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FEATURED ARTICLE

The overnight reduction of amyloid β 1-42 plasma levels is diminished by the extent of sleep fragmentation, sAPP-β, and *APOE* ε4 in psychiatrists on call

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Abstract

Introduction: In mice there might be an association between sleep deprivation and amyloid β plasma levels. Hence, we examined whether amyloid plasma levels are associated with sleep duration or fragmentation in 17 psychiatrists on-call.

Methods: Amyloid β (Aβ)42, Aβ40, and soluble amyloid precursor protein β (sAPP-β) plasma concentrations were measured at the beginning and end of 90 on-call nights, and analyzed using generalized linear models.

Results: In on-call nights, a 10.7% reduction of Aβ42 was revealed overnight. Every single short sleep interruption diminished this reduction by 5.4%, as well as every pg/mL of sAPP-β by 1.2%, each copy of *APOE* ε4 by 10.6%, and each year of professional experience by 3.0%.

Discussion: The extent of sleep fragmentation diminishes the physiological overnight reduction of Aβ42 but not Aβ40 plasma levels in the same direction as the enzyme for Aβ42 production, the genetic risk factor for Alzheimer's disease (AD), and on-call experience. Might on-call duty and sleep fragmentation in general alter the risk for AD?

KEYWORDS
Alzheimer's disease, amyloid plasma levels, on-call duty, psychiatrists, sleep, sleep fragmentation

1 | INTRODUCTION

The increase in brain amyloid is an early and important step in the development of Alzheimer's disease (AD).[1] Although amyloid β 40 (Aβ40) is the predominant form of Aβ in the brain, Aβ42 is the principal Aβ peptide in Alzheimer's plaques and its plasma levels are altered in AD (for an overview see Molinuevo et al[2]). Aβ and soluble amyloid precursor protein β (sAPP-β) are produced as a result of sequential cleavage of the amyloid precursor protein (APP) by the β-site amyloid precursor protein cleaving enzyme 1 (BACE1)[2]; and the plasma levels of BACE1[2] and sAPP-β[3] are altered in AD. The apolipoprotein E (*APOE*) ε4 allele is regarded the major genetic risk factor for sporadic AD; and many associations between *APOE* ε4 and AD biomarkers have already been reported.[2]

One hypothesis is that an impaired clearance of Aβ contributes to cerebral amyloid deposition.[4] It was shown that amyloid plasma levels

in humans are subject to circadian rhythms.[5] In mice, sleep deprivation increases amyloid levels in the interstitial fluid[6] and in the brain.[7,8] In addition, sleep deprivation increases BACE1 levels.[8] In rats, sleep deprivation also increases amyloid, BACE1, and sAPP-β levels in the brain.[9] Clearance of molecules such as Aβ from the brain is higher in sleeping than in awake mice because of the increase of the interstitial space.[10]

In healthy subjects, sleep fragmentation and low sleep efficiency are associated with reduced cognitive function.[11-13] Impaired sleep quality predicts cognitive decline.[14] In elderly subjects, shorter self-reported sleep duration is associated with increased brain amyloid load[15] and worse subjective sleep quality, with abnormal cerebrospinal fluid (CSF) Aβ42/Aβ40 ratios.[16] To date, the impact of sleep duration and sleep fragmentation on amyloid plasma levels in humans has not been studied.

Physicians who are on call represent shift working with the utmost socioeconomic significance. Therefore, an impact of on-call duty on the amyloid cascade, being crucially involved in the pathogenesis of AD, would be of great socioeconomic interest, as on-call duty may lead to physicians having a higher risk of developing AD.

Hence, we examined whether (1) Aβ42 is reduced overnight in psychiatrists on call and (2) the reduction of Aβ42 plasma levels is associated with sleep duration or the number of sleep interruptions, as a measure of sleep fragmentation. In addition, the specificity of associations were further investigated by (1) comparing Aβ42 plasma levels of normal nights spent at home and by (2) comparing to Aβ40 plasma levels and to the Aβ42/A40 ratio. Factors associated with baseline Aβ42 plasma levels were also explored.

RESEARCH IN CONTEXT

1. Systematic review: The authors reviewed the literature on the associations between sleep fragmentation and dementia using PubMed. All relevant citations are appropriately cited.
2. Interpretation: The extent of sleep fragmentation diminishes the physiological overnight reduction of amyloid β (Aβ)42 but not Aβ40 plasma levels in the same direction as the driving enzyme for the production of Aβ42, the genetic risk factor for Alzheimer's disease (AD), and years of professional on-call. Hence, on-call duty and sleep fragmentation in general might be associated with an altered risk of developing AD.
3. Future directions: Due to the high socioeconomic impact of such an association, further studies elucidating the interplay between sleep fragmentation and AD are urgently necessary.

2 | METHODS

2.1 | Participants and assessments

Psychiatrists on call from the university hospital Klinikum rechts der Isar, Technical University of Munich (Munich, Germany) were included. Written informed consent was obtained from all participants. The study protocol was submitted to the ethics committee of the Technical University of Munich Faculty of Medicine. No objections were raised, and all clinical investigations were conducted in accordance with the principles of the sixth revision of the Declaration of Helsinki.

Venous blood was collected from all participants at the beginning of their call shift at 5 pm ("evening") and at the end of their call shift at 8 am ("morning"). A maximum of eight consecutive night shifts were studied for each participant and compared with results from two nights spent at home. The rated nights at home were not adjacent to nights on call to avoid any interactions. After collection and centrifugation, plasma samples were immediately stored in aliquots at −80°C until further analyses. Aβ42 and Aβ40 plasma concentrations were measured in quadruplicate with a commercially available enzyme-linked immunosorbent assay (ELISA; Innotest β-amyloid 1-42 RUO; IBL Amyloid-beta 1-40 HS) in accordance with the manufacturer's instructions (Innogenetics, Ghent, Belgium; IBL, Gunma, Japan). The minimum detection limit was 4 pg/mL. sAPP-β was determined in plasma with the commercially available ELISA (sAPP-β wild-type HS, IBL, Gunma, Japan; detection range 0.78 to 50 ng/mL). Apolipoprotein E (*APOE*) εX genotypes of those participants who additionally provided written informed consent for genetic testing were determined through analysis of blood samples using standard polymerase chain reaction (PCR) and restriction enzyme digestion as described previously.[17]

Participants completed sleep protocols documenting the time of falling asleep and wake-up times. We distinguished between sleep interruptions of less (eg, phone calls/advice) or more (eg, consultations) than 15 minutes. Participants were requested to wear actometers to verify documentation. The total time of demand and the professional experience of the psychiatrist were reported in order to account for the individual level of stress.

2.2 | Statistical analyses

Sample size calculation was performed based on dynamics of amyloid plasma levels in normal sleep-wake cycles of mice[6] in advance. A moderate (between- subject) correlation of $r > 0.58$ for continuously distributed data reaches statistical significance when there are 12 paired observations (=12 participants). Likewise, within subject correlations of $r > 0.40$, statistical significance is reached when at least three on-call shifts are assessed for these 12 participants.

To adequately take into account the variability of the information of all nights per participant (dependent data) and all participants (independent data) generalized estimation equations (GEEs) with exchangeable correlation structure were set up to account for repeated measurements.

In the following, each statistical analysis is introduced in a separate paragraph. The first analysis was calculated to test whether the Aβ42 plasma levels decrease significantly overnight both for the nights on call and for the nights at home (see 3.2. for results). The second analysis looks at the effects of sleep duration and number of sleep interruptions on Aβ42 (see 3.3. for results). The third analysis seeks a possible effect of the on-call duty setting on Aβ42 (compare 3.4. for results). The fourth analysis aims to identify variables associated with the baseline levels of Aβ42 (see 3.5. for results). In addition to these analyses of interest, several additional analyses were carried out to assess the specificity of the results of the previous analyses (see 3.6. for results). These comprised analyses of Aβ40, sAPP-β, and Aβ42/Aβ40 plasma level changes with the same models as the analyses of Aβ42 plasma changes.

To test whether Aβ42 plasma levels decrease significantly overnight, a GEE was set up with the outcome variable the relative reduction of Aβ42 (median levels of (Aβ42 evening – Aβ42 morning)/Aβ42 evening). The model included an intercept only. This analysis was calculated separately for nights on call and nights at home.

To assess the effects of sleep duration and number of sleep interruptions on Aβ42 reductions, another GEE was set up with the outcome variable being the relative reduction of Aβ42 (median plasma levels of (Aβ42 evening – Aβ42 morning)/Aβ42 evening), the factor sex, and the covariates the number of sleep interruptions ≤15, and >15 minutes, total time of demand, years of professional experience, age, median levels of Aβ42 evening, median levels of sAPP-β evening, sleep duration, and sleep duration the previous night, including solely on-call nights. This analysis was repeated including the additional covariate *APOE* ε4 frequency in those participants who underwent genotyping.

To evaluate a possible effect of the on-call duty setting (eg, stress, unfamiliar sleep environment) a GEE was set up with the outcome variable as the relative reduction of Aβ42, the factor sex and on-call/at-home, and the covariates number of sleep interruptions ≤15 and >15 minutes, years of professional experience, age, median plasma levels of Aβ42 evening, sleep duration, and, sleep duration the previous night, including both nights on call duty and at home.

To identify variables that are associated with baseline levels of Aβ42 (plasma levels of Aβ42 evening), a GEE was set up with the outcome variable levels of Aβ42 evening, the factor sex, and the covariates years of professional experience, age and, sleep duration previous night, and median levels of sAPP-β evening, including both nights on call and at home. This analysis was repeated including the additional covariate *APOE* ε4 frequency in those participants who underwent genotyping.

2.2.1 | Analyses of specificity of the above findings

To test whether Aβ40 plasma levels decrease significantly overnight, GEEs with exchangeable correlation structure were set up to account for repeated measurements. The outcome variable was the relative reduction of Aβ40 (median levels of (Aβ40 evening - Aβ40 morning)/Aβ40 evening). The model included an intercept only. This analysis was calculated separately for on-call nights and nights at home.

To assess whether sleep duration and number of sleep interruptions also influence Aβ40 reductions, another GEE was set up with the outcome variable of relative reduction of Aβ40 (median plasma levels of (Aβ40 evening – Aβ40 morning)/Aβ40 evening), the factor sex, and the covariates number of sleep interruptions ≤15 and >15 minutes, total time of demand, years of professional experience, age, median levels of Aβ40 evening, median levels of sAPP-β evening, sleep duration, and sleep duration the previous night, including solely on-call nights.

To test whether sAPP-β plasma levels decrease significantly overnight, GEEs with exchangeable correlation structure were set up to account for repeated measurements. The outcome variable was relative reduction of sAPP-β (median levels of (sAPP-β evening – sAPP-β morning)/sAPP-β evening). The model included an intercept only. This analysis was calculated separately for on-call nights and for nights at home.

The ratio of Aβ/Aβ40 was recently suggested to match amyloid load measured by positron emission tomography (PET) in cognitively normal subjects.[18] Therefore, the following analyses were calculated using the Aβ/Aβ40 ratio consistent to the analyses using Aβ42.

To test whether Aβ42/Aβ40 plasma levels decrease significantly overnight, a GEE was set up with the outcome variable being the relative reduction of Aβ42/Aβ40 (median levels of (Aβ42/Aβ40 evening – Aβ42/Aβ40 morning)/Aβ42/Aβ40 evening). The model included an intercept only. This analysis was calculated separately for nights on call and nights at home.

To assess the effects of sleep duration and number of sleep interruptions on Aβ42/Aβ40 reductions, another GEE was set up with the outcome variable being the relative reduction of Aβ42/Aβ40 (median plasma levels of (Aβ42/Aβ40 evening – Aβ42/Aβ40 morning)/Aβ42/Aβ40 evening), the factor sex, and the covariates the number of sleep interruptions ≤15 and >15 minutes, total time of demand, years of professional experience, age, median levels of Aβ42/Aβ40 evening, median levels of sAPP-β evening, sleep duration, and sleep duration the previous night, including solely on-call nights. This analysis was repeated including the additional covariate *APOE* ε4 frequency in those participants who underwent genotyping.

To identify variables that are associated with baseline levels of Aβ42/Aβ40 (plasma levels of Aβ42/Aβ40 evening), a GEE was set up with the outcome variable levels of Aβ42/Aβ40 evening, the factor sex, and the covariates years of professional experience, age and sleep duration previous night, and median levels of sAPP-β evening, including both nights on call and at home. This analysis was repeated including the additional covariate *APOE* ε4 frequency in those participants who underwent genotyping.

For all tested hypotheses, the level of significance was set to 0.05. All analyses were calculated in R (version 3.3.1; The R Foundation for Statistical Computing; www.r-project.org/foundation/)

3 | RESULTS

3.1 | Characteristics of patient sample

Seventeen psychiatrists participated in the study wherein a total of 90 call nights and 27 nights spent at home were analyzed. Participants' characteristics are shown in Table 1 and for the sub-set who underwent genotyping, in Supplementary Table S1.

3.2 | Reduction of Aβ42 plasma levels overnight

The changes of Aβ42 before and after every on-call duty of all participants are shown in Figure 1; the changes averaged across nights in Supplementary Figure S1.

The GEE solely including the relative reduction of Aβ42 as outcome revealed a significant reduction of Aβ42 overnight (estimate 0.107, $P < 0.001$) during call nights. During nights spent at home, Aβ42 was also significantly reduced (estimate 0.114, $P = 0.001$).

3.3 | Association between relative reduction of plasma Aβ42 overnight and sleep durations and interruptions

During on-call nights, each sleep interruption ≤15 minutes significantly decreases the relative reduction of Aβ42 overnight by 5.4%. Relative reduction of Aβ42 was also significantly decreased by every additional year of professional experience by 3.0%, by every additional pg/mL of sAPP-β by 1.2% (and by every additional copy of the *APOE* ε4 allele by 10.6%). In contrast, every additional year of age of the psychiatrist significantly increases the relative reduction of Aβ42 overnight by 3.9% as well as female sex by 15.2%. A summary of all effects on the relative reduction of Aβ42 overnight is displayed in Figure 2; truncated scatterplots of the individual estimates are shown in Figure 3. Details of this analysis including estimates and *P*-values of all parameters are provided in Supplementary information.

3.4 | Effect of the on-call duty setting on relative reduction of Aβ42

In the GEEs with the outcome variable relative reduction of Aβ42, the estimate for differences in nights on call and at home was 0.009 ($P = 0.842$). This indicates that Aβ42 overnight was reduced by an additional 0.9% during nights at home as compared to nights on call in the present data. However, there was no evidence that could justify a generalization of this effect.

TABLE 1 Characteristics of participants

Demographics	
Numbers	17
Males:females	10:7
	Mean/SD/range
Age (years)	34/4.3/26 - 49
Professional experience with on-call duty (years)	5 /4.4 /0 - 18
Characteristics of nights with on-call duty	
Nights, total numbers	90
	Mean/SD/range
Nights per participant	3.7/2.2/1 – 8
Sleep duration, previous night (hours)	7.4/0.5/3.5 – 10.0
Sleep duration (hours)	5.4/1.7/1.4 – 9.5
Number of sleep interruptions ≤15 minutes	0.4/1.0/0 – 6
Number of sleep interruptions >15 minutes	0.3/0.6/0 – 2
Number of sleep interruptions in total	0.7/1,1/0 – 6
Time of demand (minutes)	220/123/20 – 680
Time of blood draw, evening	5:12 pm/00:22/4:30 pm – 6:45 pm
Time of blood draw, morning	08:19 am/00:27/07:45 am – 10:45 am
Aβ42 plasma levels, evening (pg/mL)[a]	30.2/31.4/0.0 – 112.4
Aβ42 plasma levels, morning (pg/mL)[a]	27.3/29.7/0.0 – 106.7
Aβ42 plasma levels, absolute differences (pg/mL)[a] (Aβ42 morning – Aβ42 evening)	-2.9/2.8/-9.3 – +0.7
Aβ42 plasma levels, relative decrease[*] (Aβ42 morning – Aβ42 evening)/Aβ42 evening)	−10.2%/12.2%/-36.6% – +6.7%
Aβ40 plasma levels, evening (pg/mL)[a]	155.3/98.7/29.1 – 398.4
Aβ40 plasma levels, morning (pg/mL)[a]	134.2/90.8/25.4 – 375.0
Aβ40 plasma levels, absolute differences (pg/mL)[a] (Aβ40 morning – Aβ40 evening)	−21.7/18.6/-48.9 – +18.7
Aβ40 plasma levels, relative decrease[a] ([Aβ40 morning – Aβ40 evening]/Aβ40 evening)	−10.4%/13.7%/-33.9% – +21.1%
Aβ42/Aβ40 plasma ratio, evening[a]	0.25/0.20/0.00 – 0.71
Aβ42/Aβ40 plasma ratio, morning[a]	0.28/0.27/0.00 - 1.08
Aβ42/Aβ40 plasma ratio, absolute differences[a] (Aβ42/Aβ40 morning – Aβ42/Aβ40 evening)	0.03/0.10/-0.1 – +0.37

(Continues)

TABLE 1 (Continued)

Aβ42/Aβ40 plasma ratio, relative increase* ([Aβ42/Aβ40 morning – Aβ42/Aβ40 evening]/Aβ42/Aβ40 evening)	10.9%/19.7%/-31.8% - +51.0%
sAPP-β plasma levels, evening (pg/mL)[a]	4.7/5.5/0.5 – 24.5
sAPP-β plasma levels, morning (pg/mL)[a]	4.7/5.8/0.7 – 25.2
Characteristics of nights at home	
Nights, total numbers	27
	Mean/SD/range
Nights per participant	1.5/0.5/1 - 2
Sleep duration, previous night (hours)	7.2/1.5/4.0 – 10.0
Sleep duration in hours	7.0/0.9/5.0 – 8.8
Number of sleep interruptions ≤15 minutes	0.3/0.6/0 – 2
Number of sleep interruptions >15 minutes	0.0/0.0/0 – 0
Number of sleep interruptions in total	0.3/0.6/0 – 2
Time of blood draw, evening	5:03 pm/00:21/4:15 pm – 5:45 pm
Time of blood draw, morning	8:32 am/00:24/8:05 am – 9:25 am
Aβ42 plasma levels, evening (pg/mL)[a]	32.3/31.3/11.5 – 105.6
Aβ42 plasma levels, morning (pg/mL)[a]	28.9/29.8/10.1 – 101.3
Aβ42 plasma levels, absolute differences (pg/mL)[a] (Aβ42 morning - Aβ42 evening)	−3.4/3.1/-9.4 – +0.4
Aβ42 plasma levels, relative decrease[a] ([Aβ42 morning - Aβ42 evening]/Aβ40 evening)	−11.8%/12.7%/-37.4% – +4.6%
Aβ40 plasma levels, evening (pg/mL)[a]	138.3/109.3/22.9 – 467.3
Aβ40 plasma levels, morning (pg/mL)[a]	110.6/92.4/19.0 – 399.6
Aβ40 plasma levels, absolute differences (pg/mL)[a] (Aβ40 morning - Aβ40 evening)	−27.6/33.3/-67.6 - +51.3
Aβ40 plasma levels, relative decrease[a] ([Aβ40 morning - Aβ40 evening]/Aβ40 evening)	−16.1%/23.2%/-39.6% - +45.2%
Aβ42/Aβ40 plasma ratio, evening[a]	0.35/0.27/0.03 – 0.84
Aβ42/Aβ40 plasma ratio, morning[a]	0.42/.0.38/0.03 – 1.41
Aβ42/Aβ40 plasma ratio, absolute differences[a] (Aβ42/Aβ40 morning - Aβ42/Aβ40 evening)	0.07/0.18/-0.10 - +0.56
Aβ42/Aβ40 plasma ratio, relative increase* ([Aβ42/Aβ40 morning - Aβ42/Aβ40 evening]/Aβ42/Aβ40 evening)	16.0%/31.3%/-47.6% - +66.0%
sAPP-β plasma levels, evening (pg/mL)[a]	5.0/5.4/0.9 – 22.5
sAPP-β plasma levels, morning (pg/mL)[a]	4.6/5.5/0.4 – 22.3
APOE genotype	
APOE genotypes 2/3: 3/3: 3/4: 4/4: n.d.	2: 9: 1: 0: 5
	Mean/SD/range
ε4 allele frequency	0.08/0.29/0 – 1

Aβ, amyloid β; *APOE*, apolipoprotein E genotype; sAPP-β, soluble amyloid precursor protein β; SD, standard deviation.

[a]Mean values of previously calculated mean values/participants are shown.

The estimates of all other variables in this model were (variable/estimate/*P*-value): sex/−0.064/0.166, number of sleep interruptions <15 minutes/−0.017/0.313, sleep interruptions >15 minutes/−0.004/0.939, age/0.019/0.074, years of professional experience/−0.012/0.272, median Aβ42 evening/−0.001/0.064, sleep duration/−0.009/0.528, sleep duration previous night/−0.023/0.214.

3.5 | Factors associated with baseline plasma levels of Aβ42

Baseline Aβ42 overnight was 29.407 pg/mL lower in females as compared to males, and increased by 22.427 pg/mL by every additional year of professional experience, and by 2.593 pg/mL by every hour of additional sleep in the previous night, and was reduced by 19.364 pg/mL by every additional year of age, by 0.594 pg/mL by every pg/mL of sAPP-β, and by 15.439 pg/mL by every additional copy of the *APOE* ε4 allele in the present data. However, there was no evidence that could justify a generalization of these effects. Details of this analysis including estimates and *P*-values of all parameters are provided in Supplementary information.

3.6 | Analyses of the specificity of the above-mentioned findings

3.6.1 | Reduction of Aβ40 plasma levels overnight

The changes of Aβ40 before and after every on-call duty of all participants are shown in Figure 4; the changes averaged across nights are shown in Supplementary Figure S2.

The GEE solely including the relative reduction of Aβ40 as outcome revealed a significant reduction of Aβ40 overnight (estimate 0.102,

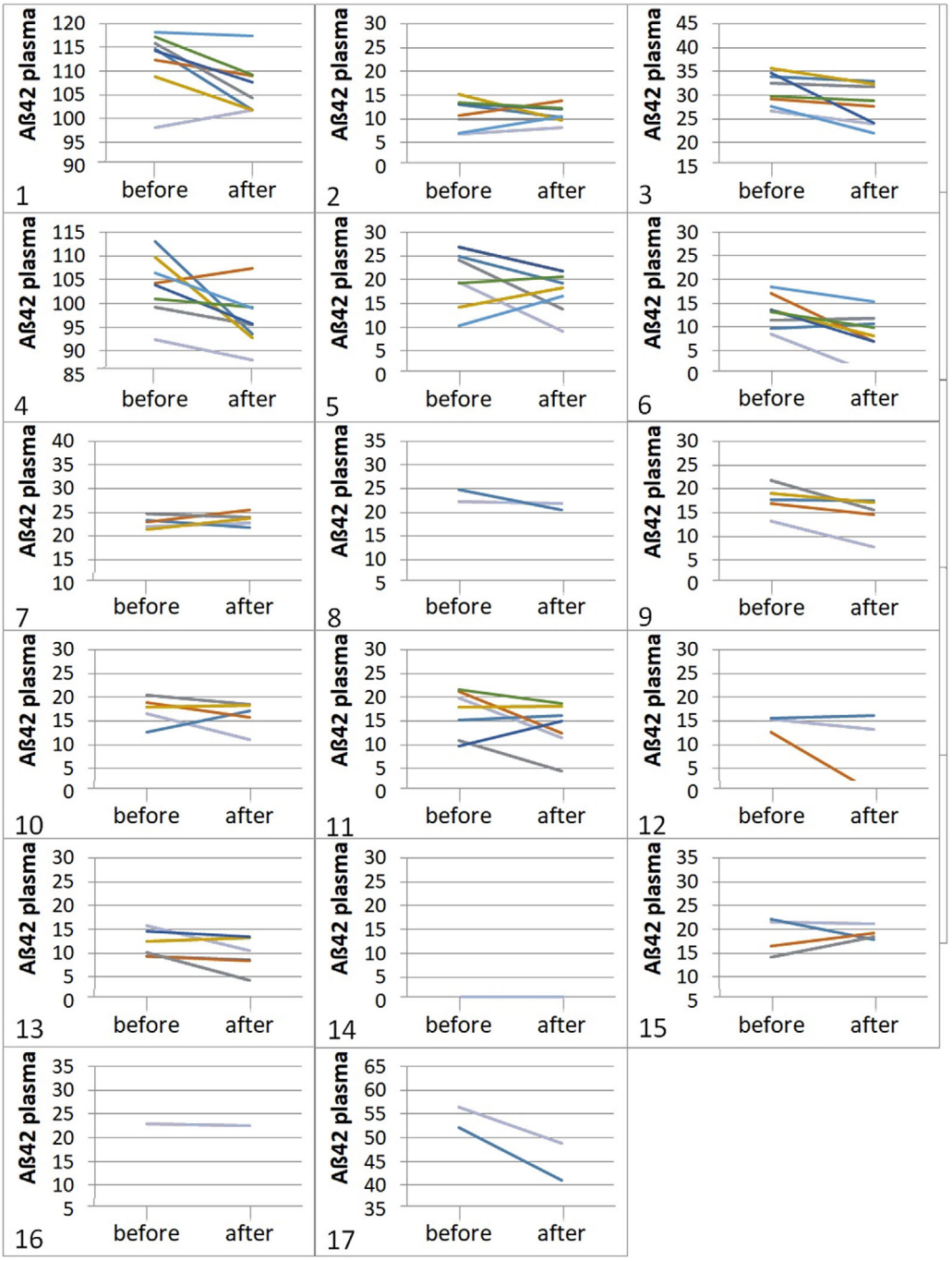

FIGURE 1 Aβ42 plasma level change before and after on-call duty of all participants. Aβ42 plasma level change in pg/mL of all nights of all participants (n = 17) before and after on-call duty. Aβ, amyloid β

$P = 0.006$) during nights on call. During nights spent at home, Aβ40 was also significantly reduced (estimate 0.186, $P < 0.001$).

3.6.2 | Association between the relative reduction of Aβ40 overnight and sleep durations and interruptions

None of the included variables were significantly associated with the reduction of Aβ40 overnight. Details of this analysis including estimates and *P*-values of all parameters are provided in Supplementary information.

3.6.3 | Reduction of sAPP-β plasma levels overnight

The GEE solely including the relative reduction of sAPP-β as outcome revealed an increase of sAPP-β overnight (estimate −0.105, $P = 0.333$) during nights on call and a decrease (estimate 0.136, $P = 0.114$) during

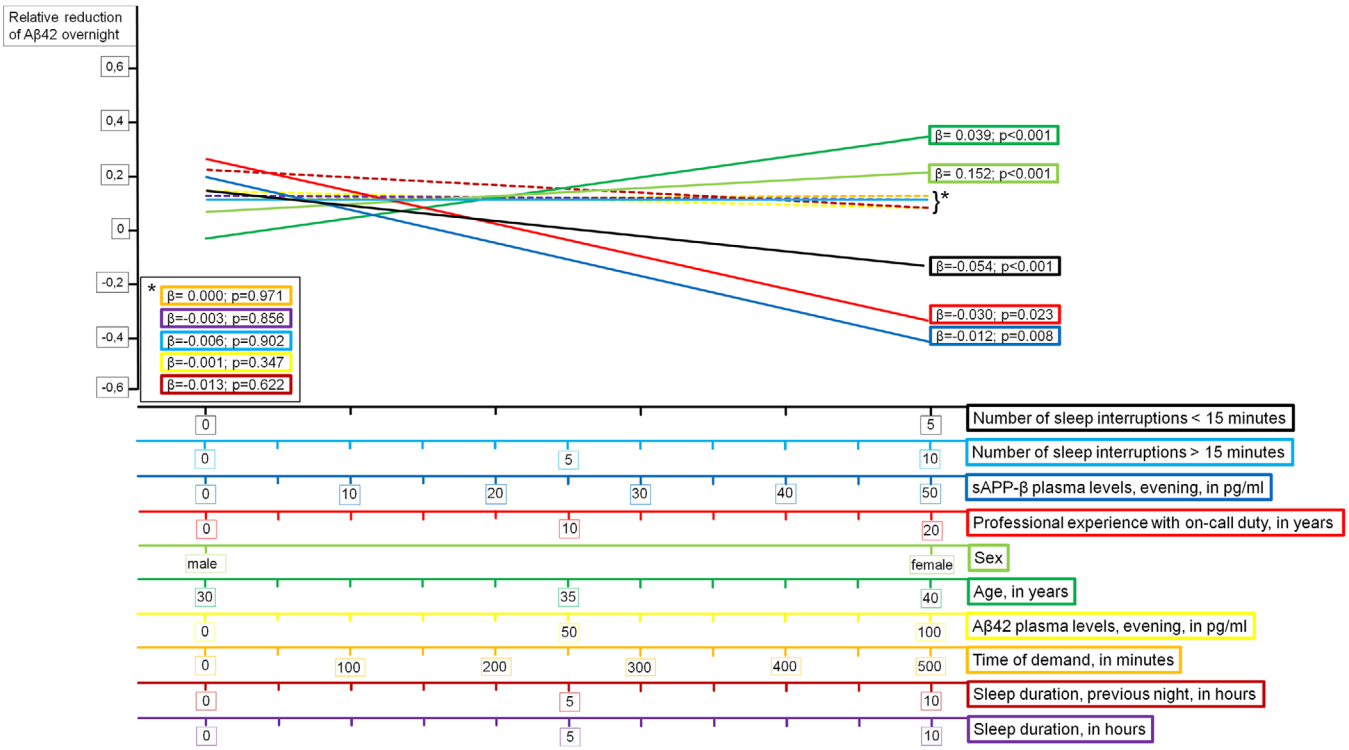

FIGURE 2 Summary of factors modifying relative reduction of plasma Aβ42 overnight during on-call duty. Estimates modifying Aβ42 plasma level reduction in pg/mL overnight during on-call duty. Aβ, amyloid β; sAPP-β, soluble amyloid precursor protein β

nights at home. However, there was no evidence that could justify a generalization of these effects.

3.6.4 | Increase of Aβ42/Aβ40 plasma levels overnight

The GEE solely including the relative reduction of Aβ42/Aβ40 as outcome revealed a significant increase of Aβ42/Aβ40 overnight (estimate 0.127, $P = 0.031$) during call nights. During nights spent at home, Aβ42/Aβ40 was also significantly increased (estimate 0.116, $P = 0.007$).

3.6.5 | Association between relative increase of plasma Aβ42/Aβ40 overnight and sleep durations and interruptions

During on-call nights, each sleep interruption ≤15 minutes significantly further increased the relative increase of Aβ42/Aβ40 overnight by 6.4%. Relative increase of Aβ42/Aβ40 was also significantly further increased by every additional year of professional experience by 8.1%, by every additional pg/mL sAPP-β by 6.3%, and by every +1.0 increase of Aβ42/Aβ40 evening ratio by 94%. In contrast, every additional year of age of the psychiatrist significantly decreased the relative increase of Aβ42/Aβ40 overnight by 10.0%. Details of this analysis including estimates and *P*-values of all parameters are provided in Supplementary information.

3.6.6 | Factors associated with baseline plasma levels of Aβ42/Aβ40

Baseline Aβ42/Aβ40 overnight was 0.063 higher in females as compared to males, increased by 0.009 by every additional year of age, increased by 0.002 every hour of additional sleep in the previous night, and by 0.001 by every pg/mL of sAPP-β, and was reduced by −0.018 by every additional year of professional experience, and by 0.005 by every additional copy of the *APOE* ε4 allele in the present data. However, there was no evidence that could justify a generalization of these effects. Details of this analysis including estimates and *P*-values of all parameters are provided in Supplementary information.

4 | DISCUSSION

This is the first study focusing on the associations between Aβ42 plasma levels and sleep fragmentation in physicians on call. Aβ42 plasma levels decrease significantly overnight; however, this decrease is significantly reduced by the number of sleep interruptions, years of professional experience, levels of plasma sAPP-β, and copies of the *APOE* ε4 allele. This association is specific for

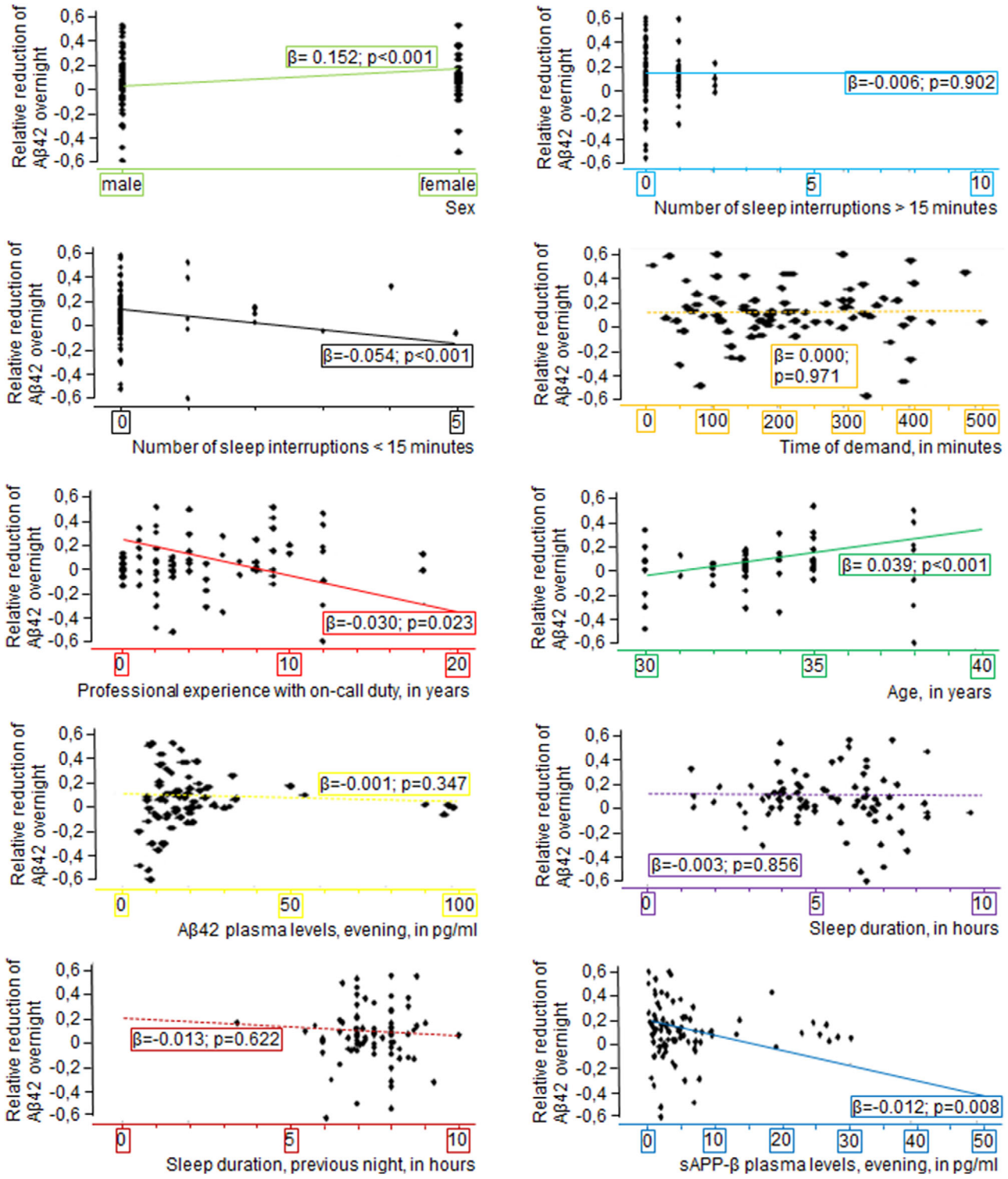

FIGURE 3 Individual factors modifying relative reduction of plasma Aβ42 overnight during on-call duty. Truncated scatterplots of estimates modifying Aβ42 plasma level reduction in pg/mL overnight during on-call duty. Aβ, amyloid β; sAPP-β, soluble amyloid precursor protein β

Aβ42, as it was not observed for Aβ40 despite Aβ40 also decreasing overnight.

Our result of a significant decrease of 10.7% of Aβ42 during nights on call and of 11.4% during nights at home is in accordance with previous work in healthy subjects that showed a mean decrease of 7.6%,[5] and is compatible with the results from a recent study demonstrating a decrease of CSF Aβ42 levels in young healthy volunteers.[19] Hence, a decrease of Aβ42 overnight can be considered a physiological process. The decrease of Aβ40 overnight has not been reported previously to the best of our knowledge.

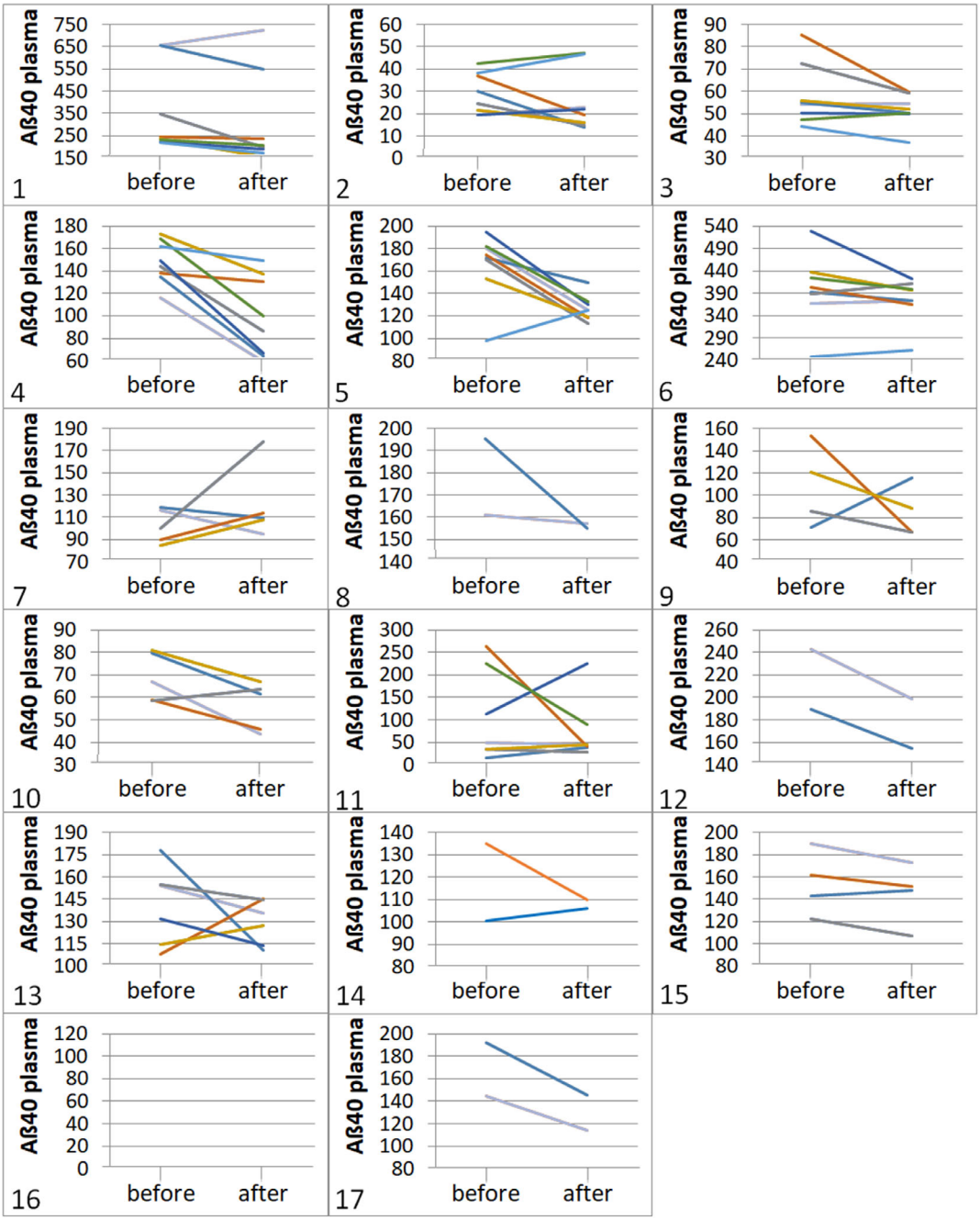

FIGURE 4 Aβ40 plasma level change before and after on-call duty of all participants. Aβ40 plasma level change in pg/mL of all nights of all participants (n = 17) before and after on-call duty. Aβ, amyloid β

The result of this study that the decrease of Aβ42 overnight is reduced by the number of sleep interruptions is compatible with further recent work: An association was demonstrated between the extent of sleep deprivation and Aβ in the interstitial fluid in wild-type and transgenic APP mice.[6] A lower decrease of Aβ42 in CSF[19] and a numerical but not significantly lower decrease of Aβ42/Aβ40 ratio[20] were shown in healthy humans with complete sleep deprivation as compared to unimpaired sleep, and worse subjective sleep quality was associate with abnormal, that is, AD typical, CSF Aβ42/Aβ40 ratios.[16] It would certainly be of great scientific interest to compare the changes of Aβ in plasma directly with other measures of amyloid such as CSF Aβ42 or amyloid PET. However, repetitive lumbar punctures or amyloid PETs before and after every night on call was not feasible in our participants for obvious reasons.

Of interest, the reduced decrease of Aβ42 is statistically significant explained by the extent of sleep fragmentation, as measured by the number of interruptions, but not by the duration of sleep, the time of demand, or environmental factors that differ between sleep at home or at hospital. One could consider the number of sleep interruptions in this naturalistic study as relatively low and speculate that the

statistically significant effect of the extent of sleep fragmentation in this study ($P < 0.001$) might be even more pronounced in an experimental design with a higher number of sleep interruptions.

The effect was selectively observed for the AD pathognomonic Aβ42 but not for Aβ40, pointing to a possible association between sleep fragmentation and the AD cascade. This hypothesis is further strengthened by the fact that both sAPP-β ($P = 0.008$), one of the enzymes responsible for producing Aβ42, and the copies of the *APOE* ε4 alleles ($P = 0.043$) significantly diminish the reduction of Aβ42 overnight in the same direction as does sleep fragmentation. Of interest, the years of professional experience also significantly diminish the reduction. It is tempting to speculate that a harmful effect of on-call duty cannot be ruled out. However, decreased plasma Aβ42 levels have been observed in amyloid PET–positive subjects.[21] Hence, further studies are needed to investigate the causes of the selective effect of sleep fragmentation on Aβ42 only and the causes of decreased plasma Aβ42 levels in amyloid PET positives.

In addition, a significant ($P < 0.001$) effect of age was noted: Every additional year of age reduces Aβ42 overnight by an additional 3.9%. However, in another study,[5] Aβ42 overnight was substantially less decreased in older normal controls as compared to younger controls. Thus, this finding should be interpreted with caution. There was also a different effect depending on sex. Among female participants, the reduction of Aβ42 overnight was increased by 15.3%. This effect could not be explained by the current study data. To the best of our knowledge, no other study has compared gender differences in the effect of sleep fragmentation to the reduction of Aβ42 overnight. Therefore, future studies are needed to further elucidate this important topic. Furthermore, there may be interactions between the independent variables age and sex, and professional experience on the reduction of Aβ42 overnight that were not looked into in the current experiment, as the number of independent observations is not sufficient to address this thoroughly. This important question has not yet been addressed in other studies, and must therefore be addressed in future studies with larger cohorts of participants with on-call duty.

In addition to the high number of measured nights and the control of many cofactors, another strength of the current study is the measurement of the less-amyloidergic Aβ40 in the analyses. Although Aβ40 was also significantly reduced overnight, it was not influenced by the cofactors as was Aβ42, pointing to the specificity of the reported associations for Aβ42 and hence for AD-specific pathophysiology. Of interest, in a sample of young Chinese volunteers, a night of complete sleep deprivation resulted in a significant, 52% increase in plasma Aβ40. This points to a differential effect on plasma Aβ40: The time of waking seems to increase Aβ40, whereas sleep fragmentation, as in our study, does not alter the reduction of Aβ40 overnight, but specifically seems to diminish the reduction of Aβ42 overnight.[22]

In addition to sleep fragmentation, we carefully controlled for other factors that could influence the Aβ42, among them on-duty setting, time of demand, and years of professional experience. It is notable that years of professional experience was a significant predictor that diminished the decrease of Aβ42 in the same direction as sleep fragmentation.

The ratio of Aβ/Aβ40 was recently suggested to match amyloid load measured by PET in cognitively normal subjects.[18] It appears reasonable to normalize the amyloidergic Aβ42 to a reference peptide (here Aβ40) to control for inter-individual differences in amyloid production and to improve diagnostic accuracy. However, in our study, intra-individual changes of Aβ42 have been compared. Therefore, normalizing for inter-individual differences in production rates appears negligible. In addition, separately calculating the effects of sleep fragmentation on both Aβ42 and Aβ40 allowed disentangling of the effect of sleep fragmentation on both peptides. Nevertheless, analyses using Aβ42/Aβ40 have been calculated. Compared to the significant reduction of Aβ42 overnight, the Aβ42/Aβ40 ratio increased overnight, as the significant relative decrease of Aβ40 outweighed the significant relative decrease of Aβ42 but all estimates of the factors associated with the relative change overnight or the baseline ratio pointed in the same direction as in the analysis using Aβ42.

The causative mechanism underlying the reduced decrease of Aβ42 by the extent of sleep fragmentation remains to be elucidated in further studies, for example, altered clearance rates. Altered production rates appear less likely causative, as sAPP-β, the product of BACE1 cleavage, was not significantly altered by the extent of sleep fragmentation (data not shown). sAPP-β was numerically but not significantly increased during on-call nights. This finding is compatible with a significant increase of BACE1 activity after sleep restriction in mice[8] or after sleep deprivation in rats,[9] as well as with a significant increase of sAPP-β in rats.[9] Obviously, complete sleep deprivation induces stronger effects on sAPP-β as compared to the sleep fragmentation in our experiment.

The reduction of the decrease of Aβ42 appears also not dependent on the *APOE* genotype that influences several aspects of the amyloid pathophysiology. However, there was almost no *APOE* ε4 allele in the included cohort. It is notable that there was a numerical non-significant association between the reduction of CSF Aβ42 and the extent of experimentally disrupted slow-wave activity during sleep in a previous study.[23] Hence, it is tempting to speculate that sleep fragmentation decreased slow wave sleep and increased synaptic activity, with increased Aβ production rates leading to less decrease of soluble Aβ overnight, as detected in this study.

Obviously participants have substantial differences in baseline Aβ42. Despite thorough analyses, these differences cannot be explained by the data of the current study: Sex, years of professional experience, age, sleep duration of the previous night, sAPP-β, as well as *APOE* ε4 frequency did not significantly explain the variability of baseline Aβ42. Therefore, further work is required to identify factors responsible for such differences.

Sleep fragmentation as a result of on-call duty induced pathophysiological changes in amyloid metabolism in our study. On-call duty does not only apply to psychiatrists, but also to many other civil service employees. In view of recent evidence that complete sleep deprivation results in an increase in amyloid burden measured by PET in the hippocampus of healthy adults,[24] the question arises whether on-call duty alters the risk of developing AD and whether the frequency and extent of on-call duty needs to be re-evaluated.

CONCLUSION

The fact that sleep fragmentation diminishes the reduction of Aβ42 plasma levels overnight raises the question whether on-call duty and sleep fragmentation in general might be associated with an altered risk of developing AD. Due to the high socioeconomic impact of such an association, further studies elucidating the association between sleep fragmentation and AD are urgently needed.

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CONFLICT OF INTEREST

The corresponding author certifies for all authors that there is no actual or potential conflict of interest in relation to this article. Outside the submitted work, Dr. T. Grimmer reported having received consulting fees from Actelion, Biogen, Eli Lilly, Iqvia/Quintiles, MSD, Novartis, Quintiles, and Roche Pharma; lecture fees from Biogen, Lilly, Parexel, and Roche Pharma; and grants to his institution from Actelion and PreDemTech.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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