SLEEPJ, 2022, 1–13

https://doi.org/10.1093/sleep/zsab241
Advance Access Publication Date: 24 September 2021
Original Article

ORIGINAL ARTICLE

Sleep duration, sleep problems, and perceived stress are associated with hippocampal subfield volumes in later life: findings from The Irish Longitudinal Study on Ageing

Céline De Looze[1,*], Joanne C. Feeney[1], Siobhan Scarlett[1], Rebecca Hirst[1], Silvin P. Knight[1], Daniel Carey[1], James F. Meaney[2] and Rose Anne Kenny[1,3]

[1]The Irish Longitudinal Study on Ageing, Trinity College Dublin, Dublin, Ireland, [2]The National Centre for Advanced Medical Imaging (CAMI), St James's Hospital, Dublin, Ireland and [3]Mercer's Institute for Successful Ageing (MISA), St James's Hospital, Dublin, Ireland

*Corresponding author. Céline De Looze, The Irish Longitudinal Study on Ageing (TILDA), Trinity College Dublin, 152-160 Pearse St, Dublin 2, Ireland. Email: deloozec@tcd.ie.

Abstract

Study Objectives: This study examines the cross-sectional and 2-year follow-up relationships between sleep and stress and total hippocampal volume and hippocampal subfield volumes among older adults.

Methods: Four hundred seventeen adults (aged 68.8 ± 7.3; 54% women) from the Irish Longitudinal Study on Ageing completed an interview, a questionnaire, and multiparametric brain magnetic resonance imaging. The relationships between self-reported sleep duration, sleep problems, perceived stress, and total hippocampal volume were examined by using ordinary least squares regressions. Linear mixed-effects models were used to investigate the relationships between sleep duration, sleep problems, perceived stress, changes in these measures over 2-years, and hippocampal subfield volumes.

Results: No cross-sectional and follow-up associations between sleep and total hippocampal volume and between stress and total hippocampal volume were found. By contrast, Long sleep (≥9–10 h/night) was associated with smaller volumes of molecular layer, hippocampal tail, presubiculum, and subiculum. The co-occurrence of Short sleep (≤6 h) and perceived stress was associated with smaller cornu ammonis 1, molecular layer, subiculum, and tail. Sleep problems independently and in conjunction with higher stress, and increase in sleep problems over 2 years were associated with smaller volumes of these same subfields.

Conclusion: Our study highlights the importance of concurrently assessing suboptimal sleep and stress for phenotyping individuals at risk of hippocampal subfield atrophy.

Statement of Significance

Our study shows that Long sleep (≥9–10 h/night), Short sleep (≤6 h) in conjunction with higher stress, and sleep problems may represent clinically relevant markers for phenotyping individuals at risk of hippocampal structural atrophy in older age. It supports an intertwined relationship between sleep and stress whereby both may synergistically contribute to hippocampal subfield damage and structural changes. Our findings highlight the importance of concurrently assessing suboptimal sleep and stress in neuropsychiatric research for the development of targeted interventions aiming at reducing the risk of late-life hippocampal atrophy.

Key words: sleep duration; disturbance; stress; hippocampal subfields

Submitted: 1 June, 2021; **Revised:** 15 September, 2021

Introduction

Aging is often associated with volume reduction of the hippocampus (HP). There is marked heterogeneity in the degree to which the HP is affected by age across individuals. The underlying causes and mechanisms of these differences are still poorly understood besides the well-established association between hippocampal atrophy and memory impairments in older age and the highly predictive value of hippocampal volume for the development of Alzheimer's disease (AD) [1]. Other risk factors for hippocampal atrophy other than age may also contribute to individual differences in hippocampal volume, encompassing genetic, environmental, and lifestyle factors.

Sleep disturbances and sleep dissatisfaction, including nighttime awakenings, earlier waking, and increased daytime sleepiness, are common complaints with advancing age [2, 3]. Negative associations between hippocampal volume and sleep deprivation severity or duration have been reported in chronic primary insomnia [4, 5], sleep apnea [6, 7], narcolepsy [8] and depression [9, 10], disorders characterized by sleep disruption. In healthy older adults, reduced hippocampal volume was associated with short sleep [4] and sleep disturbance [4, 11]. Longitudinal associations in a large sample of cognitively normal participants (18–90 years) from the European Lifebrain consortium showed 0.22% greater annual hippocampal volume loss for those with worse sleep quality, problems, and daytime tiredness [12]. In animal studies, chronic sleep restriction and disruption were associated with impaired neurogenesis, reduced cell proliferation, altered synaptic plasticity, and dendritic atrophy in the HP [13–16]. These processes may result in hippocampal shrinkage and impaired hippocampal function. Several rodent studies support the causal role of prolonged sleep restriction and disruption in reducing hippocampal volume [17]. Chronic sleep restriction to 4 hours per day for a month resulted in a reduction of the HP by 10% [17]. Cumulative evidence, therefore, suggests that sleep may be an important risk factor for hippocampal atrophy.

Chronic stress and elevated glucocorticoid levels have also been associated with lower hippocampal volume and hippocampal dysfunction in aged rats [18, 19], Cushing's syndrome [20, 21], post-traumatic stress disorder (PTSD) [22, 23], AD [24], and healthy older adults [25]. Stress signaling is initiated through the hypothalamic-pituitary-adrenal (HPA) axis via secretion of the glucocorticoid hormone cortisol. Cortisol is essential in responding and adapting to stressful events but prolonged elevated glucocorticoid levels may lead to maladaptive response to stressors [26]. The HP controls the cortisol-induced feedback inhibition of the HPA axis [27, 28]. It contains a high concentration of glucocorticoid and mineralocorticoid steroid receptors, which makes it particularly vulnerable to permanent neuronal and synaptic damage from prolonged stress exposure [28, 29]. With chronic stress, cortisol-induced damage of the HP may result in the remodeling of its structure and function, which in turn may lead to HPA axis hyperactivity, raised baseline cortisol levels, and circular damage to the HP [30]. Chronic stress may therefore be considered as another important driver of reduced hippocampal volume.

The evidence of a relationship between poor sleep and hippocampal volume and between chronic stress and hippocampal volume is however mixed as other human studies report no association [12, 31–34]. One reason for these inconsistent findings may be due to the low sensitivity of a global hippocampal volumetric approach, which may obscure hippocampal subfield distinct vulnerability to sleep deprivation and disruption and prolonged exposure to stress. Evidence from animal studies suggests that hippocampal subfields are distinctively vulnerable to these effects [28, 35, 36]. The HP is not a homogeneous structure but consists of multiple subfields with distinct histology, connectivity, and functional specialization. Experimental sleep deprivation and fragmentation in rodents have consistently been associated with impaired neurogenesis and reduced cell proliferation in the dentate gyrus (DG) [13, 35]. The hippocampal subfield Cornu Ammonis-1 (CA1), Cornu Ammonis-3 (CA3), and DG were also found to be particularly vulnerable to the neurotoxic effects of glucocorticoid exposure or chronic stress [20, 28, 29, 36]. In-vivo human studies, albeit scarce, showed that sleep disturbance was associated with lower CA1 volume in chronic primary insomnia [5] and with smaller CA3 and DG in PTSD [37]. In addition, perceived stress was negatively correlated with CA2/CA3 and CA4/DG volumes in healthy older adults [38]. Higher cortisol levels were associated with smaller right CA1-3 in healthy older adults [39] and smaller left CA1-3 in major depressive disorder [39].

Investigating the effects of sleep and stress on the hippocampal components may provide better insights into the pathophysiological mechanisms that are involved in hippocampal atrophy. However, to date, human imaging studies examining the impact of sleep disturbance and chronic stress on hippocampal subfield volumes are scarce and have not examined the combined effect of sleep and stress on hippocampal subfield volumes [4, 12, 31, 32, 40, 41]. It is not entirely clear whether these factors have independent, additive, or mediating effects on the HP. There is however biological and psychological evidence for an interconnected relationship between these two factors [42]; they may contribute to pathophysiological dysfunction in each other and may partly explain the individual differences in hippocampal subfield volumes observed in older age. Although some animal studies support the distinct contribution of sleep deprivation and acute stress on hippocampal structural plasticity [43], a frequently raised issue is whether the observed effect of sleep on hippocampal structure and function is mediated through stress, induced by forced wakefulness; or, conversely, whether chronic stress leads to reduced sleep quality and duration and ultimately to hippocampal damage. Sleep disturbance and shorten sleep duration correlate with higher awakening and evening cortisol levels [44, 45] and induction of high doses of cortisol has been shown to increase wakefulness [46], suggesting an active synergy between these two factors. On one hand, chronic sleep deprivation may cause baseline glucocorticoid levels to rise, leading to HPA dysregulation. On the other hand, a disruption in HPA regulation, resulting from prolonged exposure to stress, may disrupt the circadian rhythm and cause sleep disturbances. The detrimental effects of stress on sleep are well described in animal studies using stress-inducing paradigms (e.g. immobilization, foot shock, water submersion) [47]. These interconnected relationships, when dysregulated, may worsen pathophysiological changes in each other, gradually leading to circular maladaptive responses [48].

Unresolved questions, therefore, remain on how poor sleep and chronic stress interrelate and contribute to hippocampal volume reduction with advancing age, and evidence from in-vivo human studies is lacking and inconclusive. In this study, we investigate whether measures of self-reported sleep, perceived psychological stress, their changes over a 2-year period,

and their interaction are related to individual differences in total hippocampal volume and hippocampal subfield volumes among community-dwelling older adults aged 50 and over. We hypothesized that short and long sleep duration, higher levels of sleep problems, and higher levels of perceived stress and their combined effect would be specifically associated with lower volume of CA1, CA3, CA4, and DG. We examine these effects in healthy adults aged 50 years and over. Midlife represents a critical inflection point of hippocampal change, from plateauing to early decline [49], and sleep and psychological stress in midlife have been associated with risk of cognitive decline and incident dementia in later life [40, 41, 50–53]. Exploring dementia-related risk factors in this age group is important as it can help establish early pathways leading to dementia-related changes, such as hippocampal atrophy.

Methods

Ethics statement

This study was approved by the Trinity College Faculty of Health Sciences Research Ethics Committee, Dublin, Ireland. Protocols conformed with the Declaration of Helsinki. Signed informed consent was obtained from all respondents prior to participation. Additional ethics approval was received for the magnetic resonance imaging (MRI) sub-study from the St James's Hospital/Adelaide and Meath Hospital, Inc. National Children's Hospital, Tallaght (SJH/AMNCH) Research Ethic Committee, Dublin, Ireland. Those attending for MRI also completed an additional MRI-specific consent form.

Design and study sample

Cross-sectional analyses included data from Wave 3 of the Irish Longitudinal Study on Ageing (TILDA), a nationally representative prospective cohort study of community-dwelling older adults aged 50 and over, resident in the Republic of Ireland. Two-year follow-up analyses included data from Wave 2 and Wave 3. Wave 2 took place between 2012 and 2013 and Wave 3 was carried out between 2014 and 2015. TILDA's random sampling procedure and study design have been described elsewhere [54]. Briefly, the population was randomly sampled using the RANSAM procedure. Household addresses were drawn from the Irish National Geodirectory. At each wave, participants completed a Computer Assisted Personal Interview which collected information on their social, health, and financial situations. At Wave 3, participants were offered to take part in an extensive nurse-administered physical and cognitive health assessment ($N = 4309$), and a subset of the respondents were also randomly selected to undergo brain MRI ($N = 578$). Participants aged 65 and over were initially recruited using a random sampling procedure, as they were hypothesized to show the more pronounced age-associated structural changes (compared with younger participants). Those aged 50–64 were then later invited to participate to increase the overall number and match the age range of the parent sample. Research nurses informed them of the MRI protocol following their health assessment and screened for MRI contraindications [54]. Eighteen did not complete MRI due to MRI contraindication ($N = 4$) or claustrophobia/nervousness ($N = 14$). Data from participants with Parkinson's disease ($N = 1$), history of stroke ($N = 4$), transient ischemic attack ($N = 3$), and data with evidence of image artifact ($N = 33$) were excluded. Of the 519 participants with adequate volumetric measures, 464 had both self-reported sleep and perceived stress data available at Wave 3. Forty-seven individuals with no self-reported sleep and perceived stress data available at Wave 2 were excluded, resulting in a final sample of 417 individuals. Supplementary Figure S1 presents the flowchart of the study sample.

Self-reported sleep measures

Self-reported total sleep duration was generated using the interview question "*Approximately how many hours do you sleep on a weeknight?*". Participants were asked to round to the nearest hour when asked to self-report their average sleep. Self-reported total sleep duration at Wave 2 and Wave 3 was categorized as Short when ≤6 h and Long when ≥10 h and participants were aged ≤64 or when ≥9 h and participants were aged >65, with the remainder as Normal sleep [55]. A six-level 2-year sleep duration change variable was generated by using total sleep duration categories from Wave 2 and Wave 3; Normal-Stable, Short-Stable, and Long-Stable corresponds to cases when the sleep duration categories (Normal, Short, Long) did not change between Wave 2 and Wave 3; Normal-Change, Short-Change, and Long-Change corresponds to cases when the sleep duration categories (Normal, Short, Long) changed between Wave 2 and Wave 3.

Self-reported sleep problems were based on three questions: (1) Participants were asked how likely they are to doze off during the day, with responses ranging from "would never doze" to "high chance of dozing" on a four-point Likert scale; (2) Participants were also asked how often they have trouble falling asleep, and (3) how often they have trouble waking up too early and not being able to fall asleep again, with responses ranging from "rarely or never" to "most of the time" on a three-point Likert scale [56]. A composite sleep score was derived at Wave 2 and Wave 3 by summing the question responses, total ranging from 0 to 7, with higher scores representing more sleep problems. A 2-year sleep problem change score was generated by subtracting Wave 2 from Wave 3 composite sleep score. A higher sleep problem change score indicates that sleep problems increased from Wave 2 to Wave 3.

Perceived stress measures

Perceived stress over the previous month was recorded using the four-item version of the Perceived Stress Scale (PSS-4) [57]. The PSS, originally developed as a 14-item questionnaire, is a measure of chronic stress associated with global stress perception. The PSS-4 includes four questions, answered from "Never" (0) to "Very Often" (4) on a 5-point Likert scale. A composite stress score at Wave 2 and Wave 3 was derived by summing the four question responses, with higher scores representing more stress. A two-year stress change score was generated by subtracting Wave 2 from Wave 3 PSS scores. A higher stress change score indicates that perceived stress increased from Wave 2 to Wave 3.

MRI protocol and T1-weighted acquisition

The MRI sampling procedure has been previously described in detail [58]. Briefly, scanning was completed at Wave 3 at

the National Centre for Advanced Medical Imaging (CAMI), St James's Hospital, Dublin, via 3T Philip's Achieva system and 32-channel head coil. The protocol included a variety of scans, including a T1-weighted (T1w) MR image acquired using a 3D Magnetization Prepared Rapid Gradient Echo sequence (TR/TE = 6.7/3.1 ms; FA = 8°; FOV = 240 × 240 × 162 mm [3]; voxel size = 0.8 × 0.8 × 0.9 mm^3; SENSE = 2; total scan duration = 5:24). The MRI data were obtained with a mean (SD) delay of 62 (40) days after the health assessment.

MRI data inspection

All T1w images were analyzed using FreeSurfer software version 6.0 [59]. The technical details of FreeSurfer procedures have been described elsewhere [59–62]. All unprocessed input volumes were inspected for evidence of image artifact and white matter lesions.

Volumetric measures

Estimated total intracranial volume (eTIV) was calculated using the FreeSurfer recon-all processing pipeline. The hippocampal subfield option, described previously [63], was used to segment the HP and its subfields: the parasubiculum, presubiculum, subiculum, CA1, CA2/3, CA4, the granule cells in the molecular layer of the dentate gyrus (GC-ML-DG), the molecular layer of the HP, fimbria, hippocampal fissure, Hippocampus-Amygdala-Transition-Area, and Hippocampal Tail. Briefly, the procedure uses a probabilistic atlas derived from a Bayesian model of a tetrahedral mesh, trained on ex-vivo manual segmentations of high-resolution hippocampal scans and in-vivo T1w images. Segmentations were checked by trained operators [64]. All datasets had hippocampal segmentations that fell within expected tissue boundaries; none of the recon-all procedures yielded any report of the error.

Covariates

Covariates included age, sex, education (none/primary, secondary, or tertiary/higher); eTIV; location of residence (urban/rural); marital status (married/never married/separated, or divorced/widowed); employment status (employed/retired or not employed). Medications were classified using the Anatomical Therapeutic Chemical (ATC) classification codes Questionnaire. Sleep medications included ATC codes N05A (antipsychotics), NO5B (anxiolytics), N05C (hypnotics and sedatives), and R06A (antihistamines); Antihypertensive medication included ATC codes C02 (antiadrenergic agents), C03 (diuretics), C07 (β blockers), C08 (calcium-channel blockers), and C09 (angiotensin-converting enzyme inhibitors); Antidepressant medication was classified by ATC code N06A; A dichotomous variable was generated to indicate usage of sleep, cardiovascular and antidepressant medications respectively. Pre-existing self-reported physician-diagnosed cardiovascular diseases and events (CVDEs) included history of angina, heart attack, congestive heart failure, stroke, transient ischemic attack, and atrial fibrillation. Data were pooled to create a dichotomous CVDE measure, for absence (CVDE free) or presence (≥1) of CVDEs. Diabetes mellitus was represented as a dichotomous variable for presence or absence of the disease; two seated systolic and diastolic blood pressure measurements were obtained separated by 1 min using an OMRON digital automatic blood pressure monitor (Model M10-IT); the averaged systolic and diastolic blood pressure measures were used for analysis. Self-reported long-term disease was represented as a dichotomous variable for presence or absence of long-term diseases (or any health problem, illness, disability, or infirmity which has troubled the respondent over a period of time). Self-reported chronic conditions included lung disease, asthma, arthritis, osteoporosis, cancer, Parkinson's disease, stomach ulcer, varicose ulcer, liver disease, thyroid disease, or kidney disease. Participants were categorized as having no chronic conditions or chronic condition ≥1. Smoker status was defined as never, past, or current. The CAGE questionnaire [65] was used as the measure of problematic alcohol consumption and is represented as a binary variable. Physical activity was recorded through the International Physical Activity Questionnaire (IPAQ [66]; short-form) and is represented as a three-level variable (low/moderate/high). Body mass index (BMI) was calculated by dividing the participants' weight in kilograms by height in meters squared. Participants' height was measured to the nearest 0.01 m (Seca 240 Stadiometer, Seca Ltd, Birmingham, UK), weight to the nearest 0.1 kg (Seca 861 Electronic Scales, Seca Ltd, Birmingham, UK). Symptoms of depression were assessed using the Centre for Epidemiological Studies Depression Scale (CESD [67]) and are represented as a continuous variable.

Covariates were selected based on previous literature showing the impact of these factors on tissue volumes, sleep, and/or stress, representing potential confounders of the relationship between hippocampal subfield volumes, sleep, and stress measures [68–72]. eTIV was included to control for individual differences in head size.

Missing data and nonresponse

Given the sample size of the sample under analysis, dummy categories were generated for the missing observations of the CAGE and IPAQ variables to reduce the loss of statistical power.

Statistical analyses

All statistical analyses were performed using R software version 3.5.2 (R Foundation for Statistical Computing, Vienna, Austria [73]).

Data descriptives. The observed sample was first characterized by total sleep duration categories, sleep problems, and perceived stress. Continuous variables were described as unadjusted means with SDs; categorical variables were given as percentages (%). For descriptive purposes, bivariate associations were investigated by means of chi-squared tests, ordinary least square, and Poisson regressions (where appropriate). To estimate selection bias, the observed sample was also compared to the rest of the cohort who attended the center-based health assessment at Wave 3 but did not undergo an MRI. Independent *t*-tests and chi-squared tests were used to assess differences between the two groups.

Cross-sectional analyses. TOTAL HIPPOCAMPAL VOLUME. Separate ordinary-least square regressions were used to examine the relationship between sleep duration (Model 1a), sleep problems

(Model 2a), stress (Model 3a), their respective interaction (Models 4a and 5a), and total hippocampal volume (outcome) at Wave 3.

Hippocampus subfield volumes. Separate linear mixed-effects regression analyses were carried out to explore the relationship between sleep duration, sleep problems, stress, and hippocampal subfield volumes at Wave 3. Mixed-effects regression models are a more parsimonious alternative to running separate ordinary-least square regression models with each hippocampal subfield as a separate outcome. In each mixed-effect model, hippocampal subfield volumes were the dependent variable. Model 1b included as fixed effects sleep duration (Normal category set as the reference level) and hippocampal subfields (the parasubiculum set as the reference level) with an interaction term. Fixed effects for Model 2b were sleep problems and hippocampal subfields with an interaction term. In Model 3b, fixed effects included perceived stress and hippocampal subfields with an interaction term. Model 4b included as fixed effects sleep duration, perceived stress, and hippocampal subfields with a three-way interaction. Model 5b included as fixed effects sleep problems, perceived stress, and hippocampal subfields with a three-way interaction. The sleep/stress variables by hippocampal subfield interaction terms were included to allow the potential for the association of the sleep/stress variables with hippocampal subfield volume to differ by different subfield regions. In all the models, participants constituted the random intercept. Models had therefore a nested structure such that the different hippocampal subfields were nested within each participant.

Two-year follow-up analyses. TOTAL HIPPOCAMPAL VOLUME. Separate ordinary-least square regressions were used to investigate the relationship between sleep duration change (**Model 6a**), sleep problems change (**Model 7a**), and stress change (**Model 8a**) between Wave 2 and Wave 3 and total hippocampal volume (outcome) at Wave 3.

HIPPOCAMPUS SUBFIELD VOLUMES. Separate linear mixed-effects regression analyses were carried out to explore the relationship between sleep duration change, sleep problem change, stress change between Wave 2 and Wave 3, and hippocampal subfield volumes at Wave 3. **Model 6b** included as fixed effects sleep duration change status (Normal-Stable category set as reference level) and hippocampal subfields with an interaction term. **Model 7b** included as fixed effects sleep problems change and hippocampal subfields with an interaction-term. Fixed effects in **Model 8b** were perceived stress change and hippocampal subfields with an interaction term. In all the models, the dependent variable was the hippocampal subfield volumes; participants constituted the random intercept.

All models were adjusted for head size (eTIV), age, sex, education, employment status, local residence, marital status, sleeping, cardiovascular and antidepressant medications, self-reported CVDEs, diabetes mellitus, systolic and diastolic blood pressure, long-term diseases, chronic diseases, smoking, alcohol intake, physical exercise, BMI, depression, and delay (in days) between the MRI scanning and the Computer Assisted Personal Interview (when the data on sleep and stress was collected) at Wave 3. Longitudinal models were further adjusted for the time period (in days) between the date of the interviews at Wave 2 and the date of interviews at Wave 3. Model 7a/b and Model 8a/b were further adjusted for sleep problem and perceived stress at baseline (Wave 2), by adding the interaction sleep problem × subfield and perceived stress × subfield as covariate, respectively, to account for the level of sleep problem and perceived stress of origin. In cross-sectional and longitudinal analyses, significance level was set to $p = .006$ (0.05/8 models).

Results

Characteristics of the observed sample

Table 1 provides descriptive statistics of the study sample in comparison with the rest of the cohort (excluded participants) at Wave 3. Supplementary Table S1 provides descriptive statistics (unadjusted means and SDs) for the study sample overall and by sleep duration categories at Wave 3. Mean age was 68.8 (SD = 7.3); 54% of the participants were female and 45% had attained tertiary level of education. Individuals within the Short sleep duration category (compared to Normal sleep) were older ($p = .02$), more likely to live in an urban area ($p < .01$), have one or more long-term diseases ($p < .01$) and be past smokers ($p < .01$). Depressive symptoms ($p < .001$), perceived stress scores ($p = .03$), and sleep problems ($p < .001$) were also higher for Short sleep. Individuals within the Long sleep duration category (compared to Normal sleep) were older ($p < .001$). There were tendencies toward taking more sleeping, cardiovascular and antidepressant medications, having long-term and chronic diseases, and low levels of physical activity with Long sleep, however, differences with Normal sleep did not reach significance.

Supplementary Table S2 provides descriptive statistics (unadjusted means and SDs) for the study sample by sleep problems. Higher sleep problems were more prevalent among older individuals ($p = .01$), individuals with third level of education ($p = .01$), divorced ($p = .05$) or widowed ($p = .02$), taking sleeping medications ($p = .06$), with diabetes ($p = .04$) and individuals with long-term ($p < .0001$) and chronic diseases ($p = .006$). Higher sleep problems were also associated with higher depressive symptoms ($p < .0001$) and perceived stress ($p < .0001$).

Supplementary Table S3 provides descriptive statistics (unadjusted means and SDs) for the study sample by perceived stress. Higher stress levels were more prevalent among younger individuals ($p = .002$), women ($p < .001$), individuals with third level of education ($p = .004$), divorced ($p < .001$) or widowed ($p = .07$), taking sleeping ($p < .001$) and antidepressant medications ($p < .0001$), with history of CVD or diabetes ($p = .07$), currently smoking ($p < .001$), having alcohol problems ($p = .01$) and more depressive symptoms ($p < .0001$).

Cross-sectional analyses

Sleep duration, sleep problems, perceived stress levels, and total hippocampal volume. There was no cross-sectional association between hippocampal volume and sleep duration, sleep problems, or stress. The interaction between sleep duration and stress was not significant ($p > .05$). The interaction between sleep problems and stress marginally improved the fit of the fully adjusted model, relative to the fully adjusted model with no interaction term ($\chi^2_{(1)} = 3.48$, $p = .05$). There was a tendency for an additive increase in sleep problems and perceived stress levels to be associated with smaller total hippocampal volume

Table 1. Characteristics of the study sample (N = 417) compared to the rest of the cohort (those excluded from analyses N = 6270) at Wave 3

	Study sample (N = 417)	Rest of the cohortWave 3 (N = 6270)	
Age (mean ± SD)	68.8±7.3	66.5±9.7	*
Sex: female (%)	54.2	56.1	
Education (%)			*
Primary/none	19.9	26.4	
Secondary	34.7	39.7	
Third level or higher	45.3	33.8	
Location: urban (%)	47.9	51.9	
Retired/not employed (%)	72.9	66.9	*
Marital status (%)			
Married	74.5	69.0	
Never married	6.5	8.5	
Separated/divorced	4.8	7.2	
Widowed	14.2	15.2	
On sleeping medication (%)	7.6	9.2	
On cardiovascular medication (%)	41.0	43.6	
On anti-depressant medication (%)	6.7	9.5	
Cardiovascular condition ≥ 1 (%)	5.3	9.9	*
Diabetes (%)	7.4	8.9	
Systolic blood pressure (mean ± SD)	134.5 ± 18.8	134.1 ± 19.5	
Diastolic blood pressure (mean ± SD)	79.7 ± 10.4	80.8 ± 10.7	
Long-term diseases ≥ 1 (%)	38.1	43.5	*
Chronic conditions ≥ 1 (%)	58.5	53.1	*
Smoking (%)			*
Never	51.6	45.0	
Past	42.9	41.6	
Current	5.5	13.3	
Problematic alcohol (%)	10.3	9.8	
Physical exercise (%)			*
Low	31.8	38.4	
Mid	38.8	32.2	
High	24.4	24.4	
BMI (mean ± SD)	27.7 ± 4.6	28.5 ± 5.1	*
Depressive symptoms (mean ± SD)	3.3 ± 3.3	4.2 ± 3.9	*
Sleep duration (%)			
Normal	61.1	60.2	
Short	29.2	32.4	
Long	9.5	7.4	
Sleep disturbance (mean ± SD)	2.0 ± 1.5	2.2 ± 1.6	*
Perceived stress (mean ± SD)	3.6 ± 2.8	4.2 ± 3.1	*
CAPI—MRI delay, days (mean ± SD)	62.1 ± 39.8	—	
Time period between W2 and W3 CAPI, days (mean ± SD)			

t-tests and chi-squared tests (where appropriate) were used to assess differences between the two groups.
CAPI, Computer Assisted Personal Interview.
*($p < .05$) indicates significant differences.

($B = -0.01$, 95% CI = −2.3 to 0.6), however, the association was not significant after correction for multiple comparisons.

Sleep duration and hippocampal subfield volumes. Table 2 provides the marginal estimates (95% CI) of hippocampal subfield volumes across Short, Normal, and Long sleep duration categories. See Supplementary Table S4 for full model output. The interaction between sleep duration and subfield significantly improved the fit of the fully adjusted model, relative to the fully adjusted model with no interaction term ($\chi^2_{(22)} = 61.6$, $p < .0001$). In the fully adjusted model, significant sleep duration × subfield interactions emerged for hippocampal tail ($p < .0001$) and molecular layer ($p = .004$), with smaller volumes for Long sleep compared to Normal sleep. Similar tendencies were observed for the GC-DG, presubiculum, and subiculum, however, the associations were not significant after adjustment for multiple comparisons. There was also a tendency for Short sleep to be associated with smaller volumes of hippocampal tail, although not significant after adjustment for multiple comparisons. Supplementary Figure S2 shows the marginal estimates and 95% CI of the hippocampal subfields volumes as a function of sleep duration category.

Sleep problems and hippocampal subfield volumes. Table 3 provides the marginal estimates (95% CI) of hippocampal subfield volumes across sleep problems scores. See Supplementary Table S5 for full model output. The interaction between sleep problems and subfield significantly improved the model fit ($\chi^2_{(11)} = 27.63$, $p = .003$). In the fully adjusted model, higher sleep problems were associated with smaller volume of CA1 ($p = .006$), hippocampal tail ($p = .0001$), and molecular layer ($p = .002$). Similar tendencies were observed for the subiculum, however,

the associations were not significant after adjustment for multiple comparisons. Figure 1 shows the marginal estimates and 95% CI of the hippocampal subfields volumes as a function of sleep problems.

Perceived stress and hippocampal subfield volumes. The interaction between perceived stress and subfield did not significantly improve the model fit ($\chi^2_{(11)} = 12.4$, $p = .33$). Perceived stress was not associated with hippocampal subfield volumes.

Sleep duration × perceived stress and hippocampal subfield volumes. Supplementary Table S6a provides the marginal estimates (95% CI) of hippocampal subfield volumes for the interaction sleep duration category × perceived stress. See Supplementary Table S6b for full model output. The three-way interaction (sleep duration × perceived stress × subfield) was significant ($\chi^2_{(22)} = 84.37$, $p < .0001$). In the fully adjusted model, Short sleep with higher perceived stress was associated with smaller volume of CA1 ($p < .0001$), hippocampal tail ($p < .0001$), molecular layer ($p < .0001$) and subiculum ($p < .001$). Similar tendencies were observed for the presubiculum and the GC-DG, however, the associations were not significant after adjustment for multiple comparisons. Supplementary Figure S3 shows the marginal estimates and 95% CI of the hippocampal subfields volumes as a function of sleep duration and perceived stress.

Sleep problems × perceived stress – hippocampal subfield volumes . Supplementary Table S7a provides the marginal estimates (95% CI) of hippocampal subfield volumes for the interaction sleep problems × perceived stress. See Supplementary Table S7b for full model output. The three-way interaction (sleep problems × perceived stress × subfield) significantly improved model fit ($\chi^2_{(11)} = 34.2$, $p < .0001$). In the fully adjusted model, the interaction sleep problems × perceived stress was negatively associated with the CA1 ($p < .001$) volume. Similar tendencies were observed for the molecular layer and subiculum volumes; however, the associations were not significant after adjustment for multiple comparisons. Supplementary Figure S4 shows the

Table 2. Marginal estimates (95% CI) of hippocampal subfield volumes across Short, Normal, and Long sleep duration categories

Sleep duration	Short	Normal (REF)	Long
Subfields			
CA1	615.2 (609.4 to 620.9)	610.3 (606.3 to 614.3)	608.0 (598.0 to 618.1)
CA2/3	206.3 (200.6 to 212.1)	200.7 (196.8 to 204.7)	200.6 (190.8 to 210.7)
CA4	244.0 (238.2 to 249.8)	238.9 (234.9 to 242.9)	235.2 (225.1 to 245.2)
DG	284.1 (278.3 to 289.9)	279.4 (275.4 to 283.4)	273.1 (263.0 to 283.1)
Molecular layer	540.0 (534.3 to 545.8)	536.6 (532.6 to 540.6)	**524.7 (514.6 to 534.7)**
Hippocampal tail	486.3 (480.5,492.1)	489.2 (485.2 to 493.2)	**465.2 (455.2 to 475.3)**
HATA	64.4 (58.6 to 70.1)	59.3 (55.3 to 63.2)	63.2 (53.1 to 73.2)
Hippocampal fissure	182.7 (176.9 to 188.5)	175.3 (171.3 to 179.3)	185.7 (175.7 to 195.7)
Fimbria	82.8 (77.1 to 88.6)	79.1 (75.1 to 83.1)	79.9 (69.9 to 90.0)
Presubiculum	286.7 (280.9 to 292.5)	286.6 (282.6 to 290.6)	277.7 (267.7 to 287.8)
Subiculum	410.7 (404.9 to 416.5)	406.9 (402.9 to 410.9)	399.6 (389.6 to 409.7)
Parasubiculum	64.5 (58.7 to 70.3)	60.1 (56.1 to 64.1)	64.3 (54.3 to 74.4)

Marginal estimates are adjusted for head size (eTIV), age, sex, education, employment status, local residence, marital status, sleeping, cardiovascular and antidepressant medications, self-reported cardiovascular diseases and events, diabetes mellitus, systolic and diastolic blood pressure, long-term diseases, chronic diseases, smoking, alcohol intake, physical exercise, BMI, depression, and CAPI-MRI delay. Bold text indicates significant association in fully adjusted model ($p < .006$). See Supplementary Table S6 for full model output.

Table 3. Marginal estimates (95% CI) of hippocampal subfield volumes across sleep problems scores (from 0 to 7)

Sleep problems	0	2	4	5	7
Subfields					
CA1	**615.3 (610.0 to 620.5)**	**611.6 (608.5 to 614.7)**	**607.9 (602.8 to 613.0)**	**606.6 (599.1 to 613.0)**	**602.4 (591.5 to 613.2)**
CA2/3	204.5 (199.2 to 209.8)	202.4 (199.3 to 205.5)	200.3 (195.2 to 205.5)	199.3 (192.4 to 206.2)	197.2 (186.4 to 208.0)
CA4	241.3 (236.0 to 246.5)	240.1 (237.0 to 243.2)	238.8 (233.8 to 244.1)	238.4 (231.4 to 245.3)	237.2 (226.4 to 248.1)
DG	281.9 (276.7 to 287.2)	280.2 (277.1 to 283.3)	277.6 (273.4 to 283.6)	277.6 (270.7 to 284.5)	275.9 (265.0 to 286.7)
Molecular layer	**541.1 (535.8 to 546.3)**	**536.6 (533.5 to 539.6)**	**532.1 (526.9 to 537.2)**	**529.8 (522.9 to 536.7)**	**525.3 (514.5 to 536.2)**
Hippocampal tail	**492.3 (487.1 to 497.6)**	**486.2 (483.1 to 489.2)**	**480.0 (474.9 to 485.1)**	**476.9 (470.0 to 483.8)**	**470.7 (459.9 to 481.6)**
HATA	61.0 (55.7 to 66.3)	61.1 (58.1 to 64.2)	61.3 (56.1 to 66.4)	61.3 (54.4 to 68.2)	61.4 (50.6,72.3)
Hippocampal fissure	177.3 (172.1 to 182.6)	178.5 (175.4 to 181.5)	179.6 (174.4 to 184.7)	180.1 (173.2 to 187.1)	181.2 (170.4 to 192.1)
Fimbria	80.5 (75.2 to 85.7)	80.3 (77.2 to 83.4)	80.1 (75.0 to 85.2)	80.0 (73.1 to 87.0)	79.9 (69.0 to 90.7)
Presubiculum	286.6 (281.3 to 291.8)	285.8 (282.7 to 288.8)	285.0 (279.9 to 290.1)	284.6 (277.7 to 291.5)	283.8 (272.9,294.6)
Subiculum	410.2 (405.0 to 415.5)	407.4 (404.3 to 410.4)	404.4 (399.4 to 409.7)	403.1 (396.2 to 410.0)	400.3 (389.4 to 411.1)
Parasubiculum	59.7 (54.5 to 65.0)	61.7 (58.7 to 64.8)	63.7 (58.6 to 68.9)	64.7 (57.8 to 71.7)	66.7 (55.9 to 77.6)

Marginal estimates are adjusted for head size (eTIV), age, sex, education, employment status, local residence, marital status, sleeping, cardiovascular and antidepressant medications, self-reported cardiovascular diseases and events, diabetes mellitus, systolic and diastolic blood pressure, long-term diseases, chronic diseases, smoking, alcohol intake, physical exercise, BMI, depression, and CAPI-MRI delay. Bold text indicates significant association in fully adjusted model ($p < .006$). See Supplementary Table S7 for full model output.

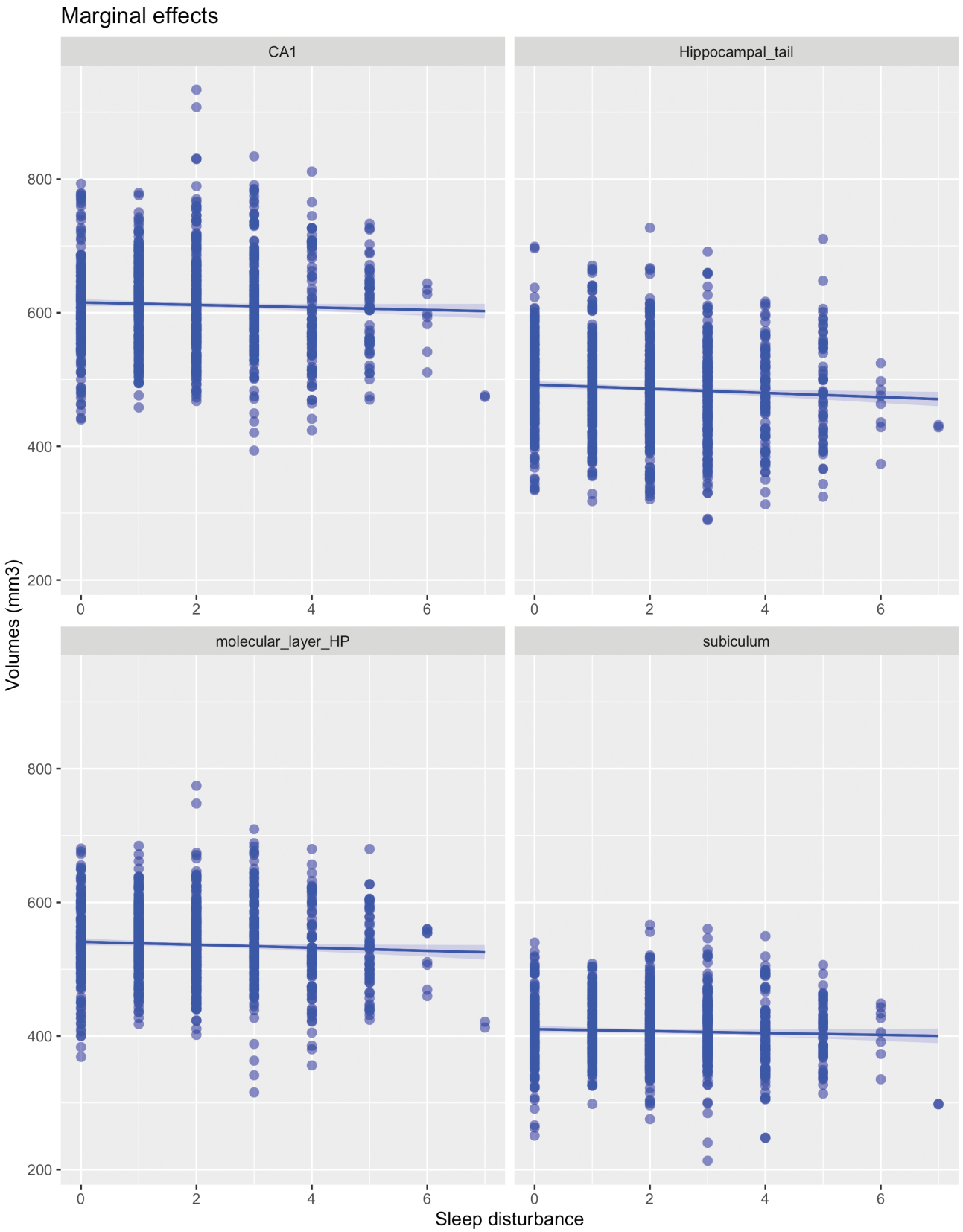

Figure 1. Marginal estimates (blue line) and 95% CI (light blue band) of the hippocampal subfields CA1, hippocampal tail, molecular layer, and subiculum volumes (mm^3) as a function of sleep problems. The overlaid scatters represent observed participant-wise data. Marginal estimates were calculated from fully adjusted models. Significant (CA1, tail, and molecular layer) and marginally significant (subiculum) associations are displayed.

marginal estimates and 95% CI of the hippocampal subfields volumes as a function of sleep problems and perceived stress.

Two-year follow-up analyses

Sleep duration change, sleep problems change, perceived stress change, and total hippocampal volume. There was no association between sleep duration change, sleep problems change, or perceived stress change between Wave 2 and Wave 3, and total hippocampal volume at Wave 3 ($p > .05$).

Sleep duration change and hippocampal subfield volumes. Supplementary Table S8a provides the marginal estimates (95% CI) of hippocampal subfield volumes across sleep duration change. The sleep duration change × subfield interaction significantly improved model fit ($\chi^2_{(55)} = 143.6$, $p < .0001$). In the fully adjusted model, individuals with Long sleep duration at Wave 2 and Wave 3 (Long-Stable) had smaller hippocampal tail ($p<.0001$), molecular layer ($p<.0001$), presubiculum ($p = .001$), and subiculum ($p = .0004$) compared to Normal-Stable. Similar tendencies (although not significant) were observed for the GC-DG (marginal significance, $p = .01$) and CA1 ($p = .05$). Individuals with Long sleep duration at Wave 2 who shortened their sleep at Wave 3 (Long-Change sleep) had smaller hippocampal tails compared to Normal-Stable ($p < .0001$). Those with Normal sleep at Wave 2 who changed at wave 3 also had smaller hippocampal tails (marginal significance, $p = .01$). When comparing Long-Stable versus Long-Change, our analyses show that a decrease in sleep duration at Wave 3 tends to be associated with larger volume of molecular layer (marginal significance, $p = .02$). See Supplementary Table S8b for full model output.

Sleep problems change and hippocampal subfield volumes. Supplementary Table S9a provides the marginal estimates (95% CI) of hippocampal subfield volumes across sleep problems change. See Supplementary Table S9b for full model output. The sleep problem change × subfield interaction significantly improved the model fit ($\chi^2_{(11)} = 25.79$, $p = .006$). In the fully adjusted model, an increase in sleep problems from Wave 2 to Wave 3 was associated with smaller volumes of CA1 ($p = .001$), hippocampal tail ($p = .001$), and molecular layer ($p = .001$) (marginal significance for the subiculum, $p = .01$; CA3 and GC-DG, $p = .05$).

Stress change and hippocampal subfield volumes. The stress change × subfield interaction only marginally improved the model fit ($\chi^2_{(11)} = 18.2$, $p = .07$). Increased stress change between W2 and W3 was marginally associated with smaller volume of hippocampal tail ($p = .01$).

Discussion

A relatively large imaging sample of older Irish adults was examined in this study with the purpose of assessing the cross-sectional and 2-year follow-up effect of sleep and stress on total hippocampal volume and hippocampal subfield volumes. No separate associations were found with total hippocampal volume. There was a tendency for a cross-sectional additive effect of sleep problems and perceived stress on total hippocampal volume, however, the association was not significant after Bonferroni correction.

By contrast, cross-sectional analyses revealed associations between sleep duration and specific hippocampal subfield volumes. First, Long sleep was characterized by lower volumes of the molecular layer and hippocampal tail. Similar tendencies were observed for the DG, presubiculum, and subiculum, although not significant. Follow-up analyses examining sleep duration change over a 2-year period also showed an association between Long-Stable sleep and smaller molecular layer, hippocampal tail, presubiculum, and subiculum. Marginal associations were observed for the DG and CA1. The associations between Long sleep and smaller hippocampal subfield volumes are consistent with the sleep duration—cognitive function association observed in other studies [7, 74]. Long sleep duration may therefore represent a clinically relevant marker for the identification of individuals with structural atrophy in the hippocampal molecular layer, presubiculum, subiculum, and tail. A systematic review and meta-analyses of prospective cohort studies by Fan et al. [75] showed that Long sleep duration was associated with a 77% increased risk of all-cause dementia and a 63% increased risk of AD. Our findings suggest that a potential mechanism underlying these associations may be related to hippocampal subfield volume atrophy and support the evidence that Long sleep is a risk factor for dementia.

Our findings further revealed that individuals with Normal sleep who changed their sleep patterns within a 2-year period had smaller volumes of hippocampal tail compared to individuals with Normal-Stable sleep. Various mechanisms may explain this relationship, two of which might be (1) an increase in engaging in a more sedentary behavior when sleeping periods or time spent in bed are lengthened [76] and (2) an increase in stress levels potentially leading to shortened or more disturbed period of sleep. Larger volumes of molecular layer for those with Long sleep who reduced their sleep within a 2-year period would support the first speculation. However, these individuals also had smaller volumes of hippocampal tail compared to individuals with Normal-Stable sleep.

Furthermore, our cross-sectional and 2-year follow-up analyses showed higher sleep problem levels and a 2-year sleep problem increase to be associated with lower volumes of subfields CA1, molecular layer, and tail (marginal for the subiculum, CA3, and DG). The sleep-CA1 association is consistent with the results reported in chronic primary insomnia [5]. Contrary to what was observed in PTSD [37], the associations with subfields CA3 and DG were only marginal in our study.

Perceived stress was not cross-sectionally associated with hippocampal subfield volumes but an increase in perceived stress over 2 years was marginally associated with smaller hippocampal tails. Our findings contrast with the results of Zimmerman et al.'s study that has shown cross-sectional associations between CA2/CA3 and CA4/DG volumes and perceived stress levels among a cohort of 116 older adults [38]. The null finding in our study may stem from lower levels of perceived stress in our study sample (PSS4 mean = 3.6 ± 2.8 vs. Zimmerman's PSS14 = 15.76 ± 7.85) —which could have obscured an effect of severe stress levels on hippocampal subfield volumes. Different findings may also be related to the use of different automatic subfield segmentation methods. Zimmerman et al. used FreeSurfer original subfield segmentation method [77] and focused on the CA1, CA2/CA3, CA4/ DG, subiculum and enthorinal cortex.

Finally, the inclusion of a sleep × stress interaction term in our cross-sectional analyses revealed that individuals with Short Sleep and higher stress levels had smaller CA1, molecular layer, subiculum, and hippocampal tail volumes. Similar tendencies were observed for the DG and presubiculum, although not significant. The co-occurrence of higher sleep problems and higher stress was also negatively associated with CA1 volumes, with marginal associations for the molecular layer and subiculum volumes. Up to 17% subfield volume difference between individuals with high level of sleep disturbance and stress was observed compared to those with high level of sleep disturbance and low stress levels. This compares to a 2%–5% subfield volume between-person difference in the sleep models without an interaction term. Larger effects for the sleep-stress interaction, therefore, support an active synergy between these two factors [42] and suggest larger structural differences when disrupted sleep and chronic stress co-occur. One plausible biological explanation of these sleep × stress – hippocampal subfields volume associations is that elevated levels of circulating glucocorticoids arising from a dysregulation of the HPA activity, directly caused by chronic stress or induced by prolonged wakefulness, may lead to a cascade of damaging effects (e.g. suppressed neurogenesis, diminished dendritic branching, reduced synaptic and neuronal plasticity), which in turn contribute to hippocampal subfield structural changes. It is not possible from the present observational study to infer any direction of causality and it may in effect be that smaller hippocampal volumes constitute a risk factor for the development of chronic stress and sleep disturbance. The effects of specific molecular mechanisms on volume change can but be speculative too, given that self-reported sleep and perceived stress are very different from sleep deprivation and chronic stress experimentally induced in animal studies. It might also be the case that the reporting of sleep duration/problems and stress perception may be related such that individuals who feel more stressed might be more inclined to report poorer sleep. However, the co-occurrence of Short sleep and higher stress levels may be of clinical relevance for the identification of individuals with potential hippocampal subfield atrophy; as opposed to Short sleepers with no perceived stress, who may represent a distinct profile of individuals with relatively preserved volumes. Consistent with this interpretation, in our study sample, individuals with Short sleep duration and no perceived stress tend to have larger hippocampal subfield volumes than individuals with Normal sleep duration, whereas, with the co-occurrence of moderate and high-stress levels, smaller volumes are observed. In the Whitehall II cohort study [78], persistent Short sleep duration at age 50, 60, and 70 compared to persistent Normal sleep duration was associated with a 30% increased dementia risk, independently of sociodemographic, behavioral, cardiometabolic, and mental health factors (depressive symptoms). By contrast, the systematic review and meta-analysis by Fan et al. [75] showed no association between Short sleep and dementia risk. Our findings suggest that the co-occurrence of Short sleep and perceived stress independently of other dementia risk factors may be damaging to the hippocampus subfields. Examining the co-occurrence of sleep duration and perceived stress (in addition to depressive symptoms) may therefore be a route of investigation to better understand the exact mechanisms through which Short sleep duration may become a critical factor for increased dementia risk.

In summary, our findings support the accumulative evidence for separate associations between sleep duration, sleep problems, and sleep-stress interaction with the volume of the hippocampal subfields CA1 and DG [5, 37, 38], although these associations were only marginal for the DG. Given that sleep problems were highly correlated with perceived stress in our study sample, our findings may therefore support a specific CA1 vulnerability to stress exposure. Our study also reveals additional associations with the molecular layer and hippocampal tail, with marginal associations for the subiculum and presubiculum, which is consistent with some other studies, although evidence is limited. Smaller molecular layer volumes were observed with higher sleep disruption among young adults with history of maltreatment [79]. Lower volumes of subiculum and presubiculum were associated with childhood trauma [80] and were more prevalent among individuals with obstructive sleep apnea [6].

The hippocampal subfields CA1, subiculum, presubiculum, and molecular layer were found to be lower by 10% to 30% in Subjective Cognitive Decline, amnestic Mild Cognitive Impairment (MCI), and AD [81, 82]. Recent reviews have implicated chronic sleep disruption and stress as potential risk factors in the development of AD and accumulative evidence suggests impaired physiological responses to chronic stress, elevated cortisol levels, disrupted circadian rhythm, and a higher prevalence of sleep problems in MCI and AD [83–86]. Our group separately showed that smaller volumes of CA1, CA2/3, CA4, molecular layer, and DG [64], higher perceived stress levels [41], higher hair glucocorticoids levels [40], Long sleep duration and higher sleep problems [74] were associated with lower performance on tests of verbal episodic memory and global cognition among healthy older adults. These observations, together with the animal literature, support the view of an intertwined relationship between sleep and stress whereby both may synergistically contribute to distinct hippocampal subfield damage and increase the risk of cognitive impairment and neurodegenerative conditions. Our study, therefore, highlights the importance of concurrently assessing and controlling sleep duration, sleep quality, and stress levels in neuropsychiatric research when aiming at identifying risk profiles of structural atrophy and accelerated cognitive decline.

Limitations

In this study, we used self-reported measures of perceived stress, sleep duration, and sleep problems, with no complementary objective measures such as salivary, hair cortisol, or actigraphy-based sleep estimations. Perceived stress was representative of the past month whereas sleep duration and sleep problems were assessed overall. The reliability of self-reported measures has come into question due to potential misperception and influence of other factors including age and cognitive function. The decision not to include hair sample and accelerometer data, although available for a subsample of the TILDA cohort, aimed at keeping a relatively large sample size for analysis. It is noteworthy that the validity of self-reported measures and complementary objective measures have been demonstrated in population-based studies of older adults [87]. Second, TILDA did not collect information on sleep disorders such as sleep apnea, or any treatments for disorders beyond capturing use of sleep medication. Third, the automatic segmentation of the hippocampal subfields in our study was based on T1-weighed (T1w) images. Criticisms have been recently levied against hippocampal subfield segmentation

based on T1w images [88] due to their low resolution and therefore inefficiency for automatic segmentations to accurately distinguish some specific subfields, such as the molecular layer [63, 88]. Iglesias et al. [63] advise to use a combination of T1w and T2w images as input for an optimal segmentation and Wisse et al. [88] recommend ensuring the validity of the automatic segmentation against high-reliability manual segmentation (bronze-standard approach) or direct comparison to histological samples (gold standard approach). In our MR protocol, the T1w and T2w images had considerably different in-plane resolutions (T1w were 0.9 mm isotropic; T2w were 0.58 × 0.72 × 4), which precluded combining both images to achieve a better overall segmentation. Validating FreeSurfer segmentation against manual segmentation was neither possible due to its labor-intensive nature given our relatively large dataset (N = 417). Manual delineation of hippocampal subfields in high-resolution images is estimated at approximately 50 h per case [63]. While we recognize that our approach departs from optimal standards and that our findings should be interpreted with caution, our relatively large sample size and the consistency of our methodology with respect to other large cohort studies (e.g. UK Biobank) afford the opportunity for our results to be replicated in future studies. Fourth, we used listwise deletion. Loss to follow-up and missing data means that the study sample was overall healthier than those excluded, however, selection of healthier participants generally leads to underestimate associations. Fifth, the number of participants who reported Long sleep at Wave 2 and Wave 3 was low. Replication of this study with larger sample size would therefore be needed to confirm our findings. Given the design of this study, another limitation is the inability to fully infer the plausible causal link between sleep duration, sleep quality, and stress with hippocampal subfield volumes and underpinning pathophysiological mechanisms. Finally, our investigation focused on the hippocampal structure, however, associations with other regions such as the amygdala, thalamus, and prefrontal cortex may also be expected and remain an interesting direction for future work.

Strengths

To the best of our knowledge, this is the first study to concurrently investigate hippocampal subfield volumes in association with self-reported sleep and perceived stress cross-sectionally, and changes in sleep/stress over a 2-year period, in a relatively large cohort of healthy older adults. Few studies have examined these associations, however separately, using global hippocampal volumetric measurements only, relatively small sample sizes, and/or based on patient populations (e.g. chronic insomnia, PTSD). Our models were also controlled for a large range of confounding covariates including head size, age, sex, education, employment status, local residence, marital status, sleeping, cardiovascular and antidepressant medications, self-reported cardiovascular diseases and events, diabetes mellitus, systolic and diastolic blood pressure, long-term diseases, chronic diseases, smoking, alcohol intake, physical exercise, BMI and depression, suggesting that these factors do not account for the observed findings.

Conclusion

The present study showed that self-reported Long sleep duration, higher sleep problems, and perceived stress and their changes over a 2-year period were associated with smaller volumes of specific hippocampal subfields and that their co-occurrence leads to larger structural between-person differences. Together, our study contributes to our understanding of the risk factors and underpinning pathways associated with hippocampal subfield atrophy, highlighting the importance of concurrently assessing and controlling sub-optimal sleep and stress levels in neuropsychiatric research for the development of targeted interventions aiming at reducing the risk of late-life hippocampal atrophy.

Supplementary Material

Supplementary material is available at *SLEEP* online.

Funding

Funding for The Irish Longitudinal Study on Ageing (TILDA) is provided by the Irish Government, the Health Research Board (HRB), The Atlantic Philanthropies, and the Irish Life PLc.

Acknowledgments

The authors would like to acknowledge the continued support and feedback of Dr. Wilby Williamson and John O'Connor of the TILDA research team. The authors would also like to acknowledge the continued commitment and cooperation of the TILDA participants and research team. MRI data collection was supported by the National Centre for Advanced Medical Imaging (CAMI). A specific acknowledgment to Jason McMorrow in CAMI.

Disclosure Statement

Financial and Nonfinancial Disclosure: None.

References

1. De Toledo-Morrell L, *et al*. From healthy aging to early Alzheimer's disease: in vivo detection of entorhinal cortex atrophy. *Ann N Y Acad Sci*. 2000;**911**:240–253.
2. Scullin MK, *et al*. Sleep, cognition, and normal aging: integrating a half century of multidisciplinary research. *Perspect Psychol Sci*. 2015;**10**(1):97–137.
3. Ohayon MM, *et al*. Meta-analysis of quantitative sleep parameters from childhood to old age in healthy individuals: developing normative sleep values across the human lifespan. *Sleep*. 2004;**27**(7):1255–1273. doi:10.1093/sleep/27.7.1255.
4. Noh H, *et al*. The relationship between hippocampal volume and cognition in patients with chronic primary insomnia. *J Clin Neurol*. 2012;**8**:30–38.
5. Joo EY, *et al*. Hippocampal substructural vulnerability to sleep disturbance and cognitive impairment in patients with chronic primary insomnia: magnetic resonance imaging morphometry. *Sleep*. 2014;**37**(7):1189–1198. doi:10.5665/sleep.3836.
6. Macey PM, *et al*. Brain morphology associated with obstructive sleep apnea. *Am J Respir Crit Care Med*. 2002;**166**(10):1382–1387.

7. Kim H, *et al*. Effects of long-term treatment on brain volume in patients with obstructive sleep apnea syndrome. *Hum Brain Mapp*. 2016;**37**(1):395–409.
8. Joo EY, *et al*. Hippocampal volume and memory in narcoleptics with cataplexy. *Sleep Med*. 2012;**13**(4):396–401.
9. Campbell S, *et al*. Lower hippocampal volume in patients suffering from depression: a meta-analysis. *Am J Psychiatry*. 2004;**161**(4):598–607.
10. Videbech P, *et al*. Hippocampal volume and depression: a meta-analysis of MRI studies. *Am J Psychiatry*. 2004;**161**(11):1957–1966.
11. Liu YR, *et al*. Sleep-related brain atrophy and disrupted functional connectivity in older adults. *Behav Brain Res*. 2018;**347**:292–299.
12. Fjell AM, *et al*. Self-reported sleep relates to hippocampal atrophy across the adult lifespan: results from the Lifebrain consortium. *Sleep*. 2020;**43**(5). doi:10.1093/sleep/zsz280.
13. Guzman-Marin R, *et al*. Sleep deprivation suppresses neurogenesis in the adult hippocampus of rats. *Eur J Neurosci*. 2005;**22**:2111–2116.
14. Raven F, *et al*. The role of sleep in regulating structural plasticity and synaptic strength: implications for memory and cognitive function. *Sleep Med Rev*. 2018;**39**:3–11.
15. Kreutzmann JC, *et al*. Sleep deprivation and hippocampal vulnerability: changes in neuronal plasticity, neurogenesis and cognitive function. *Neuroscience*. 2015;**309**:173–190.
16. Kent BA, *et al*. Sleep and hippocampal neurogenesis: implications for Alzheimer's disease. *Front Neuroendocrinol*. 2017;**45**:35–52.
17. Novati A, *et al*. Chronic sleep restriction causes a decrease in hippocampal volume in adolescent rats, which is not explained by changes in glucocorticoid levels or neurogenesis. *Neuroscience*. 2011;**190**:145–155.
18. Sapolsky RM, *et al*. Prolonged glucocorticoid exposure reduces hippocampal neuron number: implications for aging. *J Neurosci*. 1985;**5**(5):1222–1227.
19. Landfield PW, *et al*. Hippocampal aging and adrenocorticoids: quantitative correlations. *Science*. 1978;**202**(4372):1098–1102.
20. Toffanin T, *et al*. Volumetric MRI analysis of hippocampal subregions in Cushing's disease: a model for glucocorticoid neural modulation. *Eur Psychiatry*. 2011;**26**(1):64–67.
21. Patil C, *et al*. Brain atrophy and cognitive deficits in Cushing's disease. *Neurosurgical Focus*. 2007;**23**(3):1–4.
22. Karl A, *et al*. A meta-analysis of structural brain abnormalities in PTSD. *Neurosci Biobehav Rev*. 2006;**30**(7):1004–1031.
23. Woon FL, *et al*. Hippocampal volume deficits associated with exposure to psychological trauma and posttraumatic stress disorder in adults: a meta-analysis. *Prog Neuropsychopharmacol Biol Psychiatry*. 2010;**34**(7):1181–1188.
24. T O'Brien J, *et al*. Clinical and magnetic resonance imaging correlates of hypothalamic–pituitary–adrenal axis function in depression and Alzheimer's disease. *Br J Psychiatry*. 1996;**168**(6):679–687.
25. Lupien SJ, *et al*. Cortisol levels during human aging predict hippocampal atrophy and memory deficits. *Nat Neurosci*. 1998;**1**(1):69–73.
26. McEwen B. Neurobiological and systemic effects of chronic stress. *Chronic Stress*. 2017;**1**:1–17.
27. de Kloet ER, *et al*. Stress and the brain: from adaptation to disease. *Nat Rev Neurosci*. 2005;**6**(6):463–475.
28. McEwen BS. Physiology and neurobiology of stress and adaptation: central role of the brain. *Physiol Rev*. 2007;**87**(3):873–904.
29. McEwen BS. Protective and damaging effects of stress mediators: central role of the brain. *Dialogues Clin Neurosci*. 2006;**8**(4):367–381.
30. Frodl T, *et al*. How does the brain deal with cumulative stress? A review with focus on developmental stress, HPA axis function and hippocampal structure in humans. *Neurobiol Dis*. 2013;**52**:24–37.
31. Spiegelhalder K, *et al*. Insomnia does not appear to be associated with substantial structural brain changes. *Sleep*. 2013;**36**(5):731–737. doi:10.5665/sleep.2638.
32. Sexton CE, *et al*. Poor sleep quality is associated with increased cortical atrophy in community-dwelling adults. *Neurology*. 2014;**83**(11):967–973.
33. Singh-Manoux A, *et al*. No evidence of a longitudinal association between diurnal cortisol patterns and cognition. *Neurobiol Aging*. 2014;**35**(10):2239–2245.
34. Coluccia D, *et al*. Glucocorticoid therapy-induced memory deficits: acute versus chronic effects. *J Neurosci*. 2008;**28**(13):3474–3478.
35. Guzmán-Marín R, *et al*. Sleep deprivation reduces proliferation of cells in the dentate gyrus of the hippocampus in rats. *J Physiol*. 2003;**549**:563–571.
36. McKittrick CR, *et al*. Chronic social stress reduces dendritic arbors in CA3 of hippocampus and decreases binding to serotonin transporter sites. *Synapse*. 2000;**36**(2):85–94.
37. Neylan TC, *et al*. Insomnia severity is associated with a decreased volume of the CA3/dentate gyrus hippocampal subfield. *Biol Psychiatry*. 2010;**68**(5):494–496.
38. Zimmerman ME, *et al*. Perceived Stress is differentially related to hippocampal subfield volumes among older adults. *PLoS One*. 2016;**11**(5):e0154530.
39. Travis SG, *et al*. Effects of cortisol on hippocampal subfields volumes and memory performance in healthy control subjects and patients with major depressive disorder. *J Affect Disord*. 2016;**201**:34–41.
40. Feeney JC, *et al*. The association between hair cortisol, hair cortisone, and cognitive function in a population-based cohort of older adults: results from the Irish Longitudinal Study on Ageing. *J Gerontol A Biol Sci Med Sci*. 2020;**75**(2):257–265.
41. Feeney J, *et al*. Change in perceived stress and 2-year change in cognitive function among older adults: The Irish Longitudinal Study on Ageing. *Stress and Health*. 2018;**34**(3):403–410.
42. Phan TX, *et al*. Sleep and circadian rhythm disruption and stress intersect in Alzheimer's disease. *Neurobiol Stress*. 2019;**10**:100133.
43. Mueller AD, *et al*. Sleep deprivation can inhibit adult hippocampal neurogenesis independent of adrenal stress hormones. *Am J Physiol Regul Integr Comp Physiol*. 2008;**294**(5):R1693–R1703.
44. Kumari M, *et al*. Self-reported sleep duration and sleep disturbance are independently associated with cortisol secretion in the Whitehall II study. *J Clin Endocrinol Metab*. 2009;**94**(12):4801–4809.
45. McEwen BS. Sleep deprivation as a neurobiologic and physiologic stressor: allostasis and allostatic load. *Metabolism*. 2006;**55**(10 Suppl 2):S20–S23.
46. Chang FC, *et al*. Corticotropin-releasing hormone (CRH) as a regulator of waking. *Neurosci Biobehav Rev*. 2001;**25**(5):445–453.
47. Sanford L, *et al*. Stress, arousal, and sleep. In: *Sleep, Neuronal Plasticity and Brain Function*. Berlin, Heidelberg: Springer; 2014:379–410.

48. Golomb J, *et al*. Hippocampal formation size in normal human aging: a correlate of delayed secondary memory performance. *Learn Mem*. 1994;**1**(1):5–54.
49. Raz N, *et al*. Regional brain changes in aging healthy adults: general trends, individual differences and modifiers. *Cereb Cortex*. 2005;**15**(11):1676–1689.
50. Yaffe K, *et al*. Connections between sleep and cognition in older adults. *Lancet Neurol*. 2014;**13**(10):1017–1028.
51. van Oostrom SH, *et al*. Long sleep duration is associated with lower cognitive function among middle-age adults – the Doetinchem Cohort Study. *Sleep Med*. 2018;**41**:78–85.
52. Lo JC, *et al*. Self-reported sleep duration and cognitive performance in older adults: a systematic review and meta-analysis. *Sleep Med*. 2016;**17**:87–98.
53. Forget H, *et al*. Cognitive decline in patients with Cushing's syndrome. *J Int Neuropsychol Soc*. 2000;**6**(1):20–29.
54. Donoghue OA, *et al*. Cohort profile update: The Irish Longitudinal Study on Ageing (TILDA). *Int J Epidemiol*. 2018;**47**(5):1398–1398l.
55. Hirshkowitz M, *et al*. National Sleep Foundation's sleep time duration recommendations: methodology and results summary. *Sleep Health*. 2015;**1**(1):40–43.
56. Jenkins CD, *et al*. A scale for the estimation of sleep problems in clinical research. *J Clin Epidemiol*. 1988;**41**(4):313–321.
57. Cohen S, *et al*. Perceived stress scale. In: *Measuring Stress: A Guide for Health and Social Scientists*. New York, NY: Oxford University Press; 1994.
58. De Looze C, *et al*. Impaired orthostatic heart rate recovery is associated with smaller thalamic volume: Results from The Irish Longitudinal Study on Aging (TILDA). *Hum Brain Mapp*. 2020;**41**(12):3370–3378.
59. Dale AM, *et al*. Cortical surface-based analysis. I. Segmentation and surface reconstruction. *Neuroimage*. 1999;**9**(2):179–194.
60. Fischl B, *et al*. Whole brain segmentation: automated labeling of neuroanatomical structures in the human brain. *Neuron*. 2002;**33**(3):341–355.
61. Han X, *et al*. Reliability of MRI-derived measurements of human cerebral cortical thickness: the effects of field strength, scanner upgrade and manufacturer. *Neuroimage*. 2006;**32**(1):180–194.
62. Jovicich J, *et al*. Reliability in multi-site structural MRI studies: effects of gradient non-linearity correction on phantom and human data. *Neuroimage*. 2006;**30**(2):436–443.
63. Iglesias JE, *et al*.; Alzheimer's Disease Neuroimaging Initiative. A computational atlas of the hippocampal formation using ex vivo, ultra-high resolution MRI: Application to adaptive segmentation of in vivo MRI. *Neuroimage*. 2015;**115**:117–137.
64. Carey D, *et al*. Dissociable age and memory relationships with hippocampal subfield volumes in vivo: data from the Irish Longitudinal Study on Ageing (TILDA). *Sci Rep*. 2019;**9**(1):1–10.
65. Mayfield D, *et al*. The CAGE questionnaire: validation of a new alcoholism screening instrument. *Am J Psychiatry*. 1974;**131**(10):1121–1123.
66. Hagströmer M, *et al*. The International Physical Activity Questionnaire (IPAQ): a study of concurrent and construct validity. *Public Health Nutr*. 2006;**9**(6):755–762.
67. Radloff LS. The CES-D scale: a self-report depression scale for research in the general population. *Appl Psychol Meas*. 1977;**1**(3):385–401.
68. Itani O, *et al*. Short sleep duration and health outcomes: a systematic review, meta-analysis, and meta-regression. *Sleep Med*. 2017;**32**:246–256.
69. Jike M, *et al*. Long sleep duration and health outcomes: A systematic review, meta-analysis and meta-regression. *Sleep Med Rev*. 2018;**39**:25–36.
70. Brindley DN, *et al*. Possible connections between stress, diabetes, obesity, hypertension and altered lipoprotein metabolism that may result in atherosclerosis. *Clin Sci (Lond)*. 1989;**77**(5):453–461.
71. Bautista LE, *et al*. The relationship between chronic stress, hair cortisol and hypertension. *Int J Cardiol Hypertens*. 2019;**2**:100012.
72. Fotuhi M, *et al*. Modifiable factors that alter the size of the hippocampus with ageing. *Nat Rev Neurol*. 2012;**8**(4):189–202.
73. R Core Team. *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing; 2018.
74. Scarlett S, *et al*. Associations between cognitive function, actigraphy-based and self-reported sleep in older community-dwelling adults: findings from The Irish Longitudinal Study on Ageing. *Int J Geriatric Psychiatry*. 2020;**36**(5):731–742.
75. Fan L, *et al*. Sleep duration and the risk of dementia: a systematic review and meta-analysis of prospective cohort studies. *J Am Med Dir Assoc*. 2019;**20**(12):1480–1487.e5.
76. Wilckens KA, *et al*. Physical Activity and cognition: A mediating role of efficient sleep. *Behav Sleep Med*. 2018;**16**(6):569–586.
77. Van Leemput K, *et al*. Automated segmenta- tion of hippocampal subfields from ultra-high resolution in vivo MRI. *Hippocampus*. 2009;**19**(6):549–57. doi:10.1002/hipo.20615 PMID: 19405131; PubMed Central PMCID: PMC2739884.
78. Sabia S, *et al*. Association of sleep duration in middle and old age with incidence of dementia. *Nat Commun*. 2021;**12**(1):1–10.
79. Teicher MH, *et al*. Does sleep disruption mediate the effects of childhood maltreatment on brain structure? *Eur J Psychotraumatol*. 2017;**8**(Suppl 7):1450594.
80. Janiri D, *et al*. Hippocampal subfield volumes and childhood trauma in bipolar disorders. *J Affect Disord*. 2019;**253**:35–43.
81. Carlesimo GA, *et al*. Atrophy of presubiculum and subiculum is the earliest hippocampal anatomical marker of Alzheimer's disease. *Alzheimers Dement (Amst)*. 2015;**1**(1):24–32.
82. de Flores R, *et al*. Structural imaging of hippocampal subfields in healthy aging and Alzheimer's disease. *Neuroscience*. 2015;**309**:29–50.
83. Lim AS, *et al*. Sleep fragmentation and the risk of incident Alzheimer's disease and cognitive decline in older persons. *Sleep*. 2013;**36**(7):1027–1032. doi:10.5665/sleep.2802.
84. Beaulieu-Bonneau S, *et al*. Sleep disturbances in older adults with mild cognitive impairment. *Int Psychogeriatr*. 2009;**21**(4):654–666.
85. Naismith S, *et al*. Sleep–wake changes and cognition in neurodegenerative disease. *Brain Res*. 2011;**190**:21–52.
86. Lloret M, *et al*. Is sleep disruption a cause or consequence of Alzheimer's disease? Reviewing its possible role as a biomarker. *Int J Mol Sci*. 2020;**21**:1168.
87. Scarlett S, *et al*. Discrepancies in self-reported and actigraphy-based sleep duration are associated with self-reported insomnia symptoms in community-dwelling older adults. *Sleep Health*. 2021;**7**(1):83–92.
88. Wisse LEM, *et al*. Hippocampal subfield volumetry from structural isotropic 1 mm^3 MRI scans: a note of caution. *Hum Brain Mapp*. 2021;**42**(2):539–550.