Neurobiology of Learning and Memory 157 (2019) 106–113

Contents lists available at ScienceDirect

Neurobiology of Learning and Memory

journal homepage: www.elsevier.com/locate/ynlme

ADHD symptoms are associated with decreased activity of fast sleep spindles and poorer procedural overnight learning during adolescence

Ilona Merikanto[a,b,c,*], Liisa Kuula[a], Tommi Makkonen[a], Risto Halonen[a], Jari Lahti[a], Kati Heinonen[a], Katri Räikkönen[a], Anu-Katriina Pesonen[a]

[a] Department of Psychology and Logopedics, Faculty of Medicine, University of Helsinki, 00014 Helsinki, Finland
[b] National Institute for Health and Welfare, 00271 Helsinki, Finland
[c] Orton Orthopaedics Hospital, Helsinki, Finland

ARTICLE INFO

Keywords:
ADHD
Adolescence
EEG
Polysomnography
Procedural memory
Memory consolidation
Sleep
Spindle

ABSTRACT

ADHD and its subclinical symptoms have been associated with both disturbed sleep and weakened overnight memory consolidation. As sleep spindle activity during NREM sleep plays a key role in both sleep maintenance and memory consolidation, we examined the association between subclinical ADHD symptoms and sleep spindle activity. Furthermore, we hypothesized that sleep spindle activity mediates the effect of ADHD symptoms on overnight learning outcome in a procedural memory task. We studied these questions in a community-based cohort of 170 adolescents (58% girls, mean age = 16.9, SD = 0.1 years), who filled in the Adult ADHD Self-Report Scale (ASRS-v1.1), and underwent an overnight sleep EEG coupled with a mirror tracing task before and after sleep. Elevated ADHD symptoms were associated with weaker fast sleep spindle activity, and poorer overnight learning in the procedural memory test. However, sleep spindles, contrary to the hypothesis, did not mediate the association between ADHD symptoms and overnight learning. Our results showed that a higher level of ADHD symptoms in adolescence is associated with similar alterations in sleep spindle activity as observed in many neuropsychiatric conditions and might contribute to altered synaptic connectivity and sleep fragmentation observed in ADHD.

1. Introduction

Attention-deficit/hyperactivity disorder (ADHD) is suggested to be a dimensional rather than categorical phenomenon, as it forms a continuum from the absence of indicators to mild and severe symptoms (Coghill & Sonuga-Barke, 2012; Levy, Hay, McStephen, Wood, & Waldman, 1997). ADHD symptoms can interfere negatively with executive functioning, development and learning (Uekermann et al., 2010). Sleep disturbances, such as sleep fragmentation, frequently associate with ADHD and its subclinical symptoms, also independently of ADHD medication (Kirov & Brand, 2014; Konofal, Lecendreux, & Cortese, 2010). Noteworthy, sleep has an important role in enhancing learning (Curcio, Ferrara, & Degennaro, 2006). Accordingly, clinical ADHD has been associated with impaired overnight declarative and procedural learning (Prehn-Kristensen, Göder, et al., 2011; Prehn-Kristensen, Molzow, et al., 2011). However, there is a gap of knowledge on how sleep and its microstructures are related to overnight memory consolidation at the non-clinical range of ADHD symptoms.

Sleep spindles are brief thalamocortical oscillations seen mainly during stage 2 of non-rapid eye movement (NREM) sleep at sigma frequency of 10–16 Hz (Iber, Ancoli-Israel, Chesson, and Quan, 2007; Lüthi, 2014). They have a major roles in maintenance of sleep (Cote, Epps, & Campbell, 2000; Dang-Vu et al., 2011) and overnight memory consolidation processes (Lüthi, 2014; Ulrich, 2016). During sleep spindle activity, memory-related networks for example in thalamus, midbrain, hippocampus and cortex are activated (Fogel & Smith, 2011). The role of sleep spindles in overnight memory-processes is assumed to promote synaptic plasticity through calcium influx that induces long-term potentiation in networks active in recent past (Fogel & Smith, 2011; Ulrich, 2016). The different role of fast and slow sleep spindles in these processes is still elusive, but may relate to differences in which memory-related networks are activated (Fogel & Smith, 2011).

Individual differences in sleep spindle activity are rather stable, trait-like characteristics (De Gennaro et al., 2008). White matter connectivity, especially at the frontal regions and subcortically around the thalamus, has been shown to contribute to this individual variability

* Corresponding author at: Department of Psychology and Logopedics, Faculty of Medicine, University of Helsinki, P.O. Box 9, University of Helsinki, 00014 Helsinki, Finland.
E-mail address: ilona.merikanto@helsinki.fi (I. Merikanto).

https://doi.org/10.1016/j.nlm.2018.12.004
Received 3 May 2018; Received in revised form 26 October 2018; Accepted 11 December 2018
Available online 12 December 2018
1074-7427/

(Piantoni et al., 2013). However, it is not clear whether the individual differences stem from genetics or from experience, or both. Interestingly, white matter abnormalities have been observed in ADHD patients and their first-order relatives (Pironti et al., 2014). These abnormalities include lowered neural branching especially at the frontal brain regions and corpus callosum (Cao et al., 2013; Chen et al., 2016; Nagel et al., 2011; Silk, Vance, Rinehart, Bradshaw, & Cunnington, 2009; Wu et al., 2017). It has been suggested that abnormal white matter connectivity could underlie altered sleep spindle activity and manifestation of neurodevelopmental disorders, such as ADHD (Gruber & Wise, 2016).

To our knowledge, there are only two previous studies analyzing the association between ADHD and sleep spindles (Kiesow & Surwillo, 1987; Prehn-Kristensen, Göder, et al., 2011). The first (Kiesow & Surwillo, 1987) found no differences in the number of sleep spindles between hyperactive boys and healthy controls from age 3 to 11 in a daytime napping experiment. Both groups were exposed to sedative Chloral Hydrate, however. Neither the second study found differences in sleep spindle density between 12 ADHD cases and healthy controls from age 10 to 16 (Prehn-Kristensen, Göder, et al., 2011), although patients with ADHD displayed reduced sleep-associated consolidation of declarative memory. The current study adds to the previous literature mainly conducted in clinical context by examining the effects of sleep spindle activity on memory-consolidation with a non-clinical, dimensional approach to ADHD. These questions are explored in a community cohort of 170 adolescents, who underwent a sleep EEG and participated in an overnight memory consolidation task. First, we examined whether dimensional ADHD symptoms associate with sleep spindle activity and overnight learning, and second, whether sleep spindle activity acts as a mediator between ADHD symptoms and overnight learning.

2. Material and methods

2.1. Participants

The study sample is derived from a Finnish community-based cohort of 1049 healthy singletons born between March and November 1998 in Helsinki, Finland. The details of the cohort are described in more detail in previous reports (Kuula et al., 2016; Merikanto, Kuula, Makkonen, Bódizs, Halonen, Heinonen, & Pesonen, 2017; Pesonen et al., 2014; Raikkonen et al., 2010). We invited to the current study those cohort members who participated in the previous follow-up at age 12 in years 2014–15 and lived within the 30 km radius from Helsinki (N = 279, 77.1% of the participants of the 12-year follow-up). Of them, 197 (70.6%) participated at the age of 17 (Mean age = 16.9, SD = 0.1 years).

The analytic sample comprised 170 (98 females and 72 males) adolescents who had both complete records of an overnight sleep EEG measurement and had completed the Adult ADHD Self-Report Scale (ASRS-v1.1) (Kessler et al., 2005) at the age of 17. Of these 170 participants, 135 participated before and after sleep-EEG in a mirror-tracing task measuring procedural overnight learning. Three of the participant's self-reported depression, one panic attacks and one an eating disorder diagnosed by a doctor. None of the participants had ADHD diagnosed by a child or adolescent psychiatrist.

The analytic sample of 170 participants in this study did not differ significantly from the rest of the participants in the initial cohort (N = 879) regarding mother's age or Body Mass Index (BMI) at birth, gestational age, birthweight, length at birth or maternal alcohol or licorice consumption during pregnancy (all $P > 0.5$) in T-tests. The Ethics Committee for Children and Adolescents' Diseases and Psychiatry at the Helsinki University Central Hospital approved the study protocol. All methods were performed in accordance with the relevant guidelines and regulations. All participants gave their written informed consent.

2.2. Sleep EEG recording

Overnight polysomnographic sleep recordings (PSG) were conducted in the homes of the participants with SOMNOscreen plus (SOMNOmedics GmbH, Germany). Electroencephalography (EEG) was recorded with gold cup electrodes at 6 EEG locations (F3, F4, C3, C4, O1 and O2) and two channels for the mastoids (A1, A2) according to the standardized 10/20 system. The electro-oculogram (EOG) and the electromyogram (EMG) were measured by using disposable adhesive electrodes (Ambu Neuroline 715, Ambu A/S, Denmark), two locations for EOG and three locations for EMG. In addition, an online reference Cz and a ground electrode in the middle of forehead were used. The sampling rate was 256 Hz (the hardware filters for SOMNOscreen plus are 0.2–35 Hz). All signals were digitally offline filtered with pass band of 0.5–40 Hz (Hamming windowed sinc zero-phase FIR filter, cut-off (-6 dB) 0.25 Hz and 44.3 Hz respectively) and re-referenced to the average signal of A1 and A2 electrodes. Sleep stages from PSG data were scored manually with the DOMINO software (v2.7; SOMNOmedics GmbH, Germany) by three experienced researchers in 30-second epochs following Americal Association of Sleep Medicine guidelines (version 2.2).

2.3. Spindle analysis

Spindles were computationally extracted with the method described by Ferrarelli et al. (2010). The manually scored PSG signals were converted to EDF format in DOMINO software and then further analyzed for spindle detection by using functions of EEGlab 13.5.4b (Delorme & Makeig, 2004) running on Matlab R2015a (The Mathworks Inc., USA). We extracted spindles from EEG signal during N2 sleep, with an impedance value equal or lower than 10 kΩ during the corresponding 30-second epoch. The spindle analysis was conducted in two frequency bands (10–13 Hz, and 13–16 Hz) in order to differentiate respectively between the slow and fast spindles, which differ etiologically (Wallant, Maquet, & Phillips, 2016), temporally and in topographic distribution (Anderer et al., 2001; Schabus et al., 2007; Wallant et al., 2016). Before applying the spindle thresholding method, the preprocessed EEG data were further filtered using the above-mentioned frequency bands separately. The threshold values for finding spindle peak amplitude in each channel were defined by the mean of the channel amplitude (μV) multiplied with 2 (lower) and 8 (higher) including all valid epochs (sleep stage N2 and impedance ≤ 10 kΩ). Thus, we used channel-wise threshold definitions, taking into account that signals may vary across the channels. Furthermore, restriction for the spindle duration was set to 250 ms on both directions from the peak maximum. Signal amplitude was required to stay under the lower threshold for 78.1 ms which is approximately the duration of one period of sine at 13 Hz. This was done in order to prevent false alarms in spindle detection (Merikanto et al., 2017). The amplitude, spindle duration and density (number of spindles per minute) were measured separately for central and frontal derivations and for fast and slow spindles. A complete description of the spindle protocol has been reported (Merikanto et al., 2017). Descriptive data of the sleep spindle amplitude, duration and number by channel separately for slow and fast sleep spindles are given in Table 1.

2.4. Assessment of ADHD symptoms

ADHD symptoms were self-reported with the Adult ADHD Self-Report Scale (ASRS-v1.1) (Kessler et al., 2005), which is a 18-item questionnaire validated in adolescents (Adler et al., 2012). Each item is rated from zero to four scale with the responses as follows: 0 = never, 1 = rarely, 2 = sometimes, 3 = often and 4 = very often, and the scale was used as continuous, with Cronbach alpha of 0.89. Two subscales were calculated, inattention sum score and hyperactivity sum score, each encompassing nine items (Kessler et al., 2005) and both with a Cronbach alpha of 0.82. We also calculated a binary variable basing on

Table 1
Mean and Standard Deviation (SD) of sleep spindle characteristics by channel separately for slow and fast sleep spindles in stage 2 sleep.

Channel	Sleep spindle duration Mean (SD)	Sleep spindle amplitude Mean (SD)	Sleep spindle number Mean (SD)
Slow sleep spindles			
C4	1.3 (0.1)	25.6 (5.2)	87.4 (56.1)
C3	1.3 (0.1)	25.8 (5.8)	84.8 (50.8)
F4	1.3 (0.09)	24.0 (5.7)	183.0 (122.8)
F3	1.4 (0.08)	24.1 (5.5)	184.5 (89.1)
Fast sleep spindles			
C4	1.4 (0.09)	21.4 (5.9)	122.3 (84.6)
C3	1.4 (0.1)	21.1 (5.9)	121.5 (76.5)
F4	1.3 (0.1)	15.4 (4.5)	112.6 (128.7)
F3	1.3 (0.09)	15.0 (3.5)	100.1 (68.0)

C refers to central derivation and F refers to frontal derivation.

a 90th percentile cut-off of the total ASRS score (below 90th percentile mean score = 40.4 and above = 59.5; 14 cases above the 90th percentile, of which 71.4% girls).

2.5. Procedural memory test

We used a mirror-tracing task (Marks, 1996; Starch, 1910) for measuring procedural overnight learning. We used a digitalized task where participants drew two circles and a six-point star as mirror-image with stylus and Wacom Intuous Pen and Touch. Computerized algorithm counted the pixels drawn between the indicated lines in the mirror-images. Both in the evening prior to sleep EEG and the next morning, the participant practiced with a non-mirror image of the circle and the star first, and then drew two circles and one star as a mirror image (please see Fig. 1 illustrating mirror-tracing of the star shape in the evening and in the following morning). Success rate in the task was calculated as a percent of pixels between the lines in relation to all pixels drawn, and overnight learning was defined as the improvement in the success rate from evening to morning in the mirror-tracing task. The sum score of the overnight learning in the three mirror-images was used in the analyses.

2.6. Confounders

Sex was derived from the birth records and participant age was calculated on the day of sleep EEG recording. Sleep duration has been previously reported being shorter among children with more symptoms of ADHD in the original study sample at 8 years of age (Paavonen et al., 2009), and was added as a covariate.

2.7. Statistical analysis

One-way ANOVA and chi-square tests were used to study sex differences in the target variables. Correlation analyses were used to study the association between general sleep characteristics and ADHD symptoms as well as the association between general sleep characteristics and overnight learning. We used multiple linear regression analysis to study the associations between ADHD symptoms and sleep spindle activity. Principal component analyses (PCA) with Varimax rotation were used to reduce the number of sleep spindle variables and potential Type I error (Gruber et al., 2013) reflecting amplitude, density and duration at central and frontal derivation. PCA was performed based on z-scores of the sleep spindle variables. Analyses were performed separately for slow and fast spindles.

We continued by examining whether ADHD symptoms and sleep spindle variables were associated with the procedural learning task results. We also analyzed whether the sleep spindle activity that was significantly associated with ADHD symptoms mediated the association between ADHD symptoms and overnight learning in the procedural memory task. Here we used Process Macro (Hayes, 2018) in linear regression models employing the bootstrapping method with 5000 bootstrapping re-samples with bias-corrected Confidence Intervals (CI). All regression analyses were adjusted for sex, participant age, and sleep duration and for overnight learning analyses also with the performance rate in the previous evening. We used SPSS version 24.0 for all statistical analyses (IBM SPSS Statistics for Windows, Version 24.0).

3. Results

3.1. Principal component analyses for sleep spindle variables

Principal component analysis (PCA) using Varimax rotation produced one-factor solution for slow sleep spindle amplitude, fast sleep spindle amplitude, slow sleep spindle density, fast sleep spindle density, and fast sleep spindle duration. For slow sleep spindle duration, principal component analysis using Varimax rotation produced a two-factor solution.

One- factor solution for slow sleep spindle amplitude with correlation matrix determinant of 0.1, Kaiser-Meyer-Olkin Measure of Sampling Adequacy (MSA) of 0.72 was weighted by all the channels for central and frontal derivations, and accounting for 65.8% of the variance (component at C3 = 0.90, C4 = 0.90, F3 = 0.84, and F4 = 0.54). The factor was named as slow sleep spindle amplitude. One-factor solution for fast spindle amplitude with correlation matrix determinant of

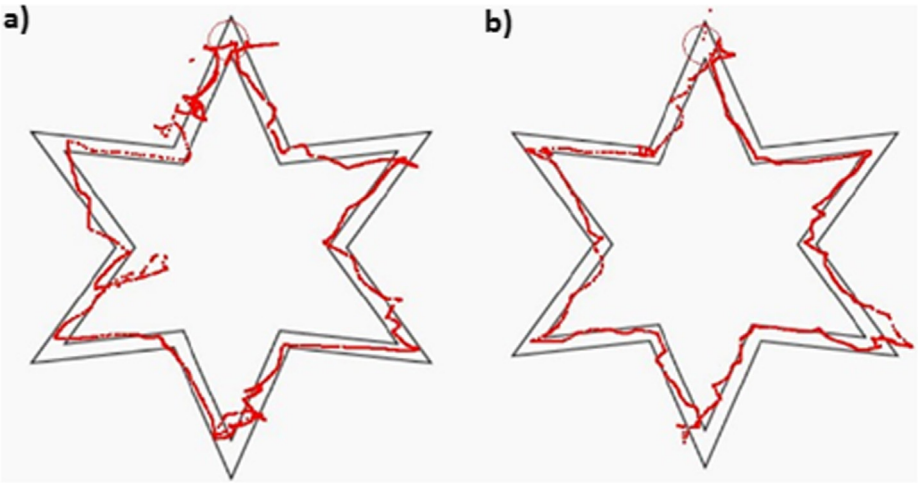

Fig. 1. Example of tracing a star shape in a mirror-tracing task (a) in the evening and (b) in the following morning.

0.05, MSA of 0.68 was weighted by all the channels for central and frontal derivations, and accounting for 67.5% of the variance (component loading at C3 = 0.92, C4 = 0.92, F3 = 0.83, and F4 = 0.57). The factor was named as fast sleep spindle amplitude.

One- factor solution for slow spindle density with correlation matrix determinant of 0.4, MSA of 0.61 was weighted by all the channels for central and frontal derivations, and accounting for 52.2% of the variance (component loading at C3 = 0.76, C4 = 0.86, F3 = 0.64, and F4 = 0.61). The factor was named as slow sleep spindle density. One-factor solution for fast spindle density with correlation matrix determinant of 0.5, MSA of 0.68 was weighted by all the channels for central and frontal derivations, and accounting for 49.1% of the variance (component loading at C3 = 0.81, C4 = 0.81, F3 = 0.67, and F4 = 0.45). The factor was named as fast sleep spindle density.

The first factor for the two-factor solution for slow sleep spindle duration, with correlation matrix determinant of 0.2, MSA of 0.58, was weighted by the channels for central derivations (rotated component matric loadings for C3 = 0.94 and C4 = 0.95) and accounted for 58.7% of the variance. The factor was named as slow central sleep spindle duration. The second factor was weighted by channels for frontal derivations (rotated component matric loadings for F3 = 0.92 and F4 = 0.87) and accounted for 28.5% of the variance. The factor was named as slow frontal sleep spindle duration. One- factor solution for fast spindle duration with correlation matrix determinant of 0.1, MSA of 0.71 was weighted by all the channels for central and frontal derivation, and accounting for 69.1% of the variance (component loading at C3 = 0.87, C4 = 0.89, F3 = 0.87, and F4 = 0.67). The factor was named as fast sleep spindle duration.

3.2. Initial analyses

As Table 2 shows, there were no differences between girls and boys regarding age, BMI, and highest education of the parents (all P > 0.05). All the participants were post-pubertal based on menstruation on girls and voice change on boys. As reported before (Merikanto et al., 2017) REM duration (P = 0.02) and stage 2 sleep duration (P = 0.03) was longer in girls than boys, while boys had longer stage 1 duration (P = 0.03; P > 0.07 for other variables). As Table 2 shows, girls scored higher in ASRS hyperactive sum than boys (P = 0.04). Participant age did not correlate significantly with ADHD symptoms (for ASRS sum $r = 0.05$, P = 0.5, for inattention sum $r = 0.02$, P = 0.8 and for hyperactivity sum $r = 0.08$, P = 0.27). As Table 3 shows, there were no significant correlations between the sleep staging, sleep duration or wake after sleep onset (WASO) minutes and the continuous ADHD scores.

Table 2
Descriptive characteristics and ADHD symptoms as continuous by sex in oneway-ANOVA or chi-square test.

	Girls (N = 98)	Boys (N = 72)	P
Age (Mean ± SD)	16.9 ± 0.1	16.9 ± 0.1	0.9
BMI (Mean ± SD)	22.7 ± 3.2	22.5 ± 3.1	0.7
Pubertal maturation[a]			
Pre-pubertal	0.0	0.0	[b]
Post-pubertal	100.0	100.0	
Highest education of the parents			
Secondary or less (%)	11.2	9.5	0.9
Vocational (%)	20.2	20.6	
University degree (%)	68.5	69.8	
Sleep duration	7:45 ± 1.06	7:27 ± 1:04	0.08
Wake after sleep onset	0:33 ± 0:30	0:40 ± 0:48	0.3
REM duration	1:41 ± 0:29	1:29 ± 0:30	0.02
NON-REM duration	6:47 ± 0:47	5:58 ± 0:52	0.4
Stage 1 duration	0:47 ± 0:22	0:54 ± 0:22	0.03
Stage 2 duration	3:15 ± 0:38	3:02 ± 0:41	0.03
Stage 3 duration	2:02 ± 0:28	2:02 ± 0:27	0.9
ASRS sum (Mean ± SD)	43.1 ± 9.6	40.4 ± 9.8	0.07
ASRS inattention sum (Mean ± SD)	23.1 ± 5.2	22.0 ± 5.3	0.2
ASRS hyperactivity sum (Mean ± SD)	20.0 ± 5.2	18.4 ± 5.3	0.04

SD refers to standard deviation.
BMI refers to Body Mass Index.
ADHD refers to Attention deficit/hyperactivity disorder.
ASRS refers to Adult ADHD Self-Report Scale (ASRS-v1.1).
[a] Pre- and post-pubertal stage was assessed based on the self-report information on menstruation in girls and voice change on boys and categorized as pre-puberty (no voice change or menstruation) and post-puberty (voice change or menstruation started).
[b] All the participants post-pubertal and no chi-square test could be calculated.

Table 3
Correlation analysis between general sleep characteristics and ADHD symptoms.

	ASRS sum *r* (P)	ASRS inattention sum *r* (P)	ASRS hyperactivity sum *r* (P)
Sleep duration	0.03 (0.7)	0.08 (0.3)	−0.03 (0.7)
Wake after sleep onset	−0.1 (0.1)	−0.2 (0.05)	−0.07 (0.3)
REM duration	−0.004 (0.9)	0.02 (0.8)	−0.03 (0.7)
NON-REM duration	0.04 (0.6)	0.1 (0.2)	−0.03 (0.7)
Stage 1 duration	−0.01 (0.9)	0.04 (0.6)	−0.06 (0.4)
Stage 2 duration	−0.02 (0.8)	0.04 (0.6)	−0.07 (0.4)
Stage 3 duration	0.1 (0.1)	0.1 (0.1)	0.1 (0.2)

ADHD refers to Attention deficit/hyperactivity disorder.
ASRS refers to Adult ADHD Self-Report Scale (ASRS-v1.1).
r refers to Pearson's correlation coefficient.

3.3. Initial analyses between overnight learning and general sleep characteristics

Participants spend on average 56.2 (SD = 46.3) seconds in tracing the star as mirror-image during the previous evening and 45.5 (SD = 21.6) seconds during the following morning ($r = -0.5$, P < 0.001 in correlation analysis). Shorter duration of tracing the mirror-image in the previous evening associated with worse performance in the evening ($r = -0.3$, P < 0.001). The duration of tracing in the morning did not correlate with overnight learning outcome ($r = -0.01$, P = 0.9).

There were no significant correlations between the sleep staging, sleep duration or wake after sleep onset (WASO) minutes and the overnight learning outcome (for sleep duration $r = -0.04$, P = 0.7, for WASO $r = 0.01$, P = 0.9, for REM duration $r = 0.006$, P = 0.9, for NREM duration $r = -0.05$, P = 0.5, for Stage 1 duration $r = -0.08$, P = 0.3, for Stage 2 duration $r = 0.02$, P = 0.8 and for Stage 3 duration $r = -0.06$, P = 0.5).

3.4. The associations between ADHD and sleep spindle activity

The associations from the regression analyses between ADHD symptoms and sleep spindle activity are presented in Table 4. Higher continuous ASRS sum score was associated with lower fast spindle amplitude (P = 0.03) and shorter fast spindle duration (P = 0.0002).

Higher continuous inattention scores were associated with lower fast spindle amplitude (P = 0.04) and shorter fast spindle duration (P = 0.001). Similarly, higher continuous hyperactivity scores were associated with lower fast spindle amplitude (P = 0.04) and shorter fast spindle duration (P = 0.001).

Those above the binary 90th percentile cut-off ASRS sum score, had shorter fast sleep spindle duration compared to those below the cut-off (B = −0.7, 95% CI = −1.3 to −0.05, P = 0.03, for other sleep spindle variables P ≥ 0.08). Fig. 2 shows the difference in fast sleep spindle amplitude and duration between those scoring above and below the

Table 4
Results from regression analysis for spindle characteristics by ADHD symptoms with sex, age and sleep duration as covariates.

Spindle characteristics	ASRS sum		ASRS inattention sum		ASRS hyperactivity sum	
	B (95% CI)	P	B (95% CI)	P	B (95% CI)	P
Amplitude (μV)						
Slow	−0.001 (−0.02 to 0.02)	0.9	0.002 (−0.04 to 0.04)	0.9	−0.006 (−0.04 to 0.03)	0.7
Fast	−0.02 (−0.04 to −0.003)	0.03	−0.03 (−0.07 to −0.001)	0.04	−0.04 (−0.07 to −0.002)	0.04
Density (number of spindles per 60 s)						
Slow	−0.001 (−0.02 to 0.02)	0.9	−0.007 (−0.04 to 0.03)	0.7	0.004 (−0.03 to 0.04)	0.8
Fast	0.004 (−0.02 to 0.02)	0.7	0.006 (−0.9 to −0.1)	0.7	0.008 (−0.03 to 0.04)	0.7
Duration (s)						
Slow in central	0.006 (−0.01 to 0.02)	0.5	0.01 (−0.02 to 0.04)	0.5	0.01 (−0.02 to 0.04)	0.6
Slow in frontal	0.01 (−0.006 to 0.03)	0.2	0.03 (−0.006 to 0.07)	0.1	0.02 (−0.02 to 0.05)	0.4
Fast	−0.04 (−0.05 to −0.02)	0.0002	−0.06 (−0.09 to −0.02)	0.001	−0.06 (−0.09 to −0.03)	0.001

ASRS refers to Adult ADHD Self-Report Scale (ASRS-v1.1).

90th percentile of ASRS sum.

3.5. ADHD symptoms, overnight learning and the mediating effect of spindle activity

Higher ASRS sum (B = −0.3, 95% CI = −0.5 to −0.01, P = 0.04) and hyperactivity score (B = −0.5, 95% CI = −0.9 to −0.04, P = 0.03) were associated with poorer overnight learning outcome in the procedural memory task. Inattention score or the 90th percentile score of total ADHD were not significantly associated with the overnight learning outcome (B = −0.04, 95% CI = −0.8 to 0.09, P = 0.1; B = −0.9, 95% CI = −9.8 to 8.1, P = 0.9). None of the sleep spindle variables was significantly associated with overnight learning outcome (all P > 0.3). Fig. 3 shows the mediation analyses where ADHD symptoms were independent, sleep spindles previously associated with ADHD symptoms as mediator, and learning outcome as the dependent variable. None of the mediation effects was statistically significant.

4. Discussion

Our study is the first to report associations between ADHD symptoms, sleep spindle activity, and overnight learning in adolescence in the largest adolescence sleep spindle data available thus far. Our results indicated that elevated ADHD symptoms were associated with lower amplitude and shorter duration of fast sleep spindles, and that elevated ADHD symptoms were associated with poorer overnight learning but that this was not mediated by the sleep spindle activity. Previous studies on ADHD and spindle activity are mainly based on clinical ADHD cases (Kiesow & Surwillo, 1987; Prehn-Kristensen, Göder, et al., 2011), and have not found compelling evidence on the relation between ADHD and spindle activity. We contributed to the literature by showing that elevated hyperactivity and inattention symptoms under the clinical range associated with weaker spindle activity. Elevated ADHD symptoms, especially regarding hyperactivity, also associated with poorer overnight procedural learning, which is in line with a small previous clinical study (Prehn-Kristensen, Molzow, et al., 2011). Our findings thus emphasize that the relation between elevated ADHD symptoms, lower spindle activity and poorer overnight learning is not exclusive to clinical cases, but are clearly observed at the subclinical range of ADHD characteristics.

Contrary to the hypothesis, weaker sleep spindle amplitude did not mediate the effect of ADHD symptoms to overnight learning in a mirror-tracing task, although previous studies have suggested that there is an association between sleep spindles and overnight learning concerning

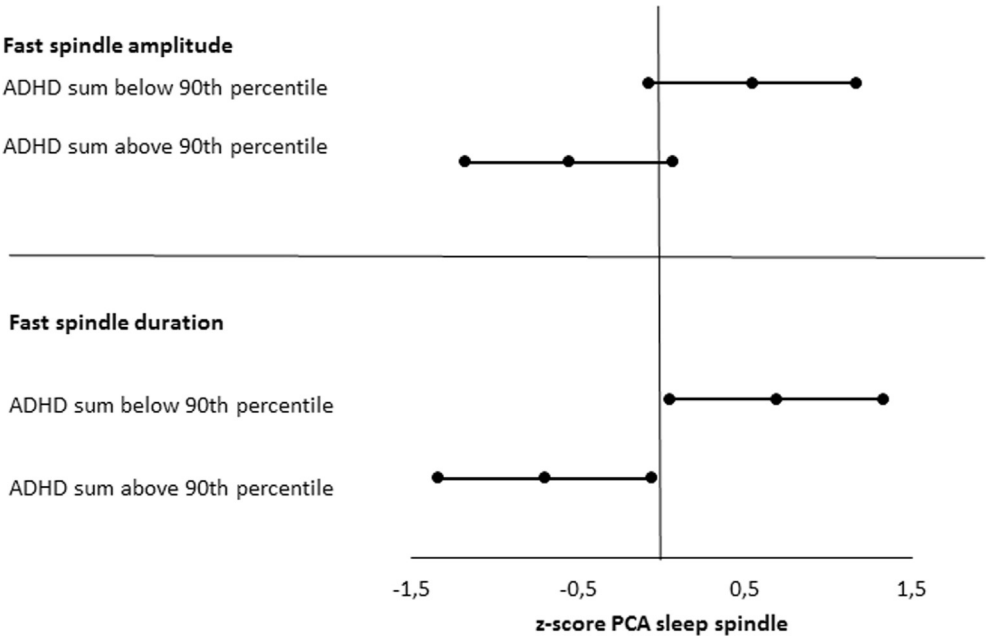

Fig. 2. Forest plot illustrating the difference in fast spindle amplitude and duration between those scoring above and below the 90th percentile. Fast spindle duration is significantly shorter among those scoring the highest ASRS sum as compared to those scoring below 90th percentile.

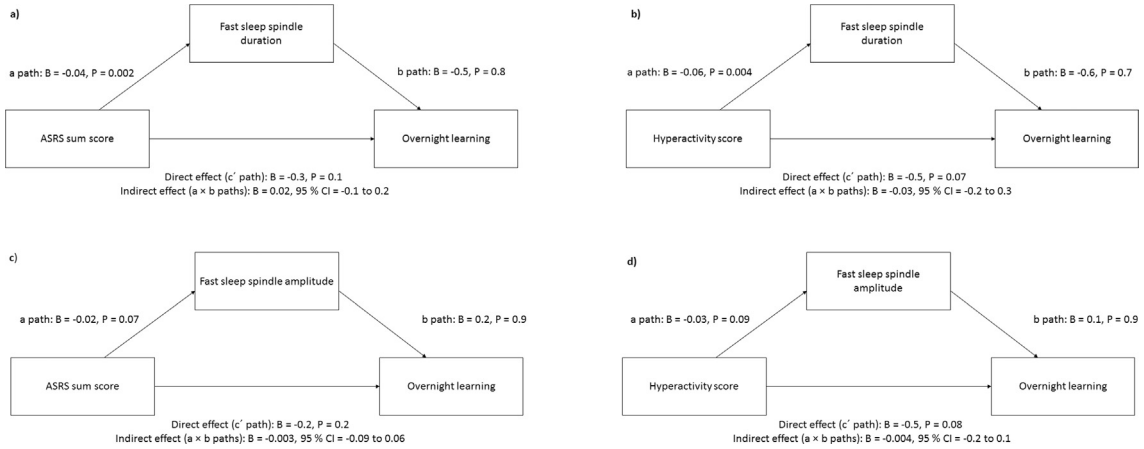

Fig. 3. Mediating effect of fast sleep spindle (a) amplitude on the association between Adult ADHD Self-Report Scale (ASRS) sum score and overnight learning, (b) duration on the association between ASRS sum score and overnight learning, (c) amplitude on the association between hyperactivity score and overnight learning and (d) duration on the association between hyperactivity score and overnight learning. The a path, b path, c path and c′ path in the mediation analyses are adjusted for sex, participant age, sleep duration and the mirror-tracing task performance rate in the previous evening. Direct effect is the association between ADHD symptoms on overnight learning while controlling for the spindle activity. Indirect effect is the measure of mediation effect.

particularly fast spindles (Fogel, Ray, Binnie, & Owen, 2015; Mölle, Bergmann, Marshall, & Born, 2011). Sleep spindle activity can, however, reflect non-adaptive processes in brain function that associates with various psychiatric diseases (Tesler, Gerstenberg, & Huber, 2013). Both altered sleep spindle activity and neurodevelopmental disorders have been associated with abnormal white matter connectivity (Gruber & Wise, 2016). Moreover, as sleep spindles have also a key role in maintenance of sleep (Cote et al., 2000; Dang-Vu et al., 2011), deficits in sleep spindle activity might explain why fragmentation of sleep is common among actual ADHD patients (Konofal et al., 2010).

Noteworthy, the association between ADHD symptoms and spindle activity was also specific to fast spindles in our study, similarly as in the recent study associating social anxiety symptoms with lower fast spindle activity (Wilhelm, Groch, Preiss, Walitza, & Huber, 2017). In terms of heritability, fast spindles have been reported to show greater heritability than slow spindles (Purcell et al., 2017). Sleep spindles appear during second postnatal month (Clawson, Durkin, & Aton, 2016) and facilitate adaptive plasticity in synaptic connections (Clawson et al., 2016; Lindemann, Ahlbeck, Bitzenhofer, & Hanganu-Opatz, 2016; Rosanova, 2005; Steriade & Timofeev, 2003). During development, both slow and fast spindle numbers increase (Chatburn et al., 2013), with slow spindles having earlier peak than fast spindles (Purcell et al., 2017). There is an increase of fast spindle activity during adolescence (Clawson et al., 2016), when synaptic pruning downsizes synapses for improved neural functioning (Rapoport et al., 2001).

Our results indicate similar deficits in sleep microstructures for elevated ADHD symptoms than reported with regard to many psychiatric conditions (Castelnovo, D'Agostino, Casetta, Sarasso, & Ferrarelli, 2016; Limoges, Mottron, Bolduc, Berthiaume, & Godbout, 2005; Manoach et al., 2014; Wilhelm et al., 2017), and even in first-degree relatives of schizophrenia patients (Manoach et al., 2014; Schilling et al., 2016). It thus seems that lower spindle activity is not restricted to one specific disorder or diagnostic condition, but is associated broadly with different types of dysfunctions (Gruber & Wise, 2016), also at the subclinical level.

4.1. Strengths and limitations

The strengths of our study include the largest sample size thus far used in adolescent sleep spindle studies, and a homogeneous age distribution. The latter increases the reliability of our analyses, as spindles are dependent on age, (Clawson et al., 2016) presenting usually a major confounding factor. We also performed the sleep spindle analyses with appropriate methodological control, and accepted only epochs with proper electrode impedance, and limited the duration of the spindle to 250 ms on both directions from the peak maximum to control for the artefacts. Finally, the participants slept according to their normal schedule at their home environment, increasing the ecological validity of the study.

As to limitations, while the Ferrarelli method (Ferrarelli et al., 2007, 2010) used in this study is widely applied, there is still lack of cross-validation studies across different sleep spindle analysis methods (Wallant et al., 2016). The literature concerning the operationalization of the mirror drawing task is also fragmentary, although it has been widely applied in prior studies on procedural learning. We acknowledge, that there is no standardized version of the digitalized, pixel-based operationalization mirror-tracing test available, limiting the comparability of the current learning outcomes. Finally, as we had only one night of sleep EEG, we could not control for any changes in sleep staging potentially related to the procedural learning task (Peters, Smith, and Smith, 2007).

4.2. Conclusions

Our study reports for the first time that elevated ADHD symptoms during adolescence are related to weaker sleep spindle activity, specifically with regard to fast spindles. This might contribute to altered synaptic connectivity and sleep problems observed in ADHD. We also found that elevated ADHD symptoms were related to poorer overnight procedural learning outcome, but that this was not mediated by sleep spindle activity.

Overall, our results indicate altered sleep microstructures and poorer overnight learning regarding procedural memory in relation to elevated ADHD symptoms. Our results show that a higher level of ADHD symptoms even in the non-clinical range is associated with similar alterations in sleep spindle activity as observed in many neuropsychiatric conditions.

Acknowledgement and conflict of interests

This study was funded by The Academy of Finland, Signe and Ane Gyllenberg Foundation and The Jalmari and Rauha Ahokas Foundation. The funders had no role in study design, data collection and interpretation, or the decision to submit the work for publication. The authors declare that there are no competing financial interests.

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