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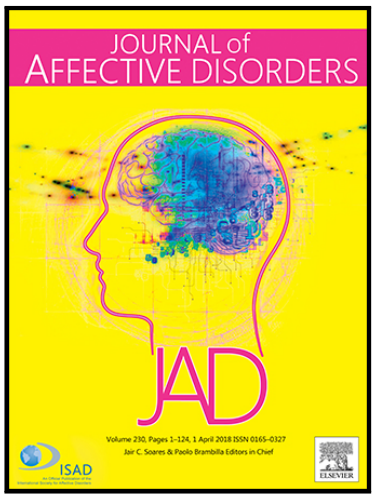

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Highlights

- REM fragmentations are short arousals during REM sleep inducing discontinuity of sleep
- Depressive symptoms and polygenic risk score (PRS) for somatic complaints are independently associated with more fragmented REM sleep
- Fragmented REM sleep may be associated with less efficient regulation of negative affect
- REM fragmentation is a conceptually distinct phenomenon from nighttime awakenings
- REM fragmentation and REM density may be distinct mechanisms lowering the quality of sleep

REM sleep fragmentation associated with depressive symptoms and genetic risk for depression in a community-based sample of adolescents

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Abstract

Introduction: Fragmented REM sleep may impede overnight resolution of distress and increase depressive symptoms. Furthermore, both fragmented REM and depressive symptoms may share a common genetic factor. We explored the associations between REM sleep fragmentation, depressive symptoms, and a polygenic risk score (PRS) for depression among adolescents.

Methods: 161 adolescents (mean age 16.9±0.1 years) from a birth cohort underwent a sleep EEG and completed the Beck Depression Inventory-II the same day. We calculated PRSes for depressive symptoms with PRSice 1.25 software using weights from a recent genome-wide association study for dimensions of depressive symptoms (negative emotion, lack of positive emotion and somatic complaints). REM fragmentation in relation to entire REM duration was manually calculated from all REM epochs. REM latency and density were derived using SomnoMedics DOMINO software.

Results: PRSes for somatic complaints and lack of positive emotions were associated with higher REM fragmentation percent. A higher level of depressive symptoms was associated with increased percent of REM fragmentation and higher REM density, independently of the genetic risks. Belonging to the highest decile in depressive symptoms was associated with a 2.9- and 7.6-fold risk of belonging to the highest tertile in REM fragmentation and density. In addition, higher PRS for somatic complaints had an independent, additive effect on increased REM fragmentation.

Limitation: A single night's sleep EEG was measured, thus the night-to-night stability of the REM fragmentation-depressive symptom link is unclear.

Conclusion: Depressive symptoms and genetic risk score for somatic complaints are independently associated with more fragmented REM sleep. This offers new insights on the quality of sleep and its relation to adolescents' mood.

Keywords: REM, sleep, adolescent, depression, polygenic risk score, EEG

Introduction

The duration and quality of Rapid Eye Movement (REM) sleep has been proposed to be related to affective and cognitive processes (Walker and van der Helm 2009). REM sleep is associated with procedural and emotional memory consolidation (Groch et al. 2013; Ackermann and Rasch 2014), although the precise function of REM sleep remains partly unresolved (Ackermann and Rasch 2014; Peever and Fuller 2017; Tempesta et al. 2018). Increasing evidence points to the role of REM sleep in emotion regulation. For example, in depressive individuals, REM sleep in specific may contribute to enhanced consolidation of negative memories (Harrington et al. 2018). REM sleep can also contribute to maintaining optimal emotional homeostasis by decreasing accumulation of negative affectivity (Gujar et al. 2011; van der Helm et al. 2011). REM sleep deprivation, in turn, is associated with increased emotional reactivity compared to NREM sleep deprivation (Rosales-Lagarde et al. 2012). At the theoretical level, it has been suggested that REM sleep promotes both reactivation of previously acquired affective experiences in the limbic system and their integration to semantic memory, leading to reduced amygdala activity and affective memory trace over time (Walker and van der Helm 2009). In support of this, it has been shown how rapid eye movements in REM sleep cause transient, time-locked activation of the amygdala (Corsi-Cabrera et al. 2016), confirming the role of limbic system in reprocessing and consolidation of emotional experiences during REM sleep.

As reviewed by Palagini (Palagini et al. 2013), REM sleep alterations, such as decrease in the interval from sleep onset to the first REM episode (REM latency), increase of total REM sleep duration, and increased REM density (the frequency of rapid eye movements per REM period) have been frequently observed in people diagnosed with major depressive disorder. It has been proposed that these changes might be prodromal and residual properties with respect to depressive episodes. For instance, shortened REM latency can persist beyond the depressive episode and increase the likelihood of relapse (Paykel 2008). Increased REM density, in turn, has been associated with increased memory consolidation of negatively-valenced emotional content (Gilson et al. 2015). Thus, these alterations may be trait markers of

depression vulnerability that are non-specific, as not all depressed individuals display REM alterations (Riemann et al. 2001). In addition, other psychiatric disorders (e.g., PTSD) are associated with different REM dysfunctions (Habukawa et al. 2018). It has also been suggested that there are underlying genetic drivers that may cause both REM sleep changes and vulnerability for depression (Palagini et al. 2013). These may equally relate to the individual vulnerability for stress.

Expanding upon traditional ways of looking at REM alterations, recent studies have examined REM sleep fragmentation (i.e., the number and duration of short arousals that disrupt the continuity of the REM period). People with chronic PTSD have more fragmented sleep, even when compared to individuals with major depressive disorder (MDD) (Habukawa et al. 2018), and this was related to the subjective experience of trauma-related nightmare intensity in those with PTSD. Thus, REM sleep continuity may function to de-potentiate emotional load; a function that is disrupted when REM is fragmented by brief arousals. Indeed, in an experimental fMRI study by van der Helm et al., (van der Helm et al. 2011) it was shown that when REM sleep was characterized by lower gamma power at the prefrontal region there was a greater overnight de-potentiation of neural and behavioral responsivity to affective stimuli. Insomnia severity and an experience of distress lasting over the night has also been associated with nocturnal hyperarousal and REM discontinuity (Wassing et al. 2016). It was suggested that hyperarousal and related REM discontinuity plays a significant role in the regulation of emotions in insomnia, depression, and PTSD, by weakening the de-potentiation of negative distress.

The current study explores the question of REM fragmentation, density and latency in relation to depressive symptoms among a community cohort of individuals born in 1998, who underwent a sleep EEG at the age of 17 years. We hypothesized to find associations between higher level of depressive symptoms and lower REM sleep quality. Given many young people experience poor sleep (Bartel et al. 2015; Crowley et al. 2018) and subthreshold depression during adolescence (Bertha and Balazs 2013; Wesselhoeft et al. 2013) this sample in this developmental period represents a good ‘model’ to test the proposed associations.

Finally, there is evidence for a genetic role linking depression and various aspects of REM sleep (see Palagini et al., 2013, for review). In the present study, we explored a new aspect of REM sleep dysregulation. We also examined whether the effect of depressive symptoms on REM sleep would be enhanced by genetic vulnerability to depression. Towards this aim, we built a polygenic risk score (PRS) derived from a recent genome-wide association study (GWAS) for depressive symptoms (Demirkan et al. 2016). The asset in polygenic scores is that they combine together many genetic variants obtained from large, already existing genome-wide association studies. Polygenic score is thus a weighted genetic index score for a risk concerning a specific trait or disease. In relation to a candidate gene approach, PRS has the advantage of representing hundreds, or even thousands of SNPs related to a complex trait in one score. This is a novel approach in studies on REM sleep, as the previous candidate gene (Chang et al. 2016) or twin studies (Markovic et al. 2018) on REM have not directly addressed the question of REM sleep quality. In sum, our study thus seeks new understanding on the quality of REM sleep in relation to individual resilience and mental health from both genetic and self-experienced perspectives.

Methods

Participants

Adolescents were from an urban community-based cohort composed of 1049 healthy singletons (Strandberg et al. 2001). The consecutive sample was born between March and November 1998 in Helsinki, Finland. We invited the participants from the cohort in the order of their 17th birthday, resulting in a very narrow age range (M = 16.9, SD = 0.1; range 16.6 to 17.2 years). We invited adolescents who had participated in the previous follow-up at the age of 12 years (Pesonen et al. 2014; Kuula et al. 2017), and who lived within a 30 kilometer radius of Helsinki and had given consent for further contact (N = 278). All in all, 196 adolescents participated (71% of the invited, 74% of those contacted by phone, 61% girls), of which 161 had valid sleep EEG, gene and questionnaire data available (61% girls), with a mean age of 16.9 years (SD = 0.1). Of the 196 adolescents, 186 underwent PSG (10 declined), and 177 had valid recordings over the entire night. Of these 161 had valid data on the BDI-II questionnaire. Due to original research interests, the flow of participation since the 8-year follow-up (Raikkonen et al. 2009; Raikkonen et al. 2017)

was weighted on those whose mothers reported higher liquorice consumption during pregnancy. The analytic sample in this study did not differ significantly from the rest of the participants in the initial cohort regarding mother's age or Body Mass Index (BMI) at birth, gestational age, birth weight, length at birth or maternal alcohol or licorice consumption during pregnancy in T-tests (all $P > 0.5$). Ethics Committee of the Children's Hospital in Helsinki University Central Hospital approved the study protocol (177/13/03/03/2014). Informed written consent was obtained from the participants.

PSG protocol and REM arousal detection

Participants underwent overnight polysomnography (PSG) in their own home, and received a monetary compensation (50€) for their time. PSG was arranged according to the participants' schedules and were conducted over the school year from January to December (excluding July due to summer holidays), and 90% of all recordings were completed on school nights. All recordings were performed using SOMNOscreen plus (SOMNOmedics GmbH, Germany). A trained research nurse attached gold cup electrodes at six EEG locations (frontal (F) hemispheres: F3, F4; central (C) hemispheres: C3, C4; occipital (O) hemispheres: O1, O2) and two for the mastoids (A1, A2). In addition to hemisphere-specific measures, we calculated overall frontal and central measures as means from both hemispheres. 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 0.2-35Hz).

All signals were digitally offline filtered with pass band of 0.5-35Hz (Hamming windowed sinc zero-phase FIR filter, cut-off (-6dB) 0.25Hz and 39.3Hz, respectively) and re-referenced to the average signal of A1 and A2 electrodes. PSG data were scored manually using the DOMINO program (v2.7; SOMNOmedics GmbH, Germany) in 30-sec epochs into Stage 1, Stage 2, SWS and REM according to AASM guidelines (The AASM Manual for the Scoring of Sleep and Associated Events). Percentages of each stage were calculated based on total sleep time.

REM parameters

REM fragmentations were scored manually during visual inspection of pre-staged REM sleep epochs within the DOMINO program. Fragmentation was defined as an arousal or an abrupt interruption of REM sleep which lasts less than 15 seconds. As a main outcome measure, we calculated REM fragmentation percent ('sum of the duration of REM fragmentation epochs/the duration of REM sleep during the night')*100). For subsequent analyses we calculated also REM fragmentation percent separately for micro arousals (duration < 3 seconds, $Mean_{micro}$ 1.5±0.7 secs) and macro arousals (duration 3-15 seconds, $Mean_{macro}$ 7.4±2.8 secs), following the threshold for REM arousals set by the American Academy of Sleep Medicine . REM latency (latency from sleep onset to the first REM epoch) and REM density (percent of rapid eye movements in relation to REM sleep duration) parameters were derived from the DOMINO software.

Self-assessment of depression. Depressive symptoms were assessed with the Beck Depression Inventory II (BDI-II) (Beck, Steer, & Carbin, 1988), which was administered on the same night as the PSG measurement. The BDI-II is a self-administered measure comprising 21 items which cover a range of depressive symptoms present over the past 2 weeks (Beck et al. 1996). Each item is rated on a 4-point scale (0–3) in terms of symptom intensity, yielding a total score ranging from 0 to 63. In this study, internal consistency (Cronbach's α) was 0.92. The sample consisted 12% of mild (≥ 14 points) and 7% of moderate (≥ 20 points) depression scores. According to many adolescent studies, the mean level varies considerably across populations in different countries, and boys usually score significantly lower than girls (Osman et al. 2008; Dere et al. 2015; Whisman and Richardson 2015). This was also seen in this sample, with only one boy included in the moderate depression group. Accordingly, in order to create a binary variable of depression risk and to correct for a potential sex and cultural bias, we used $\geq 90^{th}$ percentile score of BDI-II defined separately for boys and girls, and compared them to those scoring below the sex-based 90^{th} percentile score (Whisman and Richardson 2015).

Genotyping

Genotyping was conducted with Illumina OmniExpress Exome 1.2 bead chip at Tartu University, Estonia in September 2014 according to standard protocols. Genomic coverage was extended by imputation using IMPUTE2 software and 1000 Genomes Phase I integrated variant set (v3 / April 2012; NCBI build 37 / hg19) as the reference sample. Before imputing the following quality control filters were applied: SNP clustering probability for each genotype > 95%, Call rate > 95% individuals and markers (99% for markers with Minor Allele Frequency (MAF) < 5%), MAF > 1%, Hardy-Weinberg Equilibrium P > 1*10-6. Moreover, heterozygosity, sex check and relatedness checks were performed, and any discrepancies removed.

Polygenic risk scores for depression

We performed a weighted polygenic risk score (PRS) analysis using betas from GWAS summary statistics data of CHARGE Consortium GWAS for dimensions of depressive symptoms (Demirkan et al. 2016). The dimensions were three subscales from The Center for Epidemiologic Studies Depression Scale (CES-D), namely negative emotion, lack of positive emotion, and somatic complaints. For the PRS analysis, we used the statistical analysis software package PRSice v1.25 (Euesden et al. 2015). The PRS was calculated by summing the products of the risk allele count multiplied by the effect reported in the discovery GWAS. The additive genotype model was used for all SNPs. Before creating PRS, clumping was used to obtain SNPs in linkage disequilibrium with an r2 <0.25 within a 200bp window (Hagenaars et al., 2016).

In the clumped sets of SNPs we found 224.798 SNPs, 224.832 SNPs, 225.019 SNPs, and 225.494 SNPs from lack of positive affect, negative affect, somatic and total score GWAS summary statistics, respectively, for the whole genome region PRS constructions. The P threshold (P_T) for selecting the 'risk' SNPs from clumped sets of SNPs was set at 0.01. It resulted in 5563, 5575, 5823 and 5587 SNPs for positive affect, negative affect, somatic and total score, respectively.

Statistical analyses

As an exploratory analysis to better understand the structure of REM fragmentation across the night, we used linear mixed-model analysis with a random intercept for studying the duration of the REM fragments in relation to the time passed from individual sleep onset. We modeled the time as linear, and higher-order quadratic (time*time), and as cubic (time*time*time) terms.

Next, we performed exploratory factor analysis with Varimax rotation to extract distinct factors from BDI-II, including all factor loadings > 0.45. We applied logarithmic transformation for the depressive symptom scale to normalize its distribution.

We used partial correlations adjusted for sex to study the initial associations between PRSes and REM parameters. To study the associations between continuous depressive symptoms and REM parameters, we used linear regressions, Model 1 adjusted for sex, and Model 2 additionally for the polygenic scores, if any of the partial correlations between PRSes and REM parameters were significant in the initial analyses. We studied the effect of sex by entering an interaction term 'sex * depressive symptom scale' to the model, if the main effect between depression scale and REM parameter was significant. Similarly, we also studied the additive effects of self-reported depressive symptoms and genetic vulnerability by entering an interaction term 'depressive symptom scale*PRS' to the model. To closer study the effect of moderate depression and genetic vulnerability to depression on REM parameters, we used logistic regressions with conditional stepwise forward selection with sex, depression, and PRSes.

Results

BDI-II factors

We found the best fit for the two-factor solution of BDI-II with a cognitive (C; 5 items) and somatic-affective (SA; 13 items) component, a solution which is a slight modification, but close to the initial factor structure suggested by Beck et al., (Beck et al. 1996) and confirmed in other studies (Skule et al. 2014). These factors explained 8.5 and 43.3% of the variation of the entire BDI-II, respectively, and similar to a recent study (Skule et al. 2014), the factor C had the highest four item loadings for self-dislike, past failures, worthlessness and self-criticalness (loadings >.70). For factor SA, this was partly in line with (Skule et al.

2014), where the highest loadings were for concentration difficulty, sadness, crying, and loss of pleasure (loadings > 0.64).

Initial analyses

Descriptive sleep statistics of the sample are presented in Table 1. Boys had significantly shorter stage 2 (S2) and REM sleep duration, lower REM density and longer REM latency than girls. They also had significantly lower BDI-II scores. Older age was negatively associated with REM fragmentation (r = -0.22, P = 0.01). BDI-II score was not associated with sleep duration, REM duration, NREM duration, absolute duration or percent share of different sleep stages, sleep efficiency or wake-after-sleep-onset (WASO) minutes (Ps > 0.3s). Note that liquorice consumption during pregnancy (Raikkonen et al. 2009; Raikkonen et al. 2017) was not correlated with depressive symptoms or REM parameters (P > 0.24) and was not studied further in this study.

REM fragmentation measures

REM fragmentation did not correlate with REM density (r = 0.03, P = 0.67) or with REM latency (r = -0.04, P = 0.64). Wake-after-sleep-onset (WASO) minutes were negatively associated with REM fragmentation (r = -0.15 P = 0.06) and did not correlate with either REM density (P = 0.81) or REM latency (P = 0.23). A more prolonged total sleep duration, NREM and REM duration were associated with more frequent REM fragmentation (r = 0.24, P = 0.002; r = 0.22, P = 0.005; r = 0.17, P = 0.03). The duration of individual, average REM fragmentation epochs also increased along longer sleep duration, NREM and REM duration (rs > 0.32, P < 0.001), but decreased with increasing WASO minutes (r = -0.25, P = 0.001). The first REM fragmentation event occurred, on average, one hour after the first REM episode.

Mixed model analyses indicated that the duration of the REM fragment was dependent on the time since sleep onset with a slight increase in duration towards the morning hours (B_{linear} = 0.40, 95% CI 0.26 -0.55, P < 0.001; $B_{quadratic}$ = -0.06, 95% CI -0.19 -0.06, P = 0.34; B_{cubic}= -0.09, 95% CI -0.18 - -0.01, P = 0.04). The models had rather equal fit according to the Akaike's Information Criterion (AIC) (Figure 1). When analyzed separately for micro and macro arousals, the duration of macro arousals was significantly

associated with the time from sleep onset only in the quadratic model (B_{linear} = 0.13, 95% CI -0.05 – 0.30, P = 0.15, $B_{quadratic}$ = -0.17, 95% CI -0.32 - -0.02, P = 0.03; B_{cubic}= -0.07, 95% CI -0.17 - 0.04, P = 0.22). Duration of the micro arousals was not associated with the time from sleep onset (all P > 0.15).

Associations between depressive symptoms, PRSs and REM parameters

As Table 2 shows, higher PRS for lack of positive emotions was modestly associated with subjective rating of depressive symptoms (total scale), and both PRS for negative emotions and PRS for lack of positive emotions were modestly associated with the somatic-affective componentPRS for somatic complaints and lack of positive emotions were positively associated with more fragmented REM sleep, and were then chosen to the following analyses.

Table 3 shows the results from the linear regression analyses between self-reported ratings of depression and REM parameters. Higher scores in depressive symptoms in the continuous total scale and the somatic-affective component were associated with more fragmented REM sleep (P < 0.01) in both Models 1 and 2, and with higher REM density in Model 2. Table 3 further shows the associations with depressive symptoms the percent of REM fragmentation due to micro (< 3 seconds) and macro (3-15 s) arousals. All significant associations remained significant with macro arousals, whereas none of the associations with micro arousals were significant (P > 0.57). There were no significant interactions between depressive symptoms and sex (P > 0.55) or depressive symptoms and PRSes in predicting the REM parameters (P > 0.07).

Logistic regressions

To categorize the REM parameters for the following logistic regression analyses, we used tertiles. Table 4 shows the results from forward conditional forward stepwise logistic regression analyses, where we used sex, binary depression score, and PRSes for somatic complaints and lack of positive emotions as the independent and the highest tertile vs. other tertiles in REM fragmentation (macro arousals) or density as the dependent variables. The final model (Table 4) explained 9% of the variance in REM fragmentation, and

it shows that one SD increase in PRS for somatic complaints associated with 1.55-fold risk to belong to the highest REM fragmentation tertile, and moderate depression (belonging to the highest 90th percentile of BDI-II) further increased the risk by 2.94. Sex and PRS for lack of positive emotions were not significantly associated with REM fragmentation and were dropped from the final model. Sex and PRSes did not increase the risk for high REM density, but moderate depression explained 14% of the risk, associating with a 7.63-fold risk to belong to the highest REM density tertile.

Discussion

Adolescence is a vulnerable period for experiencing both poor sleep and low mood (Bertha and Balazs 2013; Wesselhoeft et al. 2013; Bartel et al. 2015; Crowley et al. 2018). Depression during adolescence results from insufficient sleep quantity and quality (Lovato and Gradisar 2014). Among sleep quality parameters, an increasing evidence shows that fragmentation of REM sleep affects negatively the regulation of distress (Walker and van der Helm 2009; Gujar et al. 2011; van der Helm et al. 2011; Harrington et al. 2018), and may then affect daytime mood. However, due to the logistical difficulties inherent in assessing sleep architecture in large samples of adolescents, data on the link between depressive symptoms and REM fragmentation are scarce. The current study explored how REM sleep fragmentation, density, and latency are related to self-reported depressive symptoms and to a genetic predisposition to depression, using an adolescent birth cohort and a polygenic risk score (PRS) derived from a recent genome-wide association study (GWAS) for depressive symptoms (Demirkan et al. 2016).

As assumed, a higher level of self-reported depression, especially the somatic-affective component, was associated with higher PRSes for negative emotion and lack of positive emotion. We also found that genetic vulnerability for CES-D-derived subscale of somatic complaints and lack of positive emotions associated positively with REM fragmentation. We did not find any significant associations between PRSes for depression and REM density or REM latency. On this ground, we added PRS for somatic complaints and lack of positive emotions to the model analyzing associations between depressive symptoms and REM parameters.

In line with the hypothesis, we found a significant positive, albeit modest, association between depression scores and REM fragmentation. Further analyses showed that this association was specific for the somatic-affective component of the BDI-II depression scale, whereas the cognitive component was not associated with any of the REM fragmentation parameters. Also, the somatic-affective component associated positively with REM density. The associations were unchanged when further adjusted for the PRSes for somatic complaints and lack of positive emotions. When the REM fragments were categorized as micro (< 3 secs) and macro (≥ 3-15 secs) arousals, the significant associations remained only for the macro arousals. This evidence consolidates the idea that minor arousals in REM sleep can be considered normative features of the sleep stage, whereas those fragmentations lasting longer than 3 seconds may be more disruptive REM arousals, as suggested by the 3-second criteria of the arousal classification by American Sleep Disorders Association (1992).

At the subthreshold depression risk level, the associations became more robust. An adolescent belonging to the highest decile in depression scores possessed a 2.9-fold risk for belonging to the highest tertile in REM fragmentation (macro arousals), and a 7.6-fold risk for belonging to the highest tertile in REM density. Additionally, the results showed that one SD increase in the genetic risk for somatic complaints associated with a 1.6-fold risk for belonging to the highest tertile in REM fragmentation. The actual, self-reported depression and the genetic risk for somatic complaints had thus an additive effect on REM fragmentation, together explaining 9% of its variation. This was not case for REM density, as the genetic risk did not add to the risk solely explained by the current depression scores.

Due to the cross-sectional design, no causality can be derived from the associations: it is possible that depressive symptoms cause REM fragmentation, or equally, that a genetic predisposition to fragmented REM affects mood negatively in the long-term, by causing inefficient dissipation of distress during sleep. As REM fragmentation is a form of sleep discontinuity, the latter perspective is supported by a substantial genetic overlap found between insomnia and internalizing psychopathology (Lind et al. 2017).

Our study brings novel evidence on the microstructure of sleep in relation to subthreshold depression. Previous studies have shown that depressed adolescents have shorter REM latency, and

increased REM density (Augustinavicius et al. 2014). However, effects sizes are usually small and it has been estimated that up to 63- 99% of depressed youth have similar sleep macroarchitecture to healthy controls (Augustinavicius et al. 2014). In line with previous studies among clinically depressed adolescents (Augustinavicius et al. 2014), the duration of REM sleep during the night was not associated with depressive symptoms. The current study thus suggests a new approach to explore the relation between REM sleep and mood, by shifting the focus from the traditional REM parameters to more subtle microarchitectural level of REM sleep.

Recent evidence shows that rapid eye movements in REM sleep cause transient, time-locked activation of the amygdala (Corsi-Cabrera et al. 2016), suggesting that amygdala participates in the emotional processes during REM sleep (Tempesta et al. 2018). The contribution of REM to emotion regulation may be either positive or negative. For example, it has been documented that selective REM sleep deprivation is associated with enhanced negative emotional reactivity, both at behavioral and neural levels (Rosales-Lagarde et al. 2012), emphasizing the role of REM in dissipation of negative affect and stabilizing the emotional reactivity to an adaptive level. On the other hand, it has been suggested, that REM-related memory consolidation may strengthen more negative over neutral emotions (Nishida et al. 2009), or aggravate fear conditioning, e.g., PTSD (Murkar and De Koninck 2018). As per (Riemann et al. 1994), higher REM density in the current study (i.e., more frequent rapid eye movements), was associated with a higher level of depressive symptoms. Surprisingly, REM density did not correlate with REM fragmentation. This may suggest different underlying mechanisms, with the enhanced REM fragmentation pointing to incomplete dissipation of negative affect, and higher density to more activated amygdala activity during the sleep. Future studies are encouraged to investigate these links between various REM parameters, affect and subcortical mechanisms.

From the clinical perspective, our results offer new insights into the link between sleep and subclinical depressive symptoms. First, REM fragmentation and WASO minutes correlated negatively. This provides evidence that REM fragmentation is a conceptually distinct phenomenon from the usual sleep quality definitions, which might also be compromised in low mood or distress (Augustinavicius et al. 2014).

Interestingly, in the current study, a more prolonged total sleep duration and REM duration were associated with more REM fragmentation. This might due to greater dissipation of sleep pressure towards morning with longer sleep, thus resulting in more fragmented REM. As the mean duration of one REM fragment above the 3 sec limit (=macro arousals) was only 7.4 secs, adolescents may not become fully aware of the arousal, especially when reporting it upon waking in the morning (e.g., in a sleep diary). REM fragments generally increased towards the morning hours, suggesting that 'over-sleeping' may increase poor quality REM in the morning. A question for future research is to not only observe if this finding can be replicated, but if so, to see whether restricting sleep aids REM sleep continuity, and in turn decreases depressive symptoms.

The strengths of this study include a relatively large sleep EEG sample of adolescents (N = 161), with genomic data available. The REM fragmentation was meticulously and manually coded for maximum precision and artefact recognition, allowing exploration of the REM fragmentation across the individual fragments and their duration during the night. Limitations of the study include the measurement of a single night, thus we cannot know how stable the REM fragmentation is from night to night. The risk ratios should be interpreted with caution, as the categorizing of REM parameters into tertiles was arbitrary; that is, as yet, there exists no previous information for a clinically significant level of REM fragmentation or density. As a further limitation, our sample was generally healthy and the proportion of those with high scores in BDI-II was small. Future studies are recommended to examine REM sleep associations with those formally diagnosed with mood disorders. Our findings resulted from a large group of 16-17 year olds, and thus we recommend future studies not only replicate these findings in this age group, but also other age groups (e.g., early-to-mid-adolescents).

Finally, our ability to predict depressive symptoms with SNPs or genetic risk scores is still relatively poor due to lack of powers in the current GWA studies of depressive symptoms and due to the complex nature of depression. For example, in the Demirkan et al. (2016) study with sample size of some 30.000 individuals, a somatic symptoms of depression PRS explained 0.3% of the variation of somatic symptom scores in a Dutch cohort of over 3000 individuals. Low power also reflects on the non-replication

of the depression GWAS findings. However, applying polygenic risk scores that reflect more homogenous dimensions of depression as compared to that reflecting propensity to broad-range depression symptoms, should provide more accurate estimates due to less genetic heterogeneity.

Conclusions

REM fragmentations are short arousals during REM sleep inducing discontinuity of sleep especially towards the morning hours. The main finding of the current study showed that depressive symptoms and polygenic risk score for somatic complaints are independently associated with more fragmented REM sleep. Although causal explanations cannot be concluded, the possibility exists that fragmented REM sleep is linked with less efficient regulation of negative affect. Based on the present study's findings, we also suggested that REM fragmentation and REM density may be distinct mechanisms lowering the quality of sleep and affecting adolescents' mood toward the negative.

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Figure 1. The scatter plot of the duration of REM fragments across the night in relation of the time from individual sleep onset.
Akaike's Information Criterion 15450 for linear, 15451 for quadratic, and 15449 for cubic models, with a smaller value indicating better fit.

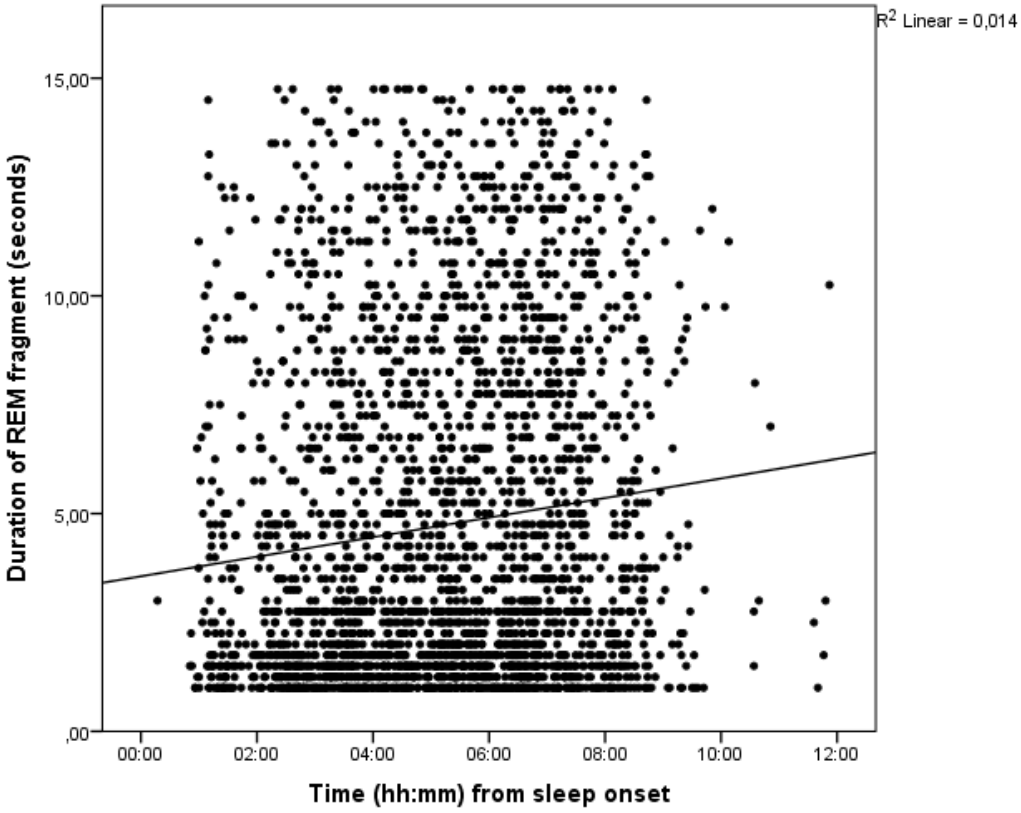

Table 1. Sample characteristics - mean, standard deviation and the significance of sex difference

	Girls (N=99)		Boys (N=62)		
	Mean	SD	Mean	SD	p
Age	16.89	0.12	16.89	0.12	.80
General sleep characteristics					
TST (hh:mm)	7:47	1:06	7:28	1:15	0.10
NREM 1 duration (hh:mm)	0:47	0:21	0:53	0:22	0.09
NREM 2 duration (hh:mm)	3:16	0:37	3:02	0:36	0.03
NREM 3 duration (hh:mm)	2:02	0:46	2:02	0:26	0.96
REM duration (hh:mm)	1:40	0:28	1:30	0:31	0.03
WASO (min)	0:16	0:19	0:20	0:16	0.14
REM sleep					
REM density percent	6.37	4.97	5.77	4.44	0.01
REM latency (hh:mm)	1:46	0:45	2:05	1:02	0.03
REM fragmentation percent	1.21	0.73	1.33	0.93	0.33
Micro fragm < 3 s percent	0.19	0.17	0.25	0.25	0.05
Macro fragm 3-15 s percent	1.02	0.64	1.08	0.86	0.60
Beck Depression Inventory II	6.82	8.67	3.68	4.49	0.009

SD = Standard Deviation. TST = Total Sleep Time. NREM = Non-REM. REM = Rapid Eye Movement. WASO = Wake After Sleep Onset.

Table 2. Partial correlations between polygenic risk scores for depression, self-reported depression and REM parameters adjusted for sex

	BDI-II	BDI-II SA	BDI-II C	REM fragmentation, all	REM micro arousals	REM macro arousals	REM latency	REM density
	β	β	β	β	β	β	β	β
PRS								
Negative emotions	0.13	0.19*	0.01	-0.02	-0.06	0.10	0.05	-0.04
Lack of positive emotions	0.20*	0.20*	0.12	0.12	-0.12	0.18*	-0.06	0.03
Somatic complaints	-0.02	-0.01	-0.04	0.22**	0.08	0.19*	0.13	-0.02
Total PRS	-0.02	-0.01	0.02	0.10	0.10	0.08	0.05	-0.01

BDI Beck Depression Inventory
SA Somatic-affective component
C Cognitive component
PRS Polygenic Risk Score

Table 3. The associations between subjective ratings of depression and REM parameters

	REM fragmentation, all		REM micro arousals		REM macro arousals		REM latency		REM density	
	β	P1/P2	β	P1/P2	β	P1/P2	β	P1/P2	β	P1/P2
BDI-II										
Total scale	0.21	0.009/0.01	0.05	0.57/0.41	0.22	0.006/0.02	-0.14	0.09/0.12	0.13	0.11/0.04
SA	0.21	0.01/0.03	0.01	0.85/0.85	0.23	0.004/0.02	-0.11	0.17/0.24	0.18	0.03/0.01
C	0.10	0.21/0.16	0.04	0.60/0.38	0.10	0.21/0.19	0.03	0.74/0.91	0.02	0.81/0.82

P1 models are adjusted for sex, and P2 additionally for the polygenic risk score for somatic complaints and lack of positive emotions
BDI Beck Depression Inventory
SA Somatic-affective component
C Cognitive component
Micro arousals > 0 and < 3 s
Macro arousals ≥3 s and < 15 s

Table 4. The odds ratio for belonging to the highest tertile in REM fragmentation and density in conditional forward stepwise logistic regressions with sex, depression and PRS for somatic complaints and PRS for lack of positive emotions

	REM fragmentation, macro arousals			REM density		
	OR (95%CI)	P	R^2	OR (95%CI)	P	R^2
Final model			0.09			0.14
Z-score for PRS somatic complaints	1.55 (1.06;2.24)	0.02	0.05	Not included	0.20	
Moderate depression (90^{th} percentile)	2.94 (1.07;8.09)	0.037	0.04	7.63 (2.56;22.89)	<0.001	0.14

PRS polygenic risk score

Note: sex and PRS for lack of positive emotions were not significantly associated with REM parameters in either models and not included in the final model