humans underlies a strong genetic influence and reaches its final size in early childhood (Schmidt *et al.*, 1995; Wetterberg *et al.*, 1983). Moreover, in addition to genetic determination one can speculate as to whether epigenetic factors, i.e. environmental conditions during pregnancy, such as light exposure at night, interindividual differences in maternal rhythmicity (e.g. morningness versus eveningness) and sleep disorders, as well as medication, might have an impact on the growth of fetal PGV, resulting in smaller pineal glands in the newborn which, again, might cause sleep disturbances in the years to come. One study in primates (Seron-Ferre *et al.*, 2002) and one in rodents (Feng *et al.*, 2012) provided evidence that certain conditions during pregnancy might influence the development of the newborn circadian system and its components. Similar findings have been demonstrated in other animal studies (reviewed in Seron-Ferre *et al.*, 1993). A compelling question within this context is why sleep problems would occur at such a late stage if the pineal gland is fully grown in early childhood (Schmidt *et al.*, 1995; Wetterberg *et al.*, 1983). Next to genetic and early-life environmental factors, an increase of dysfunctional tissue (i.e. calcification) may contribute to 'low' PGV as a predisposing factor of primary insomnia. Moreover, sleep habits change with age and sleep processes are impaired in old age (Touitou, 2001).

Adjusting for the effect of age, an explorative analysis revealed a significant correlation between PGV and REM latency in insomnia patients. Mean REM latency was shorter in insomnia patients, reflecting substantial disturbances of normal sleep processes. This finding is in line with results by Monti *et al.* (1999), who had conducted a trial on the polysomnographic effects of exogenous melatonin in insomnia patients. Compared to healthy controls, patients were characterized by reduced REM sleep latency and REM sleep time. Interestingly, REM sleep latency was increased after administration of exogenous melatonin. Kunz *et al.* (2004) inferred that REM sleep is impaired in the context of many sleep disorders. In addition, it has been stated that exogenous melatonin increases sleep propensity in healthy volunteers while decreasing both sleep onset latency and REM latency (Caldwell, 2000).

In summary, our results (i.e. significantly smaller PGV in patients and positive correlation of both PGV and REM latency and PGV and sleep quality) may suggest a linkage between PGV and sleep processes. Follow-up MRI investigations of patients suffering from primary insomnia could help to assess whether small pineal glands represent a cause or an effect of this illness.

A limitation of the present study is that it cannot resolve the degree to which pineal calcifications and cysts might have influenced the results of the PGV measurements and the correlation analyses between PGV, polysomnographic

parameters and sleep quality because no additional CT scans or further MRI sequences were performed. Therefore, it was not possible to analyse functional parenchyma instead of considering the total pineal volume. Moreover, it should be kept in mind that our findings might be influenced by a selection bias with respect to the patients we have analysed. This group sought help in a sleep clinic and may be characterized by potential risk factors for the occurrence of primary insomnia, including small pineal gland, low melatonin levels as well as poor sleep/insomnia and resulting psychological strain. Insomnia patients combining more than one of these risk factors may be more likely than others to seek help in a sleep clinic. Further studies, comprising bigger samples and performing additional CT scans, are needed to clarify this topic.

CONCLUSION

In the present study, PGV was significantly smaller in primary insomnia patients than in healthy controls and PGV was correlated significantly with sleep quality in the whole sample. An exploratory analysis of the relation of PGV to polysomnographic parameters, adjusting for the effects of age, showed a positive correlation of PGV and REM latency in insomnia patients. Our results corroborate the hypothesis that small PGV may be related to the pathophysiology of insomnia. The causal relationship, however, needs further investigation.

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AUTHOR CONTRIBUTIONS

JMB and IN performed the majority of the experiments, volumetric evaluation and statistical analyses; IN and JMB designed the study and wrote the manuscript; CS, FP and LH performed the MRI investigations; CS, MS and FP performed the polysomnographic investigations; CS, FE, LH, FP, FL, MD and MS edited the manuscript.

CONFLICT OF INTEREST

MD and his group received honoraria for lectures and consulting from Bristol-Myers Squibb, Servier and Otsuka Pharma. CS received funding by the Olympia-Morata-Foundation of the University of Heidelberg not related to this study. Other authors declare no conflicts of interest.

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