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Validating Chronotype Questionnaires in Adolescents: Correlations with Actigraphy and the Dim Light Melatonin Onset

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Paciello, Leonie Marie

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Dekan: Prof. Dr. B. Pichler

1. Berichterstatter: Privatdozentin Dr. M. Quante
2. Berichterstatter: Privatdozentin Dr. S. Diekelmann

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Für meine Familie

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List of Abbreviations

ANOVA Analysis of variance

CSM Composite Scale of Morningness

DI Distinctness/Amplitude subscale

DLMO Dim Light Melatonin Onset

ELISA Enzyme-linked immunosorbent assay

ET Evening Type

EV Evening affect subscale

FSL Functional Sensitivity Limit

IT Intermediate Type

MA Morning affect subscale

MANOVA Multivariate analysis of variance

MT Morning Type

ME Morningness-Eveningness

MEQ Morningness-Eveningness Questionnaire

MEQ-CA Morningness-Eveningness Questionnaire for Children and Adolescents

MESSi Morningness-Eveningness Stability Scale (improved)

MS Midpoint of sleep

MSF-SC Midpoint of sleep on free days (corrected for sleep debt)

PDSS Pediatric Daytime Sleepiness Scale

RIA Radioimmunoassay

rMEQ Reduced Morningness-Eveningness Questionnaire

rMEQ-CA Reduced Morningness-Eveningness Questionnaire for Children and Adolescents

SA Variable from self-assessed sleep onset and offset times

SD Standard deviation

1 Introduction

1.1 Chronotype

1.1.1 Defining Chronotype and Morningness-Eveningness

Chronotype is an individual circadian preference, which can be loosely divided into a morning type, an evening type and an intermediate type (Natale and Cicogna, 2002).

While some people go to bed early and perform at their mental and physical best in the morning, others reach their peak performance in the latter half of the day and prefer to stay awake longer (Roenneberg et al., 2003, Adan et al., 2012). The two types are often colloquially called “larks” and “owls” (Randler et al., 2017).

Chronotype and the construct of morningness-eveningness (ME) are two terms in this context that vary slightly in their definition but are often used synonymously (Zerbini and Merrow, 2017, Di Milia et al., 2013). ME describes a preference for performing certain activities during a specific time of day. While chronotype also describes a daytime-dependent peak phase in mental and physical performance, it can be more objectively defined, for example by the midpoint of sleep on free days (MSF-SC) (Roenneberg et al., 2019). The midpoint of sleep is the midpoint between the sleep onset and offset timing (Benoit et al., 1981). The MSF-SC is a corrected midpoint of sleep, which is used to correct the midpoint of sleep for influencing factors, such as sleep debt (Roenneberg et al., 2004).

The chronotype can as well be indicated by endogenous markers, such as melatonin levels and core body temperature (Kantermann et al., 2015, Lack et al., 2009, Duffy, 1999). Chronotype indices and the concept of diurnal preferences, as measured by questionnaires, correlate strongly (Zavada et al., 2005). The correct use of both terms in the literature, however, is still a subject of discussion (Bauducco et al., 2020, Goldin et al., 2020).

The circadian typology is influenced by individual and environmental factors (Adan et al, 2012). It also varies by age and sex (Randler et al., 2017). A 6-month longitudinal study on adolescent twin pairs found a high genetic influence on chronotype (Inderkum and Tarokh, 2018). Based on the knowledge of the role of

certain genes in the body's circadian clock (Lowrey and Takahashi, 2011), multiple genetic variants that might influence the phenotypical expression of circadian preference were identified in genome-wide association studies (Kalmbach et al., 2017).

The photoperiod at birth is discussed as an environmental factor in circadian preference, as children born in a decreasing photoperiod (September-October) were found to have a higher prevalence of morningness, while those born in an increasing photoperiod (March-April) were more often associated with eveningness (Caci et al., 2005). Other studies on the influence of the photoperiod showed a small effect in mostly Caucasian populations (Vollmer et al., 2012) and no effect in an Asian study population (Takao et al., 2009). Canadian researchers observed an association between circadian preference and the season at birth, but stressed that the results were only partly explained by the length of the photoperiod (Mongrain et al., 2006).

Studies also reported latitude to be a factor associated with circadian typology, as different latitudes have different daylight times and light intensity in relation to their distance from the equator (Leocadio-Miguel et al., 2017, Borisenkov, 2010, Borisenkov et al., 2012). Overall, the chronotype seems to be influenced by the season and the resulting change in daylength and photoperiod (Shawa et al., 2018).

1.1.2 Circadian preference in adolescents

Adolescents and young adults have the highest prevalence of evening orientation (Roenneberg, Kuehnle et al. 2004). In an epidemiologic study in Germany, Austria, Switzerland and The Netherlands, circadian typology was shown to be almost normally distributed, with most people being intermediate types and the evening chronotype being slightly more prevalent than the morning type (Roenneberg et al., 2007).

Children tend to be earlier, *i.e.* morning or intermediate, chronotypes (Roenneberg et al., 2007). During early adolescence, circadian preference shifts towards an evening orientation and is then progressively delayed until early adulthood (Randler et al., 2017). This process then reverses and reverts back to

an earlier sleep-wake rhythm (Roenneberg et al., 2007). This tendency towards eveningness in early adolescence is partly due to biological reasons, such as hormonal changes in puberty (Hagenauer and Lee, 2012, Carskadon et al., 1993, Jenni et al., 2005). Researchers found a progressive delay of phase preference until five years after their menarche in female adolescents (Frey et al., 2009). Further reasons are found in social and scholastic obligations (Carskadon, 2002), late-night media use (Cain and Gradisar, 2010), and psychosocial factors (Carskadon, 2002).

The peak of lateness in chronotype is reached in adolescence and the early 20s (Randler et al., 2017, Roenneberg et al., 2004).

1.1.3 Difficulties by chronotype in a social and environmental context

At this age, young adults are expected to follow an early school schedule. While morning-oriented adolescents are alert and at their performance peak during school hours, strongly evening-oriented adolescents' night sleep is cut short (Roenneberg et al., 2007). Studies showed that circadian phase preference has a substantial influence on school performance and that morning types tend to perform better in school than evening types (Zerbini and Merrow, 2017, Tonetti et al., 2015b). Morning types might have an advantage, as school starts early in most countries and exams usually take place in the morning as well. There was a significant difference in academic performance between the morning and evening hours, but not in the afternoon, in a study on the timing of examinations (van der Vinne et al., 2014). The authors hypothesized that neither chronotype was at a disadvantage at this time of day.

The need for a more flexible or delayed school start has been pointed out in several studies and reviews (Valdez, 2019, Zerbini and Merrow, 2017). Recent evidence has supported these claims. A delayed school start improved academic performance in US-American secondary school children in a pre-post research study (Dunster et al., 2018) and in a longitudinal study (Kelley et al., 2017). A flexible school start in a secondary school in Germany had a positive effect on sleep deprivation and subjective performance (Winnebeck et al., 2019). An adaption of school start times to morning, midday or afternoon corresponding to

individual circadian preference also led to higher academic performance (Goldin et al., 2020).

1.1.4 Chronotype in a health context

Studies identified having an evening chronotype as a risk factor for several physical and mental health-related issues.

In a nationally representative sample of Canadian adolescents, evening types had more back problems, headaches and unhealthy behaviors, such as the consumption of soft drinks and cigarettes (Gariépy et al., 2019). In another study in 1620 Korean adults, a significant association between evening types and metabolic syndrome and diabetes was observed (Yu et al., 2015). An evening chronotype was also repeatedly linked to a higher prevalence of obesity (Arora and Taheri, 2015, Olds et al., 2011, Cespedes Feliciano et al., 2019).

Eveningness was found to be an independent risk factor for poor mental health, as well as emotional and behavioral problems in adolescents (Li et al., 2018). An evening chronotype was independently associated with a poorer self-regulation in another study (Owens et al., 2016).

Researchers hypothesized that the higher prevalence of health-related issues in evening chronotypes is due to a chronic mismatch of their societal obligations and their biological prerequisites or, more specifically, their circadian timing (Gariépy et al., 2019, Kansagra, 2016).

1.2 Measurement of chronotype

1.2.1 Questionnaires

An efficient way of gathering information on a population’s chronotype is by self-assessment questionnaires.

A very widely used chronotype questionnaire is the Morningness-Eveningness Questionnaire (MEQ) (Horne and Ostberg, 1976). A German translation of the 19-item original version was validated against dim light melatonin onset and body temperature (Griefahn et al., 2001). In the 1990s, adaptations of the MEQ for specific use in children and adolescents were established (Ishihara et al., 1990,

Carskadon et al., 1993). A review by Tonetti et al. summarized numerous validation studies for the MEQ in children and adolescents (MEQ-CA), consisting of comparisons to actigraphy, oral body temperature and questionnaires (Tonetti et al., 2015a). The MEQ-CA contains the same number of items as the original MEQ for adults (Ishihara et al., 1990). Criticism of the length of the MEQ led to the introduction of a shortened, 5-item version (Adan and Almirall, 1991). The German version of the reduced MEQ (rMEQ) (Randler, 2013) was used in a large-scale study on the epidemiology of circadian preferences in a German population, ages 5 to 70 years. (Randler et al., 2016b). The rMEQ has been used in international adolescent populations, see for example studies from India (Haldar et al., 2020), Hungary (Urbán et al., 2011) and Finland (Kuula et al., 2018, Merikanto et al., 2017). It is unclear if these research groups used the adult version of the rMEQ or the rMEQ-CA, as only few studies explicitly stated an adaptation of the questionnaire to children and adolescents (Filardi et al., 2016).

Another widely applied measure to determine circadian preference is the Composite Scale of Morningness (CSM) (Smith et al., 1989). Its 13 item-structure is a combination of nine MEQ-items and four of a second chronotype questionnaire, the Diurnal Type Scale (Torsvall and Akerstedt, 1980). Validation studies on the adult version of the CSM showed good validity against the original Diurnal Type Scale and subjectively reported sleep-wake parameters (Kato et al., 2019), as well as against actigraphy (Thun et al., 2012) and the questionnaire-derived midpoint of sleep (Jankowski, 2015). The German version of the CSM was validated against the MEQ in a mixed-age sample of adolescents and young adults with a mean age of 15.4 ± 3 years (Randler, 2007).

The Morningness-Eveningness Stability Scale (improved; MESSi) was recently introduced as a new method of assessing chronotype (Randler et al., 2016a). It differs from previous questionnaires in that it uses three subscales, namely morningness, eveningness and distinctness/amplitude (see Material and Methods), to assess ME as a multidimensional construct. Validation studies for the MESSi consisted of a confirmatory factor analysis (Vagos et al., 2019), a validation against health and personality correlates (Díaz-Morales et al., 2017), and a cross-cultural comparison (Rahafar et al., 2017). The German version of

the MESSi was validated against actigraphy in a university student population (Faßl et al., 2019). In adolescents, it was validated against other chronotype questionnaires, such as the CSM and the Children's Chronotype Questionnaire, as well as personality and assessments of affectivity (Weidenauer et al., 2019, Demirhan et al., 2019).

The reduced version of the MEQ-CA (rMEQ-CA) was validated in a confirmatory factor analysis in Hungarian adolescents (Urbán et al., 2011), as well as against self-report questions on sleep habits (Danielsson et al., 2019). The validity of the CSM in adolescents was examined against the MEQ (Önder et al., 2013), cortisol (Randler and Schaal, 2010), and in a third study against the MSF-SC and self-reported bed and wake times (Randler, 2009). In 2015, the CSM was validated against the MSF-SC and the Munich Chronotype Questionnaire in a Polish population (Jankowski, 2015).

An extensive review of the literature on the use of chronotype questionnaires in adolescents was published in 2015 (Tonetti et al., 2015a). A 2020 literature review on pediatric sleep tools (Sen and Spruyt, 2020) completes these findings with validation studies for the rMEQ, the CSM and the MESSi. An overview of known validation studies in adolescents is shown in Table 1.

Table 1. Known validation studies of chronotype questionnaires in adolescents

Questionnaire	Study	Instrument of validation	Age (years)	Sample size (N)	Result
rMEQ-CA	(Urbán et al., 2011)[1]	Confirmatory factor analysis	No age range stated, mean age 15.3 (SD 0.56)	2565	Construct validity in Hungarian adolescents

	(Danielsson et al., 2019)[2]	Self-reported, questions on sleep habits	16-26	1000	Significant negative correlations between rMEQ and all sleep variables
CSM	(Randler, 2009)[1]	Self-reported sleep onset and offset times, MSF-SC	13-18	491	Significant correlations with sleep onset and offset times on weekends, sleep onset time on weekdays, and with the MSF-SC.
	(Randler and Schaal, 2010)[1]	Cortisol in saliva	13–16	43	Significant correlations with cortisol awakening response
	(Önder et al., 2013)[1]	MEQ, confirmatory factor analysis	15-18	543	Convergent/ discriminant validity in Turkish high school students, construct validity
	(Jankowski, 2015)[2]	MCTQ, MSF-SC	13-46 [a] 13-15 [b] 16-18	952 [a] 265 [b] 150	Construct and convergent/ discriminant validity in the Polish version
MESSi	(Weidenauer et al., 2019)[2]	CSM, CCTQ, CFA	11-17	215	Convergent/ discriminant validity in German high school students, construct validity

	(Demirhan et al., 2019)[2]	BIG-5, Subjective alertness level, PSQI, PANAS	14-47	1076	Convergent/ discriminant validity in Turkish high school students

[1] as stated in 2015 review (Tonetti et al., 2015a), [2] as stated in 2020 review (Sen and Spruyt, 2020). a/b = subsample.

BIG-5 = Big five inventory, CCTQ = Children's chronotype questionnaire, CSM = Composite Scale of Morningness, MCTQ = Munich Chronotype Questionnaire, (r)MEQ(-CA) = (reduced) Morningness-Eveningness Questionnaire (for Children and Adolescents), MESSi = Morningness-Eveningness Stability Scale, MSF-SC = Midpoint of sleep on free days, PANAS = Positive and Negative Affect Schedule, PSQI = Pittsburg Sleep Quality Index, SD = standard deviation

1.2.2 Actigraphy

There are several approaches to examine the validity of a questionnaire. One is by correlating it against other, already established questionnaires (i.e., Caci et al., 2009, Randler, 2007, Weidenauer et al., 2019). Another is by comparing the questionnaire to objectively measured behavior, such as recordings of the sleep-wake rhythms. Polysomnography is the gold standard for examining sleep related behavior in a controlled environment (Marino et al., 2013). Another useful tool for estimating sleep and wake rhythms is actigraphy. An actigraph resembles a watch and noninvasively registers body movements. These are translated into activity counts, which yield information about sleep-wake and activity patterns (Mitchell et al, 2017, Troiano et al, 2007). In multiple studies which compared actigraphy to polysomnography, actigraphy was proven to be a valid instrument to examine sleep-wake rhythms (Ancoli-Israel et al., 2003, Quante et al., 2018, Sadeh et al., 1994, Marino et al., 2013). Actigraphy is often used as an alternative in validation studies, as it is easier to use for longer study durations and more applicable in a real-world setting (Faßl et al, 2018, Thun et al, 2012, Tonetti, 2007, Werner et al, 2009, Lucas-de la Cruz et al, 2016). Actigraphic data can also be applied in clinical settings and has been stated to improve the evaluation and monitoring of treatment responses in certain sleep disorders (Morgenthaler et al, 2007). The third edition of the International Classification of Sleep Disorders

(ICSD-3) encouraged the use of actigraphy, self-assessment questionnaires and, in addition, biomarkers such as the dim light melatonin onset (DLMO) when diagnosing circadian rhythm sleep-wake disorders (Sateia, 2014).

1.2.3 Dim Light Melatonin Onset (DLMO)

1.2.3.1 Human circadian rhythm

Humans exhibit a near 24-hour circadian timing (Duffy and Wright, 2005). The inner rhythm is continuously being reset to this 24-hour period, prompted by exogenous stimuli, so-called Zeitgebers, such as light (Moore, 1997). This internal circadian rhythm is controlled by two factors: homeostatic sleep pressure and endogenous processes (Borbély, 1982). These processes are influenced by the suprachiasmatic nuclei in the hypothalamus, a region in the brain which is also called the internal circadian clock (Moore, 1997). The two factors can be described using a concept consisting of a process S and a process C. The process S describes an inner sleep drive, building up pressure during the day and resetting during sleep time. The circadian rhythmicity in the release of endogenous hormones is part of the process C (Borbély, 1982). These changes in hormone levels during the day can for instance be observed in the secretion of cortisol (Elverson and Wilson, 2005) and melatonin (Claustrat and Leston, 2015).

1.2.3.2 Melatonin

Melatonin is an endogenous hormone whose secretion from the pineal gland is suppressed by light (Lewy et al, 1980). Melatonin therefore reaches its peak level during nighttime and plays a key role in the decrease of core body temperature (Strassman et al., 1991). The internal circadian clock and melatonin concentration are strongly associated, as melatonin secretion is controlled by the suprachiasmatic nuclei (Moore, 1996).

Melatonin concentration can be measured in bodily fluids, either directly in blood plasma or saliva (Voultsios et al., 1997), or by determining its metabolite in urine (Markey et al., 1985). Absolute melatonin concentration should either be determined in plasma or indirectly using its urinary metabolite, as both are representative of the melatonin production in the pineal gland (Claustrat et al.,

2005, Markey et al., 1985). Saliva samples are, however, a valid method for measuring relative changes in melatonin levels (Laakso et al., 1990). Saliva sampling has one obvious advantage compared to plasma measurement in being non-invasive (Middleton, 2013). In addition, it allows more frequent sampling than the urinary metabolite measurement (Benloucif et al., 2008). It is, however, more susceptible to confounding factors, for example, in-mouth contamination through food or drinks (Kennaway, 2020).

Researchers are currently working on ways to enable reliable and accurate at home-saliva sample collection (Burgess et al., 2015, Burgess et al., 2016). These findings would increase the efficiency of melatonin sampling and might enable larger epidemiologic studies.

1.2.3.3 Measurement of Dim Light Melatonin Onset

The dim light melatonin onset (DLMO) was introduced in 1989 (Lewy and Sack, 1989). To date, it is considered the gold standard of circadian phase estimation (Reid, 2019). The DLMO is defined as the increase in melatonin levels in dim light conditions (Lewy and Sack, 1989). This increase is usually observed in the evening, approximately 2 to 3 hours before habitual bedtime (Benloucif et al., 2008). Onset time correlates with circadian phase preference, as evening chronotypes were observed to have a later DLMO than morning types (Goulet et al., 2007). In a longitudinal study in a younger (9-10 years) and an older (15-16 years) adolescent cohort, the DLMO phase and actigraphic sleep-wake parameters both shifted during the 2.5-year assessment, becoming later with increased age (Crowley et al., 2014).

In the first DLMO analyses, plasma melatonin concentration was measured by gas chromatographic mass spectrometry (Lewy and Sack, 1989). The latest methods introduced for research and diagnostic purposes are the third-generation immunoassays, *i.e.* commercially available radio immunoassays (RIA) or enzyme-linked immunosorbent assays (ELISA) (Kennaway, 2019).

Although DLMO is considered the gold standard of circadian phase estimation, there are several factors that are needed to be taken into consideration to achieve

accurate results. For example, posture seems to have an impact on melatonin levels, as does cotton wool in sample holders (Kennaway, 2020).

DLMO estimations can vary, as there are different methods of determining the exact time of melatonin onset. Both relative and absolute approaches have been used to measure the rise in melatonin concentration, which makes comparing published results difficult (Crowley et al., 2016, Molina and Burgess, 2011, Benloucif et al., 2008). The impact of using different threshold methods on the accuracy of the results is still being discussed (Molina and Burgess, 2011).

The DLMO has been used to validate chronotype questionnaires such as the Munich Chronotype Questionnaire and the MEQ in adults (Kantermann et al., 2015, Griefahn et al., 2001). Salivary DLMO phase estimation in adolescents was validated against self-reported sleep-wake times and the midpoint of sleep (Crowley et al., 2006).

1.3 Objective and scientific hypothesis

Adolescence is a critical phase in an individual's life. Many health-related behaviors (Spear and Kulbok, 2001) and even disorders (Bartlett et al., 2013) develop during this phase. Chronotype and diurnal preferences were consistently shown to have an influence on academic performance and many health-related issues and behaviors. It is evident that a fundamental understanding of the impact on circadian preference is crucial for acting adequately on these findings. Large epidemiologic studies are required to obtain the necessary information. In order to do this, valid and reliable instruments are needed.

Unfortunately, even for widely used and well-known questionnaires such as the rMEQ and the CSM, there are only few validation studies against objective measures in the adolescent age group (Tonetti et al., 2015a, Sen and Spruyt, 2020, Table 1). To our knowledge, the CSM and the MESSi have not yet been validated against actigraphy or DLMO in this age group. The rMEQ was used in combination with actigraphy in two adolescent studies in Finland (Merikanto et al., 2017, Merikanto et al., 2020). A literature search on comprehensive validation studies for a set of multiple questionnaires and the corresponding objective measurements only yielded results in the adult age group (Thun et al., 2012).

Thus, our objective was to validate three adolescent chronotype questionnaires (MESSi, rMEQ-CA, CSM) against actigraphy and the DLMO. To accomplish this, we recruited 55 healthy 13- to 16-year-olds who provided us with information on their sleep-wake and activity patterns through actigraphy and a sleep diary. Participants also completed the Pediatric Daytime Sleepiness Questionnaire (PDSS). We measured the evening rise in melatonin concentration in saliva samples in a sub-sample of 24 adolescents.

We hypothesized that there is a significant correlation between the questionnaires, actigraphy and the DLMO. We examined the reliability of the questionnaires and their associations with the DLMO, sleep timing, midpoints of sleep as well as with activity parameters from actigraphy (midpoints of highest and lowest activity). We explored the influence of chronotype on sleep onset and offset times and midpoints of sleep using univariate analyses. Based on the literature, we hypothesized that evening types have later sleep onset times in general and sleep offset times on free days, and therefore also later midpoints of sleep than morning types (Roenneberg et al., 2007, Thun et al., 2012).

The influence of age, sex and the interaction between age and sex on the questionnaires was examined using a multivariate analysis, as age and sex both had an effect on chronotype in a meta-analysis based on chronotype questionnaires (Randler and Engelke, 2019).

2 Material and Methods

2.1 Study population

Our study sample consisted of 55 healthy adolescents aged 13 to 16 (49.1% male, mean age ± SD = 14.4 ± 1.1 years). We recruited participants between February 2019 and February 2020 by distributing flyers at schools, word of mouth and e-mail-announcements using the University of Tübingen's e-mail distribution list. The study was approved by the Institutional Review Board of the Medical Faculty of the University of Tübingen (Ref. No. 959/2018BO1). Each adolescent and their guardian signed an informed consent before participating in the study. Participants received compensation in form of a 20 € book voucher if they

participated in both parts of the study (actigraphy and DLMO), or a 10 € voucher for participation in only the actigraphic measurements.

Inclusion and exclusion criteria

Inclusion and exclusion criteria were enquired during a preliminary phone interview. A summary of the criteria is shown in Table 2. Our intent was to eliminate factors that might have an external influence on the sleep-wake rhythm.

Table 2. Inclusion and exclusion criteria for study participation

Inclusion criteria	Exclusion criteria
- Age 13 to 16 years	- Regular intake of melatonin - Regular intake of any medication excluding oral contraceptives - Travel across more than 2 time zones in the last month - Previous diagnosis of a ▪ Sleep disorder ▪ Neurological disorder ▪ Psychotic disorder ▪ Bipolar disorder - Depression - Chronic medical condition or developmental disorder

To give a better overview, our methods and respective study samples are summarized in the following flow chart (Figure 1).

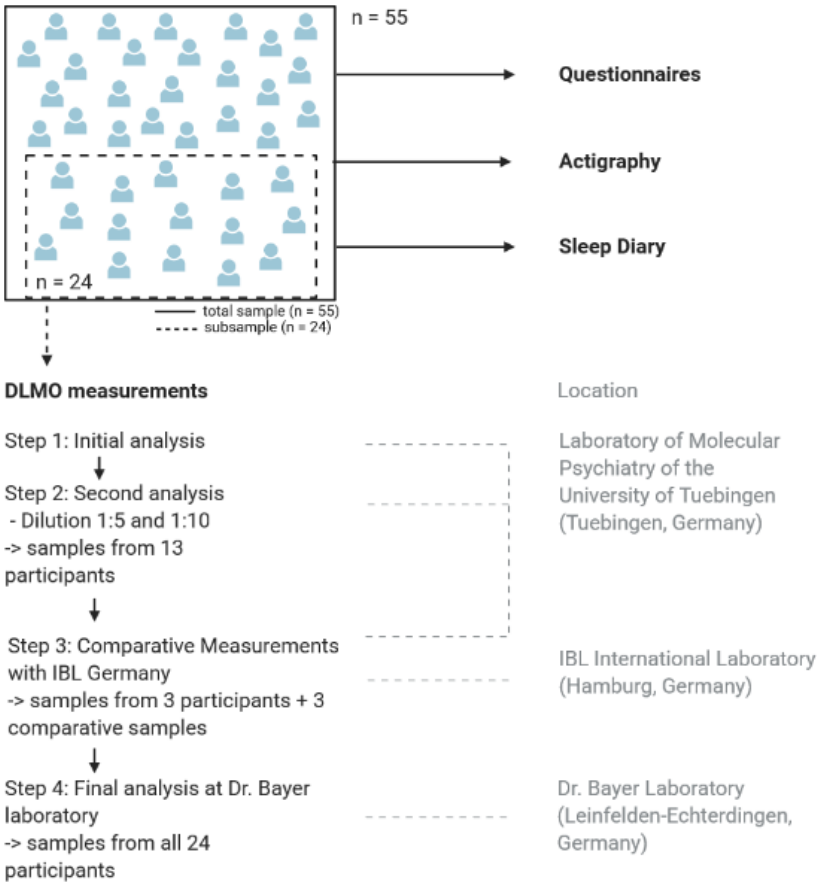

Figure 1. Overview of procedure and study samples

(Created with www.biorender.com)

2.2 Instruments

2.2.1 Questionnaires

We asked all participants to report their age, height and weight.

Participants also completed the following questionnaires, which consisted of three chronotype questionnaires and one sleepiness questionnaire:

Morningness-Eveningness Stability Scale (improved; MESSi)

The MESSi (Randler et al., 2016a) is a self-assessment questionnaire consisting of three subscales. The morning affect subscale (MA) and the eveningness subscale (EV) measure individual diurnal preference in activity, performance and mood. The distinctness/amplitude subscale (DI) assesses the stability of a

subject's circadian phasing throughout the day. Each subscale has five items, which can be answered by checking the applicable answer on a five-level scale. Four items are reverse-coded. Attainable scores range from five to 25 points on each subscale. High scores in the MA or the EV subscales reflect a higher expression of morning- or eveningness. Higher scoring in the DI indicates a higher amplitude in the individual circadian rhythm, which means more fluctuation in performance and mood during the day.

Reduced Morningness-Eveningness Questionnaire for Children and Adolescents (rMEQ-CA)

In the reduced version of the Morningness-Eveningness Questionnaire (Horne and Ostberg, 1976), chronotype is assessed by five items (Adan and Almirall, 1991). Three of them are timelines on which subjects are supposed to indicate their preferred wake time, the time they usually grow tired and their subjective peak of performance and well-being. The participants are also asked how tired they are in the first half-hour after waking up and to self-assess their chronotype. We adapted some wordings of the German version of the rMEQ (Randler, 2013) to make them more suitable for adolescents, *i.e.* changing the formal address "Sie" to the more informal "Du".

The scoring of the rMEQ-CA classification is described in the comparative table (Table 3).

We had two participants who scored 17.5 points. In this case we decided to classify them as Intermediate Types (IT).

Composite Scale of Morningness (CSM)

With the CSM (Smith et al., 1989), participants can self-assess their circadian rhythmicity by answering questions about their sleep-wake rhythm and diurnal preferences regarding academic and physical performance. We used the German version of the CSM (Randler, 2007). The questionnaire has 13 items and participants can score between 13 and 55 points. Participants can classify themselves as either morning, intermediate or evening type. Cut off scores are dependent on the individual study population, as the 90th and the 10th percentile

are used to score morning and evening types, respectively (Smith et al., 1989, Randler, 2007).

Table 3. Comparison of the three chronotype questionnaires

	MESSi	rMEQ-CA	CSM
Items	15 (5 per subscale)	5	13
Scoring	15-75 (5-25 per subscale)	4-25	13-55
Scaling	Rating scale (5 levels)	3 timelines 2 rating scales	Rating scale (4-5 levels)
Chronotype Classification	None	- 22-25 pts: Definitive MT - 18-21 pts: Moderate MT - 12-17 pts: IT - 7-11 pts: Moderate ET - 4-7 pts: Definitive ET	- ≤ 10^{th} percentile: ET - >10^{th} < 90^{th} percentile: IT - ≥ 90^{th} percentile: MT

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CSM=Composite Scale of Morningness, ET=Evening Type, IT=Intermediate Type, MESSi=Morningness-Eveningness Stability Scale, MT=Morning Type, pts = points, rMEQ-CA= Reduced Morningness-Eveningness Questionnaire for Children and Adolescents

Pediatric Daytime Sleepiness Scale (PDSS)

The PDSS is a scale that measures excessive daytime sleepiness in children (Drake et al., 2003). The scale is built up in a Likert-type format (0 = always to 4 = never). One answer is reverse-coded. We used the German version (Schneider

and Randler, 2009), which consists of eight items. Results can range from 0 to 32 points. Higher scores indicate greater sleepiness (Drake et al., 2003).

Note (concerning all questionnaires): In cases in which two answers were checked or the participant's answer on a timeline covered two or more scoring ranges, we used the mean value to score the questionnaire.

2.2.2 Habitual sleep onset and offset times

Habitual sleep onset and offset times on weekdays and weekends were self-assessed by the participants when filling out the questionnaires.

2.2.3 Actigraphy

Individual activity levels and sleep-wake rhythms were measured by actigraphy. Participants were instructed to wear an actigraph for seven consecutive days and asked to only remove the actigraph during water-based activities (*e.g.,* bathing or swimming). We used the model GT3X+ of the ActiGraph series (ActiGraph, Pensacola, FL-USA), which was validated against polysomnography for use in adolescents (Quante et al., 2018). The actigraph resembles a watch and is worn on the non-dominant wrist. A person wearing the actigraph is shown in Figure 2 for better visualization. The actigraph has a built-in accelerometer which registers body movements. The data is downloaded after measurement using the corresponding software (ActiLife, Pensacola, FL-USA).

Figure 2. A person wearing the actigraph GT3X+

An established procedure (Quante et al., 2019) was followed, *i.e.* the actigraphy was considered valid if there were at least 10 hours of valid signal during wake hours and at least 4 valid days in total, one of them being a weekend night.

Activity counts were measured in 1-minute epochs for 7 consecutive days. One recording day equaled 24 hours.

We didn't use one Saturday night for actigraphy analyses, as 13 adolescents participated in the saliva sample collection on that day.

2.2.4 Sleep Diary

A sleep diary is a daily record of an individual's sleep timing. The literature recommends the use of an additional sleep diary in actigraphy studies, as actigraphy has certain limitations in differentiating, for example, rest and inactive wake periods (Sadeh, 2011). For this reason, all participants completed a sleep diary established in clinical use (Paciello et al., 2019), which was specifically adapted for this study. The participants were asked to note their sleep onset and offset times as well as naps and awakenings during the night. They were also requested to document when the actigraph was taken off.

2.3 Data scoring

2.3.1 Sleep-wake parameters

We also followed an already established procedure in the sleep-wake rhythm scoring process using the ActiLife software (Mitchell et al., 2017). Sleep and wake periods were manually identified as sharp increases or sharp decreases in activity counts and could then be compared to the sleep timing reported in the sleep diary (Figure 3). The major sleep period (the time frame between a subject's sleep onset and offset time) and nap times during the day were scored using the combined information from actigraphy data and the sleep diary. If the times noted in the sleep diary differed too much from the actual decrease in activity measured by the actigraph (> 30 minutes), sleep periods were visually determined from changes in activity.

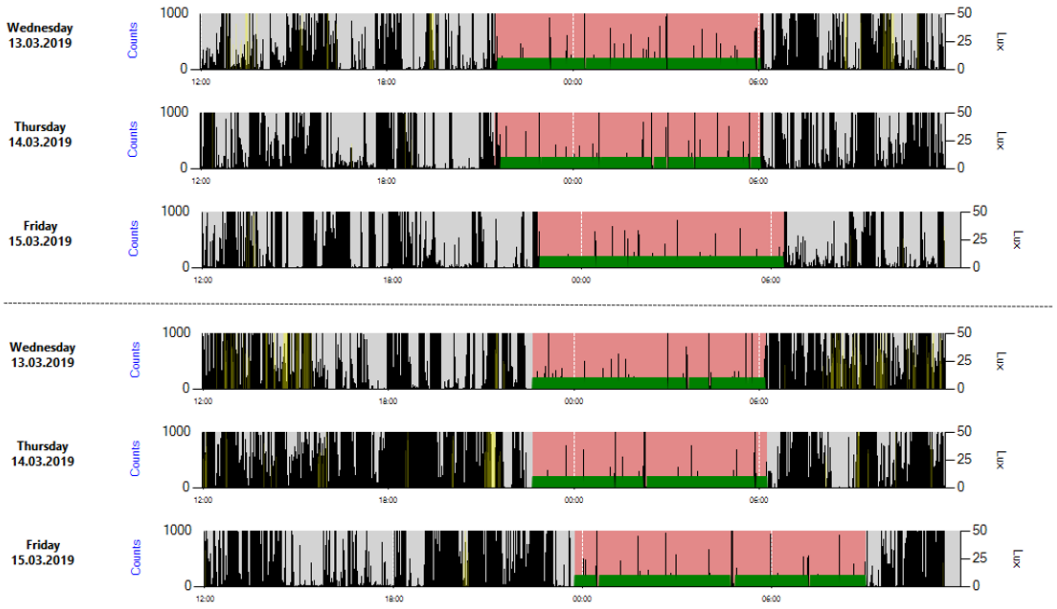

Figure 3. Scored actigraphy data from three consecutive nights (Wednesday to Friday, March 2019)

The upper figure is of a participant who scored high on the rMEQ-CA (22,5 points, Definitive Morning Type), the lower of a participant who scored low on the rMEQ-CA (9 points, Evening Type). Sleep periods are marked in red and green.

The data was then processed using the Cole-Kripke sleep-wake algorithm (Cole et al., 1992). We did a wear time validation to be able to differ between times the actigraph was worn or not worn using the Troiano algorithm (Troiano et al., 2008) and counterchecked with the information in the sleep diary.

2.3.2 Rest-activity parameters

We derived rest-activity patterns using using a publicly available algorithm (https://github.com/nsrr/actiCircadian).

The code generates rest-activity patterns following two different approaches during a during a 24-hour day (Mitchell et al., 2017). The cosinor approach uses a regression model that is based on the assumption of a known, because synchronized to a 24-hour rhythm, time period (Cornelissen, 2014). The non-parametric approach was introduced as an alternative to the cosinor analysis, because rest-activity rhythms do not completely correspond to a sinusoidal wave form (Mitchell et al., 2017). The non-parametric analysis does not require any

assumption of the waveform of the circadian rhythm (Van Someren et al., 1999). We applied both codes to MATLAB (version R 2019a).

We used the following rest-activity parameters in subsequent statistical analyses (Table 4, Figure 4):

Table 4. Cosinor and non-parametric variables

Cosinor analysis	
Acrophase	Time point of peak activity
Non-parametric analysis	
M10-midpoint	Midpoint in time and activity counts of the most active 10-hour period
L5-midpoint	Midpoint in time and activity counts of the least active 5-hour period

Definitions of Acrophase and M10- and L5-midpoint according to Mitchell et al., 2017.

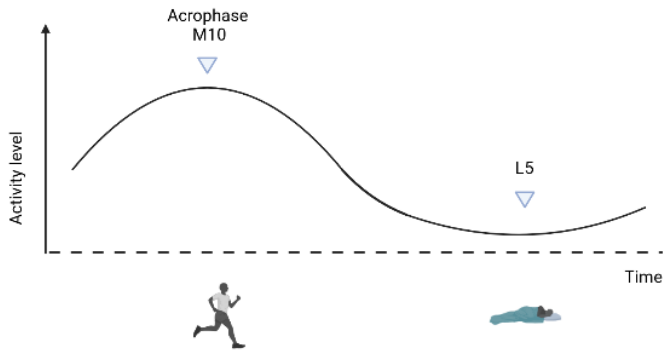

Figure 4. Schematic illustration of rest-activity parameters

(created with www.biorender.com)

2.4 Statistical analysis

IBM© SPSS© for Windows (version 25) was used to statistically analyze the data.

We calculated the respective average sleep duration and midpoint of sleep for weekdays and weekends (MS) from actigraphy and self-assessed sleep timing. The midpoint of sleep is the midpoint between the sleep onset and offset times (Benoit et al., 1981). We also calculated the midpoint of sleep on free days (MSF-

SC), which refers to the midpoint of sleep corrected for sleep debt (Roenneberg et al., 2004) from self-assessed sleep onset and offset times. We also determined the respective overall average sleep duration from actigraphy and self-assessed sleep onset and offset times (Table 5).

Table 5. Calculations of sleep midpoints during the week, on free days (MSF-SC) and overall average sleep duration

Overall average sleep duration	$\frac{(5\times weekday\ sleep\ duration+2*weekend\ sleep\ duration)}{7}$
Midpoint of sleep	$sleep\ onset\ time + (\frac{1}{2}\ sleep\ duration)$
MSF-SC	$weekend\ MS - \frac{1}{2} \times (weekend\ sleep\ duration - overall\ sleep\ duration)$

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MS = midpoints of sleep, MSF-SC = midpoint of sleep on free days.

We examined the reliability of the questionnaires using Cronbach's alpha (Cronbach, 1951) within the interpretation guidelines by George & Mallery, which range from α=<0.5 (inacceptable) to α=>0.9 (excellent) (George and Mallery, 2003). We applied Spearman correlations to assess correlations between the different questionnaires and actigraphy. Correlations were interpreted as follows: 0.00 to 0.19 as very weak, 0.20–0.39 as weak, 0.40–0.59 as moderate, 0.60–0.79 as strong and 0.80–1.00 as very strong (Swinscow, 1997).

The influence of chronotype classification on sleep-wake parameters was analyzed using univariate analysis. We examined whether age, sex, or the interaction between age and sex had an influence on questionnaire scores in multivariate analyses. Levene's test for homogeneity was interpreted using the median. We chose Bonferroni post-hoc testing for all parametric uni- and multivariate analyses. Effect sizes were calculated as partial Eta-squared ($p\eta^2$). We interpreted a partial Eta-squared from 0.1 to 0.3 as weak, 0.3-0.5 as moderate und > 0.5 as strong (Cohen, 1988). The significance level was at $p < 0.05$.

We transformed data which were not normally distributed to fit parametric univariate analyses, using the natural logarithm (ln) of the variables. We did a comparative ANOVA with non-transformed variables.

In the parametric multivariate analyses (MANOVA), there were also groups which were not normally distributed (PDSS female group, CSM 16-year-old group). The MANOVAs was, however, shown to be robust enough against violations of normal distribution (Finch, 2005).

2.5 Dim Light Melatonin Onset

2.5.1 Sample collection

In order to measure the DLMO, we collected saliva samples of our subsample of 24 participants. The sample collection took place on the evening of March 16, 2019, and followed the procedure described by Crowley et al. (Crowley et al., 2016). The participants watched two age-appropriate movies (Jumanji and Forrest Gump) in dim light (< 20 lux) during the sampling window from 8 p.m. to 12 a.m. During these 4 hours, we collected approximately 2 ml of saliva in Salivettes (Sarstedt, Nümbrecht, Germany) every 30 minutes, resulting in 9 samples per participant.

In order to avoid cross-reactivity with the melatonin-assay, participants were asked to refrain from the consumption of non-steroidal anti-inflammatory drugs (NSAIDs), alcohol, nicotine, chocolate or caffeine in the 72 hours before and during saliva collection. Consumption of bananas was also not allowed during sample collections because of a possible cross reactivity. Subjects were allowed to eat gummi-bears until 15 minutes before and to drink water until 10 minutes before each sample collection. Participants who had consumed food or beverages in the time between sample collections had to rinse their mouth 15 minutes before the next sample was taken.

If a subject needed to use the restroom during the sampling window, we ensured they wore sunglasses. We made a list of participants' restroom breaks in order to check for possible aberrations in these samples later on. Participants were not

allowed to leave their seats in the 10 minutes before each sample collection in order to minimize interference by posture changes.

Light readings were taken every 30 minutes during sample collection using a lux meter (Figure 5).

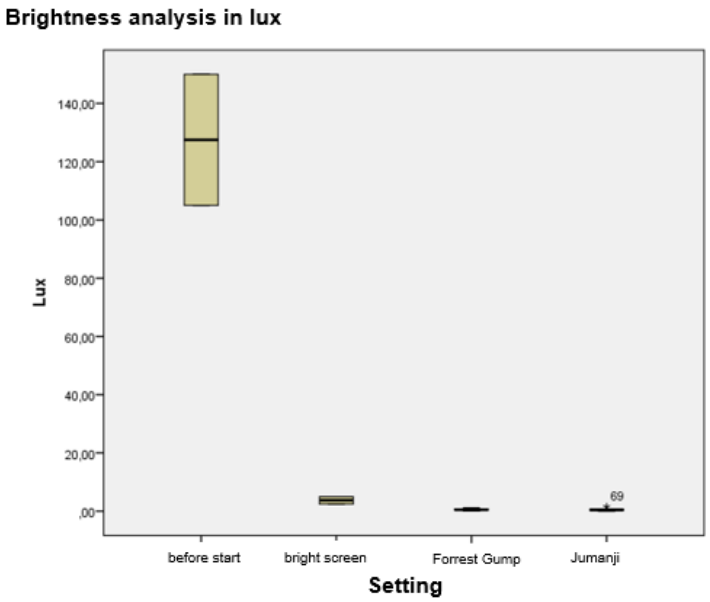

Figure 5. Light readings before sampling time and during the movies (provided by Christoph Randler).

Y-axis = Brightness in lux, x-axis = time points of light readings

After collection of the final samples, participants were either picked up by their guardian or driven home in a taxi.

The samples were then frozen at -20 degrees.

2.5.2 Sample analysis

The saliva samples were analyzed by performing enzyme-linked immunosorbent assays (ELISA). We used a commercially available kit from IBL Germany (Melatonin direct saliva ELISA, Reference No. RE54041). Following the kit's instructions, samples were first thawed and centrifuged for 10 minutes at 2000 – 3000 x g. The subsequent analysis followed the procedure described in the kit's manual (direct excerpt from the IBL Germany RE54041 manual (IBL)

downloadable at https://www.ibl-international.com/de_de/non-extraction-melatonin-saliva-elisa, last accessed on May 9, 2021, 10:36 am):

1) *"Pipette 100 µL of each Standard, Control and sample into the respective wells of the microtiter plate.*
2) *Pipette 50 µL of Antiserum solution into each well. Cover plate with adhesive foil. Shake plate carefully for 10 seconds.*
3) *Incubate 16 -20 h at 2 -8°C.*
4) *Remove adhesive foil. Discard incubation solution. Wash plate 4 x with 250 µL of diluted Wash Buffer. Remove excess solution by tapping the inverted plate on a paper towel.*
5) *Pipette 100 µL of Biotin solution into each well. Cover plate with adhesive foil.*
6) *Incubate 2 h at RT (18 -25°C) on an orbital shaker (500 rpm).*
7) *Remove adhesive foil. Discard incubation solution. Wash plate 4 x with 250 µL of diluted Wash Buffer. Remove excess solution by tapping the inverted plate on a paper towel.*
8) *Pipette 100 µL of Enzyme Conjugate into each well. Cover plate with adhesive foil.*
9) *Incubate 1 h at RT (18 -25°C) on an orbital shaker (500 rpm).*
10) *Remove adhesive foil. Discard incubation solution. Wash plate 4 x with 250 µL of diluted Wash Buffer. Remove excess solution by tapping the inverted plate on a paper towel.*
11) *Pipette 100 µL of TMB Substrate Solution into each well.*
12) *Incubate 15 min at RT (18 -25°C) on an orbital shaker (500 rpm).*
13) *Stop the substrate reaction by adding 100 µL of TMBStop Solution into each well. Shake briefly. Color changes from blue to yellow.*
14) *Measure optical density with a photometer at 450 nm (Reference-wavelength: 600-650 nm) within 15 min after pipetting of the Stop Solution."*

We first carried out an analysis of the samples from 13 participants (117 samples) at the laboratory of Molecular Psychiatry of the University of Tuebingen. In these samples, the coefficient of variation in double determinations was too high. The

coefficient of variation is a method to ensure measurement precision and reliability (Schultheiss and Stanton, 2009). It reflects the deviance of duplicate measurements in the same batch (intraassay) or the batch-to-batch consistency of the measurements (interassay) (Salimetrics, 2021). Several samples were not measurable due to being above the maximum range. Sample dilution of 1:5 and 1:10 still resulted in abnormally high values. Possible reasons for both issues will be explored in the discussion section.

To understand whether these difficulties were due to technical problems or aberrant samples, we did comparative measurements with the help of the IBL laboratory in Hamburg, Germany. The IBL laboratory performed an analysis on participants' samples that had very high values in our previous analysis. We analyzed samples with already known melatonin concentrations from IBL. Due to lower coefficients of variation and the melatonin values being within the measurable range in the IBL measurement of our samples, we decided to stop our measurements in the Tuebingen laboratory.

The final analysis of all samples took place at the Dr. Bayer laboratory (Leinfelden-Echterdingen, Germany), following the procedure described above. The results of this analysis were used in subsequent statistical analyses.

The functional sensitivity limit (FSL) of the assay is 1.0 pg/ml according to the kit's manufacturer. The FSL is defined as the minimum salivary melatonin concentration measurable with an intra-assay coefficient of variation lower than 20 % (Davies, 2013). We followed the convention that values beneath the FSL are assigned to the value of functional sensitivity (here 1.0 pg/ml) (Kennaway and Salkeld, 2017).

We did not use values for analyses if variation in double determinations was too high or if there was not enough saliva left to measure in duplicate. To our knowledge, there is no reference on how much variation is allowed in melatonin saliva measurement. For this reason, we decided to use the cortisol values defined in the 2019 guidelines of laboratory medicine, as cortisol also follows the human circadian rhythm. The upper limit of allowable variation in ring trials was 30 % (Bundesärztekammer, 2019).

The overall coefficient of variation of double determinations of the samples was 15 %. Looking only at the samples within our defined range of variation (30%), the overall coefficient of variation was 10%. Samples were measured in duplicate but not on the same plate. For this reason, it was not possible to state an intraassay coefficient of variation.

2.5.3 Dim Light Melatonin Onset calculations and statistical analysis

Currently, four ways of determining the DLMO can be found in the literature:
a) Using an absolute threshold (3 or 4 pg/ml in saliva) (Crowley et al., 2016, Benloucif et al., 2008)
b) Determining the average of three baseline values and adding 2 standard deviations (Voultsios et al., 1997)
c) Using twice the minimum detection limit of the assay (Deacon and Arendt, 1994)
d) Visually estimating the DLMO as an increase in melatonin levels (Benloucif et al., 2008)

Obviously, results from these methods vary. This was already recognized by researchers who compared the results obtained by different methods (Crowley et al., 2016, Molina and Burgess, 2011). Other researchers introduced using a consensus of a relative and an absolute method for better comparability of the results (Benloucif et al., 2008).

In this study, we used a fixed threshold (4 pg/ml) for DLMO calculations (Crowley et al., 2016). DLMO was determined in Windows Excel (version 2011) by linear interpolation of the mean values directly above and below the threshold value. We did not calculate the DLMO if all samples were above the threshold value. We were able to calculate the DLMO in 12 participants by linear interpolation of the 4 pg/ml threshold (Figure 6).

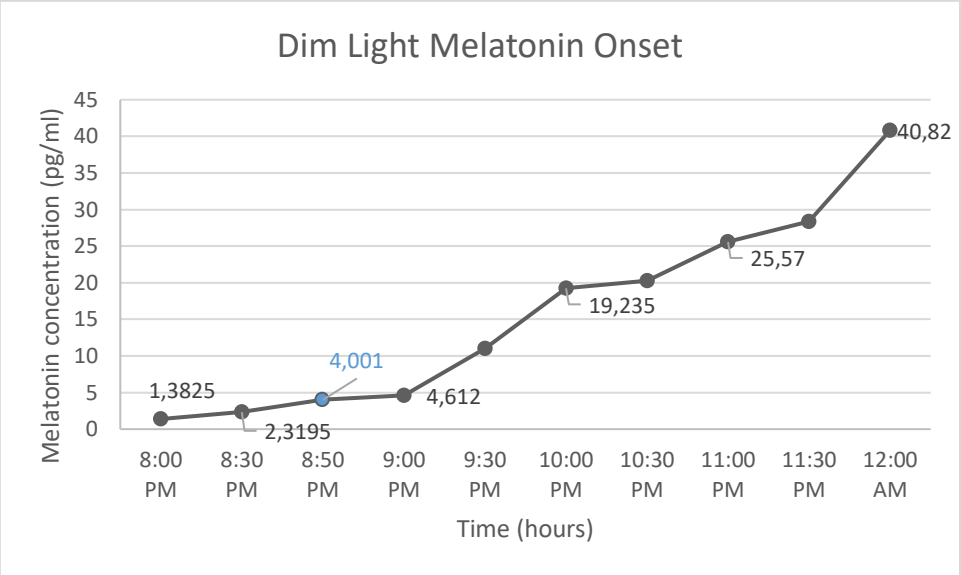

Figure 6. Example of melatonin concentration at time points of sampling

Grey = melatonin concentration at time points of sampling, blue = linear interpolation of threshold time point (*i.e.,* DLMO). Y-axis = melatonin concentration in pg/ml, x-axis = time in hours.

The resulting values were then correlated against the questionnaires using Spearman correlations and interpreted as described in section 2.4.

3 Results

Parts of the following results (demographics, convergent validity, correlations with actigraphy data) and their subsequent discussion were published in the Journal of Sleep Research in 2022 (Paciello et al., 2022).

3.1 Demographics

All participants in our sample filled out the questionnaires and the sleep diary and took part in actigraphy measurements (49.1% male, mean age + SD = 14.4 ± 1.1 years).

Three participants failed to complete the rMEQ-CA- and the CSM-questionnaire. We had to discard the actigraphy data of three subjects because of missing or insufficient actigraphy or sleep diary data.

We discarded one MESSi questionnaire answer in one participant because the answer seemed to be an obvious outlier. The participant put 10:44 am as his average wake time on weekdays, which doesn't correspond to the starting times of the German school system.

The descriptive statistics of our study sample (N=55) including demographics, questionnaire scores and sleep-wake and activity parameters are shown in Table 6.

Table 6. Descriptive overview of the demographic values, questionnaire scores and actigraphic sleep-wake and activity parameters

Demographics	**Mean (SD) or percentage**
Age (in years) [a]	14.4 (± 1.1)
Height (in cm) [a]	168.5 (± 10.1)
Weight (in kg) [a]	56.8 (± 12.4)
BMI, mean (SD) (in kg/m^2) [a]	19.8 (± 2.7)
z-score [a]	-0.22 (± 1.02)
Female (%) [a]	28 (50.9 %)
Sleep and chronotype questionnaire scores	**Mean (SD)**
MESSi-Morningness subscale [a]	17.4 (4.3)
MESSi-Eveningness subscale [a]	15.7 (3.7)
MESSi-Distinctness subscale [a]	15.3 (4.7)
rMEQ-CA [c]	14.8 (3.1)
CSM [c]	37.2 (6.7)
PDSS [a]	11.0 (7.2)
Actigraphic sleep-wake parameters	**Mean (SD)**

Average Sleep Duration (hours) [d]	8:41 (0:47)
Midpoint of Sleep Weekdays (hours) [d]	02:28 (0:29)
Midpoint of Sleep Weekends (hours) [d]	03:54 (0:49)
Actigraphic activity parameters	**Mean (SD) or Median (IQR)**
M10 Whole Week (dec. hours) [c]	14.3 (12.0,15.9)
M10 Weekdays (dec. hours) [c]	14.3 (11.9,15.7)
M10 Weekends (dec. hours) [c]	15.7 (14.4,17.6)
L5 Whole Week (dec. hours) [c]	2.7 (1.0)
L5 Weekdays (dec. hours) [c]	2.3 (1.1)
L5 Weekends (dec. hours) [c]	2.5 (1.8,3.8)
Acrophase Whole Week (dec. hours) [c]	14.3 (0.7)
Acrophase Weekdays (dec. hours) [c]	13.9 (0.7)
Acrophase Weekends (dec. hours) [c]	15.5 (1.1)
Dim Light Melatonin Onset	**Mean (SD)**
DLMO (hours) [e]	20:48 (0:12)

Partly published in Paciello et al., 2022.

a N=55, b N=53, c N=52, d N=51, e N=12, with mean (SD) or median (Q1, Q3).

BMI = Body Mass Index, dec. = decimal hours, CSM = Composite Scale of Morningness, DLMO = dim light melatonin onset, MESSi = Morningness-Eveningness-Stability Scale, PDSS = Pediatric Daytime Sleepiness Scale, rMEQ-CA = reduced Morningness Eveningness Questionnaire for Children and Adolescents, SD = standard deviation.

Subsample

A subsample of our participants (N=24) also provided us with saliva samples for DLMO analysis. We calculated the DLMO of 12 participants (25% male, mean age + SD = 14.0 ± 0.95 years) by linear interpolation of the 4 pg/ml threshold.

An exploration of differences in demographics in the subsample using a Mann-Whitney U test showed no significant difference in age, height, BMI and the BMI's z-score in the subsample with melatonin measurements compared to the overall cohort (N=12, $p \geq 0.05$). A chi-squared test indicated an almost significant difference in sex ($p=0.06$), the percentage of females in the subsample was higher (75%).

3.2 Chronotype distribution and daytime sleepiness

Chronotype distribution

In the CSM questionnaire, we used the 10^{th} and the 90^{th} percentile to classify morning and evening types. In male participants, this corresponded to ≤ 27.2 points to classify evening types and ≥ 46.8 points to classify morning types. Cutoff values were ≤ 25.6 points and ≥ 44.2 points for female participants. According to the CSM, 12 participants were classified as Morning Type (MT, 23%), 35 as Intermediate Type (IT, 67%) and 5 as Evening Type (ET, 10%).

In the rMEQ-classification, only one participant classified as a Definite Morning Type and no participant as a Definite Evening Type. For this reason, we subsumed both Morning Types and Evening Types into one respective group for the following statistical analyses. The rMEQ-CA classified seven participants as MT or definite MT (14%), 36 as IT (69%) and nine as ET (17%).

An overview of the chronotype distribution according to sex can be found in Table 7.

Table 7. Chronotype classification according to sex

Questionnaire	Morning Types	Intermediate Types	Evening Types
rMEQ-CA (N=52)	♂ N=4 ♀ N=3 (DM N=1)	♂N=15 ♀ N=21	♂N=6 ♀N=3

CSM (N=52)	♂ N=6 ♀ N=6	♂N=17 ♀ N=18	♂N=2 ♀N=3

♂= male, ♀= female.

CSM = Composite Scale of Morningness, DM = Definite Morning Type, rMEQ-CA = reduced Morningness Eveningness Questionnaire for Children and Adolescents.

In a sign test, the chronotype classification of the CSM and the subsumed classification of the rMEQ-CA differed significantly from each other ($p < 0.01$, Figure 7).

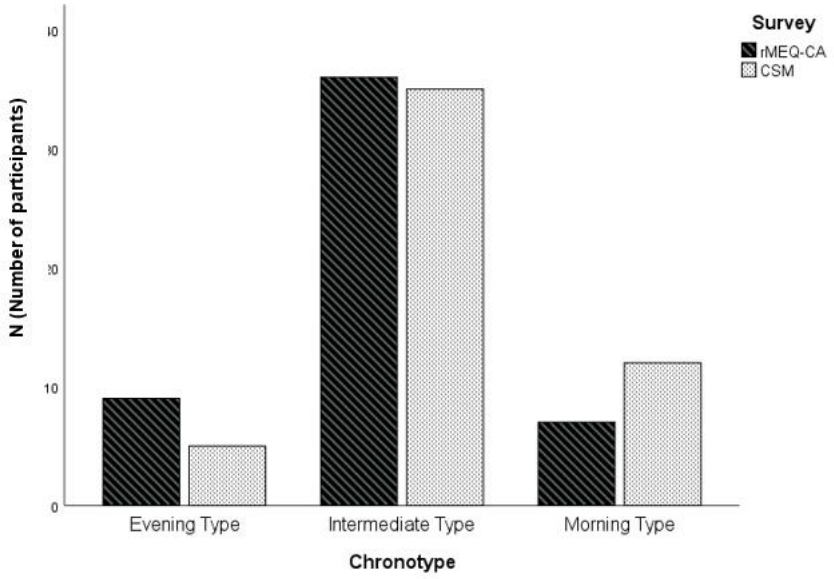

Figure 7. Comparison of chronotype classification according to rMEQ-CA and CSM questionnaires

Y-axis = Number of participants in respective group (N), x-axis = chronotype group. Morning Types and Definite Morning Types subsumed into "Morning Type" in the rMEQ-CA group.
CSM=Composite Scale of Morningness, rMEQ-CA= Reduced Morningness Eveningness Questionnaire for Children and Adolescents

The MESSi subscales measure the extent of morningness, eveningness and distinctness and don't classify into specific chronotypes. Figure 8 shows the distribution of subscale scores in relation to the rMEQ-CA chronotype classification (Figure 8). Here, it is demonstrated that a participant classified as MT scored highest on the Morningness subscale, while a participant classified as

ET scored highest on the Eveningness subscale. Scores on the distinctness scale were lower in IT.

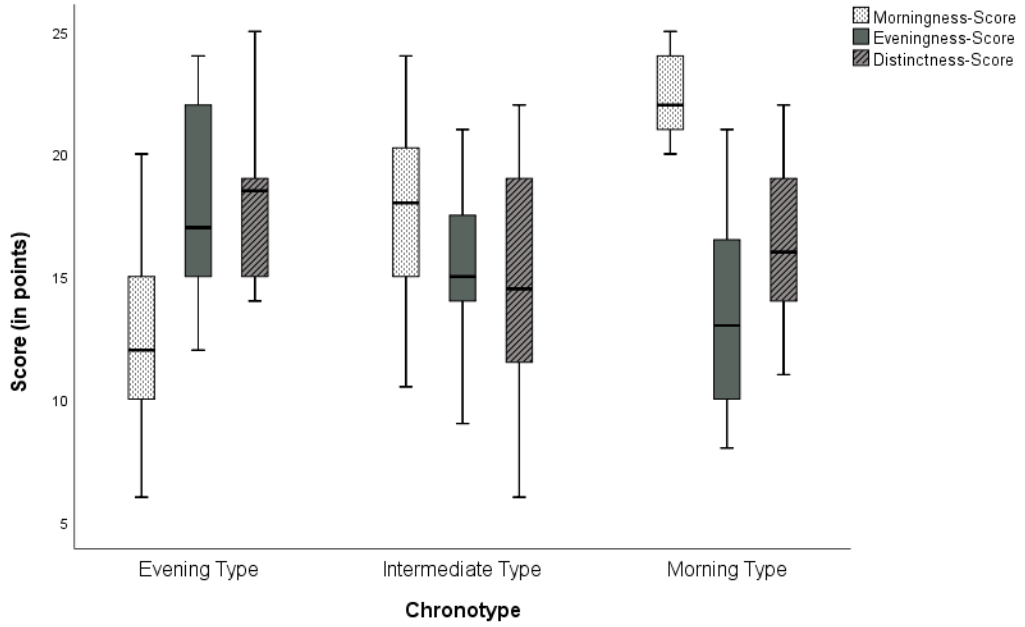

Figure 8. Distribution of MESSi-scores according to chronotype (rMEQ-CA classification)

Y-axis = Score in points, x-axis = chronotype group.

Sleepiness questionnaire

The threshold of the PDSS for conspicuous results is at > 26 points for children aged 13 years and younger and >29 points for children aged over 13 years. None of the participants scored above the threshold value for their age.

3.3 Reliability

We examined reliability in each questionnaire. Table 8 shows Cronbach's α for the rMEQ-CA, the CSM and the PDSS. As the MESSi consists of three individual subscales (MA, EV and DI), we tested reliability in every subscale. The questionnaires' respective reliability ranged from acceptable (Cronbach's α > 0.7) to excellent (Cronbach's α > 0.9).

Table 8. Reliability of the questionnaires

Questionnaire	Number of items	α
rMEQ-CA	5	0.70
CSM [d]	13	0.91
PDSS [f]	8	0.82
MESSi MA [a]	5	0.88
MESSi EV [b]	5	0.74
MESSi DI [b]	5	0.83

Published in Paciello et al., 2022.

a N=55, b N=54, c N=53, d N=52, e N=51, f N=50

CSM=Composite Scale of Morningness, DI = Distinctness/Amplitude subscale, EV = Evening affect subscale, MA = Morning affect subscale, MESSi=Morningness-Eveningness Stability Scale, PDSS = Pediatric Daytime Sleepiness Scale, rMEQ-CA= Reduced Morningness-Eveningness Questionnaire for Children and Adolescents

3.4 Spearman correlations

3.4.1 Correlations between the questionnaires

We conducted a Spearman correlation analysis between the questionnaires (Table 9). Here, the MESSi MA correlated strongly positively with the CSM (0.84) and the rMEQ-CA (0.71). Negative correlations with the other MESSi subscales were weak (EV-subscale -0.38, DI-subscale -0.37).

The MESSi EV showed moderate correlations with a lower CSM (-0.48) and a higher PDSS score (0.49) and correlated weakly negatively with the rMEQ-CA (-0.34, $p < 0.05$). Other than the described association with the MESSi MA, the MESSi DI only correlated positively with the PDSS (0.51). A higher PDSS score was also strongly correlated with a lower rMEQ-CA (-0.64), CSM (-0.78) and MESSi MA score (-0.76). The CSM and the rMEQ-CA correlated very strongly positively with each other (0.88).

Table 9. Spearman correlations between the questionnaire scores

	MESSi MA	MESSi EV	MESSi DI	rMEQ-CA	CSM	PDSS
MESSi MA	-	-0.38** [a]	-0.37** [a]	0.71** [b]	0.84** [b]	-0.76** [a]
MESSi EV	-0.38** [a]	-	0.12 [a]	-0.34* [b]	-0.48** [b]	0.49** [a]
MESSi DI	-0.37** [a]	0.12 [a]	-	-0.16 [b]	-0.25 [b]	0.51** [a]
rMEQ-CA	0.71** [b]	-0.34* [b]	-0.16 [b]	-	0.88** [b]	-0.64** [b]
CSM	0.84** [b]	-0.48** [b]	-0.25 [b]	0.88** [b]	-	-0.78** [b]
PDSS	-0.76** [a]	0.49** [a]	0.51** [a]	-0.64** [b]	-0.78** [b]	-

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a N=55, b N=52, ** p < 0.01, * p < 0.05

CSM=Composite Scale of Morningness, DI = Distinctness/Amplitude subscale, EV = Evening affect subscale, MA = Morning affect subscale, MESSi=Morningness-Eveningness Stability Scale, PDSS = Pediatric Daytime Sleepiness Scale, rMEQ-CA= Reduced Morningness-Eveningness Questionnaire for Children and Adolescents

3.4.2 Correlations with actigraphy

Correlations with sleep-wake parameters

Higher MESSi MA, rMEQ-CA and CSM scores moderately correlated with earlier bedtimes on weekdays (-0.42, -0.44 and -0.48, respectively), as well as on weekends (-0.33, -0.51, -0.52, respectively). The MESSi EV correlated positively with sleep onset (0.28) and offset times (0.28) on weekends. A higher PDSS score correlated moderately with a later sleep onset time both on weekdays (0.46) and weekends (0.56) and with a later sleep offset time on weekends (0.31). Correlations with sleep offset times on weekdays were not significant (Table 10).

Table 10. Spearman correlations of sleep-wake parameters and questionnaire scores

	Weekday sleep onset time	Weekday sleep offset time	Weekend sleep onset time	Weekend sleep offset time
MESSi MA	-0.42**[a]	-0.08[a]	-0.32*[b]	-0.28*[b]
MESSi EV	0.24[a]	0.15[a]	0.28*[b]	0.28*[b]
MESSi DI	0.21[a]	0.10[a]	0.09[b]	0.01[b]
rMEQ-CA	-0.44**[c]	-0.20[c]	-0.51**[d]	-0.45**[d]
CSM	-0.48**[c]	-0.20[c]	-0.52**[d]	-0.51**[d]
PDSS	0.46**[a]	0.16[a]	0.56**[b]	0.31*[b]

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a N=52, b N=51, c N=49, d N=48, **p < 0.01, * p < 0.05

CSM=Composite Scale of Morningness, DI = Distinctness/Amplitude subscale, EV = Evening affect subscale, MA = Morning affect subscale, MESSi=Morningness-Eveningness Stability Scale, PDSS = Pediatric Daytime Sleepiness Scale, rMEQ-CA= Reduced Morningness-Eveningness Questionnaire for Children and Adolescents

In a comparative correlation analysis, the MS of actigraphy and the MS of self-assessed sleep onset and offset times showed moderately positive correlations on weekends (0.59). Correlations between the respective weekday MS were strong (0.77, Table 11).

Table 11. Spearman correlations of the respective midpoints of sleep from actigraphy with self-assessed sleep onset and offset times

	Weekday MS Actigraphy	Weekend MS Actigraphy
Weekday MS SA	0.77**[b]	0.50**[c]
Weekend MS SA	0.52**[a]	0.59**[b]

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a N=51, b N=50, c N=49, ** < 0.01, * < 0.05.

MS = midpoint of sleep, SA = self-assessed sleep onset and offset times.

A later actigraphic MS on weekdays correlated with a lower MESSi MA, rMEQ-CA and CSM score (-0.43, -0.51 and -0.54, respectively), and a higher MESSi

EV (0.29) and PDSS (0.49) score. On weekends, the actigraphic MS showed similar correlations, a later MS was correlated with lower MESSi MA (-0,41), rMEQ-CA (-0.57) and CSM (-0.66) and with higher MESSi EV (0.38) and PDSS (0.55) scores. The weekday MS calculated from self-assessed sleep onset and offset times showed moderate to strong negative correlations with the MESSI MA, CSM and rMEQ-CA scores (-0.46, -0.62, -0.65, respectively) and positive correlations with the PDSS and MESSi EV scores (0.56 and 0.31, respectively). The weekend MS correlations with these variables were of similar strength (MESSI MA -0.48, CSM -0.58, rMEQ -0.60, MESSI EV 0.31, PDSS 0.42). The rMEQ-CA, CSM and MESSi MA scores had strong negative correlations with the MSF-SC (-0.67, -0.66 and -0.51, respectively). The correlations of the MESSi EV and PDSS scores with the MSF-SC were of similar strength compared to those of the other MS. The MESSi DI did not correlate with any MS or MSF-SC (Table 12).

Table 12. Spearman correlations of MS and MSF-SC determined by actigraphy and self-assessed sleep onset and offset times with the MESSi and other survey scores

	Self-assessed sleep onset and offset times			Actigraphy	
	Weekday MS	Weekend MS	MSF-SC	Weekday MS	Weekend MS
MESSi MA	-0.46** [b]	-0.48** [a]	-0.51** [b]	-0.43** [b]	-0.41** [c]
MESSi EV	0.31* [b]	0.31* [a]	0.36** [b]	0.29* [b]	0.38** [c]
MESSi DI	0.12 [b]	0.08 [a]	0.14 [b]	0.22 [b]	0.01 [c]
rMEQ-CA	-0.65** [e]	-0.60** [d]	-0.67** [e]	-0.51** [e]	-0.57** [f]
CSM	-0.62** [e]	-0.58** [d]	-0.66** [e]	-0.54** [e]	-0.66** [f]
PDSS	0.56** [b]	0.42** [a]	0.57** [b]	0.49** [b]	0.55** [c]

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a N=53, b N=52, c N=51, d N=50, e N=49, f N=48, ** p < 0.01, * p < 0.05.

CSM=Composite Scale of Morningness, DI = Distinctness/Amplitude subscale, EV = Evening affect subscale, MA = Morning affect subscale, MESSi=Morningness-Eveningness Stability Scale, MS = midpoint of sleep, MSF-SC = midpoint of sleep on free days, PDSS = Pediatric Daytime Sleepiness Scale, rMEQ-CA= Reduced Morningness-Eveningness Questionnaire for Children and Adolescents

Correlations of rest-activity parameters

The correlation analyses of the activity parameters Acrophase and L5-midpoint with the questionnaire and sleep-wake parameters are shown in Table 13. We analyzed the seven-day average (whole week) and separately weekday and weekend variables. The analysis of the M10-midpoint variable is displayed in the appendix (Table S1). Correlations with the M10-midpoint were not significant in all cases ($p \geq 0.05$).

We first examined correlations between the cosinor and the non-parametric approach. The full-week non-parametric L5-midpoint and M10-midpoint were strongly (M10 = 0.70) to moderately (L5 = 0.47) correlated with the cosinor variable Acrophase. Correlations became weaker when comparing the respective weekday and weekend variables.

Regarding the full-week variable, a later Acrophase timing correlated with lower rMEQ-CA (-0.38), CSM (-0.44) and MESSi MA scores (-0.31) and was positively correlated with the PDSS-score (0.42). There was no significant correlation with the other MESSi subscales. Acrophase correlated positively with sleep offset times on weekdays (0.35), but not on weekends, as well as with weekday (0.61) and weekend sleep onset times (0.59). All correlations with the actigraphic MS and MS from self-assessed sleep onset and offset times were significant and ranged from weak (weekend MS SA 0.37) to strong (weekday MS Actigraphy, 0.67).

When separated into a weekday and a weekend variable, the weekday Acrophase did not correlate with any of the other variables except weekday sleep offset times (0.34). However, Acrophase timing on weekends corresponded to

lower rMEQ (-0.34) and CSM (-0.40) scores, and higher PDSS scores (0.38). There was no correlation with the MESSi subscales. A later weekend Acrophase timing correlated with later sleep onset (0.45) and offset times (0.51) on weekends and later sleep offset times during the week (0.39). Correlations of weekend Acrophase with MS were stronger on weekends (MS Actigraphy = 0.62, MS SA = 0.49, MSF-SC SA = 0.40) than on weekdays (MS Actigraphy = 0.41, MS SA = 0.33, MSF-SC SA = 0.40).

We did not see any significant correlation regarding the L5-midpoint variables with the questionnaire scores. An exception was a weak correlation of the PDSS score with the full-week L5-midpoint (0.32).

The full-week L5-midpoint showed positive correlations with sleep onset time on weekdays (0.42) and sleep offset times on weekends (0.29), but not with any other sleep onset and offset times. The only correlation with the MS was a weak positive correlation of L5-midpoint with the actigraphic weekend MS (0.36).

Looking at the weekday and the weekend variable separately, L5-midpoint on weekdays correlated positively with sleep onset (0.29) and sleep offset times (0.46) on weekdays, as well as with sleep onset times on weekends (0.42). Both weekday MS (MS Actig. = 0.44, MS SA 0.35) and the actigraphic MS on weekends (0.34) were positively correlated with the weekday L5-midpoint.

A later weekend L5-midpoint correlated weakly with a later MS on weekends (MS Actig. = 0.29, MS SA = 0.28), and did not show any other correlations with sleep onset and offset times or MS.

Table 13. Spearman correlations of activity parameters Acrophase and L5-midpoint with questionnaire scores and sleep wake parameters

	Acrophase Whole Week hrs	Acrophase Weekdays hrs	Acrophase Weekends hrs	L5 Whole Week hrs	L5 Weekdays hrs	L5 Weekends hrs
MESSi MA	-0.31* [a]	-0.07 [a]	-0.20 [a]	-0.21 [a]	-0.12 [a]	-0.03 [a]
MESSi EV	0.17 [a]	-0.09 [a]	0.26 [a]	0.20 [a]	0.08 [a]	0.08 [a]
MESSi DI	0.13 [a]	0.18 [a]	0.07 [a]	-0.05 [a]	-0.07 [a]	-0.02 [a]

rMEQ-CA	-0.38** [d]	0.05 [d]	-0.34* [d]	-0.12 [d]	-0.25 [d]	-0.02 [d]
CSM	-0.44** [d]	0.03 [d]	-0.40** [d]	-0.27 [d]	-0.26 [d]	-0.12 [d]
PDSS	0.42** [a]	0.05 [a]	0.38** [a]	0.32* [a]	0.25 [a]	0.20 [a]
Sleep onset time Weekdays	0.61** [a]	0.13 [a]	0.39** [a]	0.12 [a]	0.29* [a]	0.09 [a]
Sleep offset time Weekdays	0.35* [a]	0.34* [a]	0.20 [a]	0.42** [a]	0.46** [a]	0.13 [a]
Sleep onset time Weekends	0.59** [b]	0.19 [b]	0.45** [b]	0.29* [b]	0.42** [b]	0.13 [b]
Sleep offset time Weekends	0.23 [b]	-0.19 [b]	0.51** [b]	0.22 [b]	0.11 [b]	0.26 [b]
MS Weekdays Actigraphy	0.67** [a]	0.22 [a]	0.41** [a]	0.27 [a]	0.43** [a]	0.13 [a]
MS Weekends Actigraphy	0.51** [b]	-0.02 [b]	0.62** [b]	0.36* [b]	0.34* [b]	0.29* [b]
MS Weekdays SA	0.54** [c]	0.07 [c]	0.33* [c]	0.19 [c]	0.35* [c]	0.17 [c]
MS Weekends SA	0.37** [b]	-0.13 [b]	0.49** [b]	0.17 [b]	0.11 [b]	0.28* [b]
MSF-SC SA	0.54** [c]	0.01 [c]	0.40** [c]	0.19 [c]	0.28 [c]	0.21 [c]
M10 hrs Week/ Weekdays/ Weekends	0.70** [a]	0.26 [a]	0.34* [a]			
L5 hrs Week/ Weekdays/ Weekends	0.47** [a]	0.30* [a]	0.28* [a]			

Correlations with Acrophase published in Paciello et al., 2022.

a N=52, b N=51, c N=50, d N=49, ** $p < 0.01$, * $p < 0.05$.

CSM=Composite Scale of Morningness, DI = Distinctness/Amplitude subscale, EV = Evening affect subscale, Hrs = hours, MA = Morning affect subscale, MESSi = Morningness-Eveningness Stability Scale, MS = midpoint of sleep, MSF-SC = midpoint of sleep on free days, PDSS = Pediatric Daytime Sleepiness Scale, rMEQ-CA= Reduced Morningness-Eveningness Questionnaire for Children and Adolescents, SA = self-assessed sleep onset and offset times.

3.4.3 Correlations with the Dim Light Melatonin Onset

The DLMO did not have any significant correlations with the questionnaires, sleep onset- and offset times, midpoints of sleep or activity parameters ($p \geq 0.05$, Table S2 can be found in the appendix).

We were able to calculate the DLMO in 50 % of our subsample (N=12). The average DLMO in our sample was at 20:48 ± 0:12.

In the other 50 % of our subsample, we did not have a lower value for interpolation because values were either

a) already above the threshold, or

b) discarded because their variations in double determination were too high. This will be explored in the discussion of the method in the following section.

An exploratory data analysis showed that early sleepers in our subsample went to bed at 22:34 ± 0:08 or 22:30 ± 0:15 (depending on chronotype classification), which is important for the discussion of sample collection timing.

3.5 Influence of chronotype in univariate analyses

Parametric analysis (ANOVA)

We analyzed the influence of chronotype classification on sleep-wake parameters and midpoints of sleep using a parametric univariate test (ANOVA). As the chronotype classifications differed significantly in the sign test, we did two separate analyses using either the subsumed rMEQ-CA or the CSM classification.

ANOVAs with transformed variables (ANOVAs 1 and 2)

Since the required normal distribution of the dependent variable was not given in all groups (Shapiro-Wilks test ≥ 0.05), we transformed the variable into its natural logarithm (ln). We did not examine a variable in these first ANOVAs if the variable could not be transformed to normal-distribution using common transformation approaches (*i.e.,* natural logarithm, square root, inverse of the variable). This was the case in the variable "sleep offset time on weekdays from actigraphy" in the analysis using the rMEQ-classification.

We controlled for homogeneity of all variables (Levene's test p ≥ 0.05). It should be noted that Levene's test was significant in the weekday MS for the self-assessed sleep onset and offset times.

In the ANOVAs on sleep onset and offset times, we could show that sleep timing differed significantly when separated by chronotype, except for sleep offset times on weekdays (Table 14). The midpoints of sleep also differed significantly when separated by chronotype (Table 15). Descriptive statistics indicated that MT had the earliest sleep onset and offset times and midpoints of sleep, followed by IT and then by ET. An exception was the sleep offset time on weekdays, which did not differ significantly between groups.

As the CSM-classified SA weekday MS turned out to be significant in the Levene's test, we compared the result of the ANOVA using the more robust Welch-ANOVA, where the influence on the MS was also significant (F (2,20.048) = 25.899, p < 0.01).

Table 14. ANOVAs 1: Influence of chronotype on sleep onset- and offset times

	Class.	MT	IT	ET	Anova F	p-value	(Partial) η^2
Sleep onset time Weekdays	CSM	21:39 ± 0:34 [c]	22:19 ± 0:50 [b]	22:56 ± 0:33 [g]	5.84	0.006	0.20
	rMEQ-CA	21:46 ± 0:36 [e]	22:11 ± 0:50 [a]	22:51 ± 0:38 [e]	3.39	0.04	0.13
	CSM+	.1.87 ± 0.04 [c]	1.91 ± 0.06 [b]	1.90 ± 0.07 [g]	1.74	0.19	0.07

Sleep offset time Weekdays	rMEQ-CA	-	-	-	-	-	
Sleep onset time Weekends	CSM	22:20 ± 0:36 [d]	23:24 ± 1:04 [b]	23:34 ± 0:19 [g]	5.75	0.006	0.20
	rMEQ-CA+	3.10 ± 0.02 [f]	3.15 ± 0.05 [a]	3.16 ± 0.01 [e]	4.82	0.01	0.18
Sleep offset time Weekends	CSM	7:52 ± 1:01 [d]	8:47 ± 1:03 [b]	9:05 ± 0:27 [g]	3.98	0.03	0.15
	rMEQ-CA	7:33 ± 1:15 [f]	8:43 ± 0:59 [a]	8:53 ± 0:56 [e]	3.72	0.03	0.14

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+ = ln-transformed variable, time in hours ± standard deviation. $p < 0.05$

a N=35, b N=32, c N=12, d N=11, e N=7, f N=6, g N=5.

Class. = classification, CSM=Composite Scale of Morningness, ET = evening type, IT = intermediate type, MS = midpoint of sleep, MSF-SC = midpoint of sleep on free days, MT = morning type, rMEQ-CA= Reduced Morningness-Eveningness Questionnaire for Children and Adolescents.

Table 15. ANOVAs 2: Influence of chronotype on midpoints of sleep

	Class.	MT	IT	ET	Anova F	p-value	(Partial) η^2
MS Weekdays Actigraphy	CSM+	0.72 ± 0.15 [e]	0.91 ± 0.18 [d]	1.03 ± 0.12 [j]	7.76	0.001	0.25
	rMEQ-CA+	0.74 ± 0.15 [h]	0.88 ± 0.19 [b]	1.02 ± 0.14 [h]	4.31	0.02	0.16
MS Weekends Actigraphy	CSM	03:06 ± 0:42 [f]	04:05 ± 0:47 [d]	04:20 ± 0:20 [j]	8.23	0.001	0.27
	rMEQ-CA	02:49 ± 0:48 [i]	04:00 ± 0:46 [b]	04:13 ± 0:35 [h]	6.935	0.002	0.24
	CSM +	0.66 ± 0.12 [f]	0.83 ± 0.15 [c]	0.94 ± 0.04 [j]	8.50	0.001	0.27

MS Weekdays SA	rMEQ-CA+	0.65 ± 0.10 [i]	0.80 ± 0.15 [b]	0.91 ± 0.15 [g]	6.05	0.005	0.21
MS Weekends SA	CSM	02:27 ± 0:25 [f]	03:00 ± 0:41 [b]	03:53 ± 0:53 [j]	7.91	0.001	0.25
	rMEQ-CA	02:26 ± 0:22 [i]	02:52 ± 0:34 [a]	03:45 ± 1:01 [g]	8.80	0.001	0.27
MSF-SC SA	CSM+	0.73 ± 0.11 [f]	0.90 ± 0.16 [c]	0.11 ± 0.10 [j]	10.12	0.001	0.30
	rMEQ-CA	02:04 ± 0:08 [i]	02:25 ± 0:22 [b]	02:52 ± 0:28 [g]	8.82	0.001	0.27

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+ = ln-transformed variable, time in hours ± standard deviation. $p < 0.05$.

a N=36, b N=35, c N=34, d N=32, e N=12, f N=11, g N=9, h N=7, i N=6, j N=5

CSM=Composite Scale of Morningness, ET = evening type, IT = intermediate type, MS = midpoint of sleep, MSF-SC = midpoint of sleep on free days, MT = morning type, rMEQ-CA= Reduced Morningness-Eveningness Questionnaire for Children and Adolescents, SA = self-assessed sleep onset and offset times.

The results of Bonferroni post-hoc tests are shown in Table 16.

In the post-hoc tests using the CSM classification, the MT group differed significantly from the ET group regarding sleep onset times on weekdays, as well as all midpoints of sleep. Differences between MT and IT were significant for sleep onset times on weekdays and weekends, as well as sleep offset times on weekends. MT and IT also differed significantly in all midpoints of sleep except for SA weekend MS. Here, differences were only significant between the IT and the ET group.

Post hoc-tests with the rMEQ-CA classification showed equally significant differences between MT and ET for weekday sleep onset time and all midpoints of sleep. The results of the rMEQ-CA classification analysis differed slightly from the CSM classification. Here, MT and ET differed significantly regarding sleep onset time on weekends. The difference between MT and IT was not significant in sleep onset time on weekdays, both weekday MS or the MSF-SC.

Table 16. Bonferroni post hoc-tests transformed ANOVAs 1 and 2

	Groups	Mean difference	Standard deviation	p-value
Weekday sleep onset time	MT to IT	[1] -0:40*	0:15	0.04
		[2] -0:25	0:19	0.59
	MT to ET	[1] -1:16*	0:24	0.008
		[2] -1:05*	0:25	0.04
	IT to ET	[1] -0:36	0:21	0.30
		[2] -0:39	0:19	0.15
Weekend sleep onset time	MT to IT	[1] -1:04*	0:19	0.007
		[2] -0.05*[+]	0.018	0.02
	MT to ET	[1] -1:14	0:30	0.06
		[2] -0.06*[+]	0.02	0.02
	IT to ET	[1] -0:09	0:27	1.00
		[2] -0.01[+]	0.02	1.00
Weekend sleep offset time	MT to IT	[1] -0:54*	0:21	0.04
		[2]-1:10*	0:26	0.04
	MT to ET	[1] -1:13	0:32	0.09
		[2]-1:19	0:33	0.07
	IT to ET	[1] -0:18	0:29	1.00
		[2] -0:09	0:25	1.00
MS Weekdays Actigraphy	MT to IT[+]	[1] -0.19*[+]	0.06	0.006
		[2] -0.14[+]	0.07	0.23
	MT to ET	[1] -0.31*[+]	0.09	0.004

		[2] -0.28*+	0.10	0.02
	IT to ET	[1] -0.12+ [2] -0.15+	0.08 0.07	0.44 0.17
MS Weekends Actigraphy	MT to IT	[1] -0:59* [2] -1:10*	0:15 0:20	0.001 0.003
	MT to ET	[1] -1:13* [2] -1:23*	0:24 0:25	0.01 0.006
	IT to ET	[1] -0:14 [2] -0:12	0:21 0:18	1.00 1.00
MS Weekdays SA	MT to IT	[1] -0.16*+ [2] -0.15+	0.05 0.06	0.004 0.06
	MT to ET	[1] -0.28*+ [2] -0.27*+	0.08 0.08	.002 .003
	IT to ET	[1] -0.12+ [2] -0.11+	0.07 0.05	0.27 0.13
MS Weekends SA	MT to IT	[1] -0:32 [2] -0:26	0:13 0:17	0.07 0.42
	MT to ET	[1] -1:25* [2] -1:19*	0:21 0:20	0.001 0.001
	IT to ET	[1] -0:52* [2] -0:53*	0:19 0:14	0.03 0.002
MSF-SC SA	MT to IT	[1] -0.17*+ [2] -0:21	0.05 0:09	0.006 0.11
	MT to ET	[1] -0.34*+	0.08	0.001

		[2] -0:48*	0:11	0.001
	IT to ET	[1] -0.17[+] [2] -0:26*	0.07 0:08	0.06 0.008

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[+] = from ln-transformed variable, * = $p < 0.05$. Mean difference in hours (non-transformed variables) or decimal hours (ln-transformed variables) ± standard deviation.

1 = CSM classification, 2 = rMEQ-CA classification.

ET = evening type, IT = intermediate type, MS = midpoint of sleep, MSF-SC = midpoint of sleep on free days, MT = morning type. SA = self-assessed sleep onset and offset times.

Comparison of back-transformed variables

We did a comparative ANOVA with the non-transformed variables. When comparing the back-transformed values to the results with the non-transformed variables, the values only differed by minutes, which can be explained by small differences in rounding the numbers (Table 17). The high correspondence of these results led us to the conclusion that the ANOVA is robust enough to analyze our non-normally distributed variables. In the following section, the results of the ANOVA with non-transformed variables will be reported and compared.

Note: While the mean values of the variables separated by chronotype corresponded well to the values generated by the ANOVA of the non-transformed values, the back-transformed standard deviation (SD) seemed to have a systematical error. Back-transformed SD were systematically at 1:01, 1:02 or 1:03 hours, which corresponds neither to the SD of the non-transformed ANOVA nor to the SD in a general explorative analysis of mean sleep onset and offset times and MS. We believe that this happened due to the program failing to turn negative logarithmic values (log ($x < 1$)) into standard deviations (which are positive). Hand-corrected values were in the range of SD generated with the non-transformed ANOVA.

Table 17. Comparison of mean sleep onset- and offset times and midpoints of sleep

	ET	IT	MT
Sleep offset time Weekdays			
Original variable	6:42	6:44	6:30
Log-transformed result	1.90	1.91	1.87
Back-transformation	6:42	6:43	6:30
Sleep onset time Weekends rMEQ-CA			
Original variable	23:33	23:17	22:06
Log-transformed result	3.16	3.15	3.10
Back-transformation	23:33	23:16	22:05
MS Weekdays Actigraphy CSM			
Original variable	02:49	02:32	02:05
Log-transformed result	1.03	0.91	0.72
Back-transformation	02:48	02:29	02:03
MS Weekdays Actigraphy rMEQ-CA			
Original variable	02:48	02:26	02:07
Log-transformed result	1.02	0.88	0.74
Back-transformation	2:46	2:24	2:05
MS Weekdays SA CSM			
Original variable	02:34	02:18	01:57
Log-transformed result	0.94	0.83	0.66
Back-transformation	2:33	2:16	1:56
MS Weekdays SA rMEQ-CA			
Original variable	02:30	02:15	01:55
Log-transformed result	0.91	0.80	0.65
Back-transformation	2:29	2:13	1:54

MSF-SC SA CSM			
Original variable	02:56	02:30	02:06
Log-transformed result	1.07	0.90	0.73
Back-transformation	2:55	2:27	2:04

CSM = Composite Scale of Morningness, ET = Evening type, IT = Intermediate type, MT = Morning type, MS = midpoint of sleep, MSF-SC = midpoint of sleep on free days, rMEQ-CA = Reduced Morningness-Eveningness Questionnaire for Children and Adolescents, SA= self-assessed sleep onset and offset times.

ANOVA with original variables (ANOVAs 3 and 4)

Homogeneity of the variables was given in all cases except the MS on weekdays of the self-assessed sleep onset and offset times of the CSM classification (Table 18).

Table 18. Levene's test of ANOVAs 3 and 4

	Levene statistic	df1, df2	p-value
Sleep onset time Weekdays Actigraphy	[1] 1.326 [2] 0.833	2.46 2.46	0.28 0.44
Sleep offset time Weekdays Actigraphy	[1] 1.466 [2] 0.350	2.46 2.46	0.24 0.71
Sleep onset time Weekends Actigraphy	[1] 2.25 [2] 2.90	2.45 2.45	0.12 0.07
Sleep offset time Weekends Actigraphy	[1] 1.42 [2] 0.34	2.45 2.45	0.25 0.71
MS Weekdays Actigraphy	[1] 0.79 [2] 0.48	2.46 2.46	0.46 0.62
MS Weekends Actigraphy	[1] 0.86 [2] 0.29	2.45 2.45	0.43 0.75
MS Weekdays SA	[1] 3.64 [2] 1.53	2.47 2.47	0.03 0.23

MS Weekends SA	[1] 0.78 [2] 2.22	2.48 2.48	0.47 0.12
MSF-SC SA	[1] 2.63 [2] 1.97	2.47 2.47	0.08 0.15

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Interpreted based on the median, p-value < 0.05.

1 = CSM-classification ANOVAs, 2 = rMEQ-CA-classification ANOVAs.

Hrs = hours, MS = midpoint of sleep, MSF-SC = midpoint of sleep on free days, SA = self-assessed sleep onset and offset times.

Similar to the ANOVAs using ln-transformed variables, the midpoints of sleep and all sleep onset and offset times except the sleep offset time on weekdays were significantly different in both chronotype classifications (Tables 19 and 20). All effects were weak ($0.1 < p\eta^2 < 0.3$). The comparative Welch-analysis of the non-homogeneously distributed MS weekday SA variable was also significant ($F(2,19.116) = 24.905$, $p < 0.01$).

Table 19. Non-transformed ANOVAs 3: Influence of chronotype on sleep onset- and offset times

	Class.	MT	IT	ET	Anova F	P-value	(Partial) η^2
Sleep onset time Weekdays	CSM	21:39 ± 0:34 [c]	22:19 ± 0:50 [b]	22:56 ± 0:33 [g]	5.84	0.006	0.20
	rMEQ-CA	21:46 ± 0:36 [e]	22:11 ± 0:50 [a]	22:51 ± 0:38 [e]	3.39	0.04	0.13
Sleep offset time Weekdays	CSM	6:30 ± 0:14 [c]	6:44 ± 0:23 [b]	6:42 ± 0:29 [g]	1.73	0.19	0.07
	rMEQ-CA	6:28 ± 0:17 [e]	6:42 ± 0:22 [a]	6:45 ± 0:25 [e]	1.30	0.28	0.05
Sleep onset time Weekends	CSM	22:20 ± 0:36 [d]	23:24 ± 1:04 [b]	23:34 ± 0:19 [g]	5.75	0.006	0.20
	rMEQ-CA	22:06 ± 0:29 [f]	23:17 ± 1:05 [a]	23:33 ± 0:18 [e]	4.52	0.02	0.17
	CSM	7:52 ± 1:01 [d]	8:47 ± 1:03 [b]	9:05 ± 0:27 [g]	3.98	.03	.15

Sleep offset time Weekends	rMEQ-CA	7:33 ± 1:15 [f]	8:43 ± 0:59 [a]	8:53 ± 0:56 [e]	3.72	0.03	0.14

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a N=35, b N=32, c N=12, d N=11, e N=7, f N=6, g N=5, * = $p < 0.05$. Time in hours ± standard deviation.

CSM = Composite Scale of Morningness, ET = Evening type, IT = Intermediate type, MT = Morning type, rMEQ-CA= Reduced Morningness-Eveningness Questionnaire for Children and Adolescents, SA = self-assessed sleep onset and offset times.

Table 20. Non-transformed ANOVAs 4: Influence of chronotype on midpoints of sleep

	Class.	MT	IT	ET	Anova F	P-value	(Partial) η^2
MS Weekdays Actigraphy	CSM	2:05 ± 0:19 [e]	2:32 ± 0:29 [d]	2:49 ± 0:22 [j]	6.46	0.003	0.22
	rMEQ	2:07 ± 0:19 [h]	2:26 ± 0:29 [b]	2:48 ± 0:24 [h]	3.85	0.03	0.14
MS Weekends Actigraphy	CSM	03:06 ± 0:42 [f]	04:05 ± 0:47 [d]	04:20 ± 0:20 [j]	8.23	0.001	0.27
	rMEQ	02:49 ± 0:48 [i]	04:00 ± 0:46 [b]	04:13 ± 0:35 [h]	6.94	0.002	0.24
MS Weekdays SA	CSM	1:57 ± 0:14 [f]	2:18 ± 0:21 [c]	2:34 ± 0:06 [j]	7.56	0.001	0.24
	rMEQ	1:55 ± 0:11 [i]	2:15 ± 0:20 [b]	2:30 ± 0:20 [g]	5.74	0.006	0.20
MS Weekends SA	CSM	02:27 ± 0:25 [f]	03:00 ± 0:41 [b]	03:53 ± 0:53 [j]	7.91	0.001	0.25
	rMEQ	02:26 ± 0:22 [i]	02:52 ± 0:34 [a]	03:45 ± 1:01 [g]	8.80	0.001	0.27
MSF-SC SA	CSM	2:06 ± 0:14 [f]	2:30 ± 0:24 [c]	2:56 ± 0:18 [j]	9.67	0.001	0.29
	rMEQ	02:04 ± 0:08 [i]	02:25 ± 0:22 [b]	02:52 ± 0:28 [g]	8.82	0.001	0.27

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a N=36, b N=35, c N=34, d N=32, e N=12, f N=11, g N=9, h N=7, i N=6, j N=5

Time in hours ± standard deviation. Class. = classification, ET = evening type, IT = intermediate type, MS = midpoint of sleep, MSF-SC = midpoint of sleep on free days, MT = morning type, SA = self-assessed sleep onset and offset times.

The post-hoc analyses of the non-transformed ANOVA mirrored the significant results of the preceding post-hoc tests of the ln-transformed ANOVA. The ANOVA was robust enough to show the same results even if the requirements of normal distribution were not given in every case.

Comparing the post-hoc tests of the two chronotype classifications, the CSM classification found significant differences between MT and IT groups regarding six sleep variables, while only three of those were significant in the rMEQ-CA analysis. Differences between IT and ET groups were significant only in the self-assessed weekend MS in the CSM analysis. IT and ET groups showed significant differences in the self-assessed weekend MS, as well as the MSF-SC, in the rMEQ post-hoc tests (Table 21).

Table 21. Bonferroni post hoc-tests of non-transformed ANOVAs 3 and 4

	Groups	Mean difference	Standard deviation	p-value
Weekend sleep onset time	MT versus IT	[1] -0:40*	0:15	0.04
		[2] -0:25	0:19	0.59
	MT versus ET	[1] -1:16*	0:24	0.008
		[2] -1:05*	0:25	0.04
	IT versus ET	[1] -0:36	0:21	0.30
		[2] -0:39	0:19	0.15
Weekend sleep onset time	MT versus IT	[1] -1:04*	0:19	0.007
		[2] -1:11*	0:25	0.02

	MT versus ET	[1] -1:14 [2] -1:27*	0:30 0:32	0.06 0.03
	IT versus ET	[1] -0:09 [2] -0:16	0:27 0:23	1.00 1.00
Weekend sleep offset time	MT versus IT	[1] -0:54* [2]-1:10*	0:21 0:26	0.04 0.04
	MT versus ET	[1] -1:13 [2]-1:19	0:32 0:33	0.09 0.07
	IT versus ET	[1] -0:18 [2] -0:09	0:29 0:25	1.00 1.00
MS Weekdays Actigraphy	MT versus IT	[1] -0:26* [2] -0:21	0:08 0:11	0.01 0.29
	MT versus ET	[1] -0:44* [2] -0:41*	0:14 0:14	0.009 0.02
	IT versus ET	[1] -0:17 [2] -0:21	0:12 0:11	0.52 0.20
MS Weekends Actigraphy	MT versus IT	[1] -0:59* [2] -1:10*	0:15 0:20	0.001 0.003
	MT versus ET	[1] -1:13* [2] -1:23*	0:24 0:25	0.01 0.006
	IT versus ET	[1] -0:14 [2] -0:12	0:21 0:18	1.00 1.00
MS Weekdays SA	MT versus IT	[1] -0:21* [2] -0:20	0:06 0:08	0.008 0.08

	MT versus ET	[1] -0:36* [2] -0:35*	0:10 0:10	0.003 0.004
	IT versus ET	[1] -0:15 [2] -0:15	0:09 0:07	0.33 0.13
MS Weekends SA	MT versus IT	[1] -0:32 [2] -0:26	0:13 0:17	0.07 0.42
	MT versus ET	[1] -1:25* [2] -1:19*	0:21 0:20	0.001 0.001
	IT versus ET	[1] -0:52* [2] -0:53*	0:19 0:14	0.03 0.002
MSF-SC SA	MT versus IT	[1] -0:24* [2] -0:21	0:07 0:09	0.009 0.11
	MT versus ET	[1] -0:50* [2] -0:48*	0:11 0:11	0.001 0.001
	IT versus ET	[1] -0:26 [2] -0:26*	0:10 0:08	0.05 0.008

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Time in hours ± standard deviation. * $p < 0.05$.

1 = CSM classification, 2 = rMEQ-CA classification.

ET = evening type, IT = intermediate type, MS = midpoint of sleep, MSF-SC = midpoint of sleep on free days, MT = morning type, SA = self-assessed sleep onset and offset times.

3.6 Effect of age and sex in multivariate analyses

Influence on questionnaires

In a multivariate analysis (MANOVA) of the influence of age and sex on the questionnaires, we found that age, sex and the interaction between age and sex, had no significant effect (Table 22).

Levene's test for homogeneity of the dependent variables was non-significant in all cases. Two groups were not normally distributed (PDSS female group, CSM 16-year-old group).

Table 22. MANOVA of the influence of age, sex and the interaction of age and sex on questionnaires

	Wilk's Lambda	F (hypothesis df, error df)	p-value	pη²
Constant term	0.01	1320.12 (6,39)	0.001*	0.10
Sex	0.83	1.38 (6,39)	0.25	0.18
Age	0.65	1.02 (18,110.79)	0.45	0.13
Age – Sex interaction	0.62	1.14 (18,110.79)	0.32	0.15

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Wilk's lambda, F-values, * p-value < 0.05, pη² = partial η².

Influence on midpoints of sleep

The MANOVA of the influence of age and sex on the midpoints of sleep showed no significant influence of sex and the interaction of age and sex (Table 23). Age had a weak effect (F (12,100.83) = 3.20, p < 0.01, pη² = 0.25, Wilk's Λ = 0.43).

Table 23. MANOVA of the influence of age, sex and the interaction of age and sex on midpoints of sleep.

	Wilk's Lambda	F (hypothesis df, error df)	p-value	pη²
Constant term	0.00	70622.96 (4,38)	.001*	1.00
Sex	0.89	1.21 (4,38)	.33	0.11
Age	0.43	3.20 (12,100.83)	.001*	0.25
Age – Sex Interaction	0.62	1.68 (12,100.83)	.08	0.15

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Wilk's lambda, F-values, * p-value < 0.05, pη² = partial Eta-squared

In the subsequent univariate analysis, age had a moderate effect on the weekday MS of actigraphy (F= 12.31, p < 0.01, pη² = 0.47), the SA weekday MS (F= 5.86, p < 0.01, pη² = 0.30) and the MSF-SC (F= 6.66, p < 0.01, pη² = 0.33), and a weak effect on the SA weekend MS (F= 4.00, p < 0.05, pη² = 0.23) (Table 24).

Table 24. Age effects on midpoints of sleep

	F	p-value	pη²
MS Weekdays Actigraphy	12.31	0.001*	0.47
MS Weekends Actigraphy	2.03	0.12	0.13
MS Weekdays SA	5.86	0.002*	0.30
MS Weekends SA	4.00	0.01*	0.23
MSF-SC SA	6.66	0.001*	0.33

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F-values, * $p < 0.05$, partial η^2.

MS = midpoint of sleep, MSF-SC = midpoint of sleep on free days, SA = self-assessed sleep onset and offset times.

16-year-olds had a significantly later actigraphic MS on weekdays compared to every other age group. 13-year-olds had a significantly earlier SA weekday MS as well as SA MSF-SC than 14- and 16-year-olds. The SA MS on weekends only differed significantly between the 13- and the 16-year-olds (Table 25).

Table 25. Bonferroni post-hoc test, influence of age on midpoints of sleep

	(I)Age	(J)Age	Mean Difference(I-J)	Standard error	p-value
MS Weekdays Actigraphy	13	14	-0:23	0:10	0.16
		15	-0:14	0:07	0.45
		16	-0:57*	0:09	<0.001
	14	13	0:23	0:10	0.16
		15	0:09	0:10	1.00
		16	-0:33*	0:11	0.04
	15	13	0:14	0:07	0.45
		14	-0:09	0:10	1.00
		16	-0:43*	0:09	<0.001
	16	13	0:57*	0:09	<0.001
		14	0:33*	0:11	0.04
		15	0:43*	0:09	0.00
MS Weekdays SA	13	14	-0:24*	0:08	0.03
		15	-0:15	0:06	0.10

		16	-0:33*	0:07	0.001
	14	13	0:24*	0:08	0.03
		15	0:08	0:07	1.00
		16	-0:08	0:09	1.00
	15	13	0:15	0:06	0.10
		14	-0:08	0:07	1.00
		16	-0:17	0:07	0.16
	16	13	0:33*	0:07	0.001
		14	0:08	0:09	1.00
		15	0:17	0:07	0.16
MS Weekends SA	13	14	-0:44	0:17	0.09
		15	-0:22	0:13	0.57
		16	-0:52*	0:16	0.02
	14	13	0:44	0:17	0.09
		15	0:22	0:16	1.00
		16	-0:07	0:19	1.00
	15	13	0:22	0:13	0.57
		14	-0:22	0:16	1.00
		16	-0:29	0:15	0.43
	16	13	0:52*	0:16	0.02
		14	0:07	0:19	1.00
		15	0:29	0:15	0.43
MSF-SC SA	13	14	-0:30*	0:09	0.02
		15	-0:17	0:07	0.10
		16	-0:38*	0:08	0.001
	14	13	0:30*	0:09	0.02
		15	0:12	0:09	1.00
		16	-0:08	0:10	1.00
	15	13	0:17	0:07	0.10
		14	-0:12	0:09	1.00
		16	-0:20	0:08	0.12
	16	13	0:38*	0:08	0.001
		14	0:08	0:10	1.00

	15	0:20	0:08	0.12

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* $p < 0.05$. Mean difference in hours ± standard error.

MS = midpoint of sleep, MSF-SC = midpoint of sleep on free days, SA = self-assessed sleep onset and offset times.

3.7 Summary of results

We examined the validity of three chronotype questionnaires by comparing them against actigraphy data from 55 healthy adolescents. Overall, we found good internal consistency as well as good convergent validity.

Chronotype classification differed by type of questionnaire. In both classifications, most participants were classified as IT. No participant showed excessive daytime sleepiness in the PDSS questionnaire. All questionnaires showed a high level of reliability.

MESSi MA subscale, CSM and rMEQ-CA scores correlated strongly with each other and showed weak to moderate negative correlations with the MESSi EV. Participants who scored higher on the MA subscale and in the CSM and rMEQ-CA had lower PDSS scores, while EV subscale and PDSS scores were positively correlated. The DI subscale only correlated moderately with higher PDSS and weakly with lower MA subscale scores. Higher MESSi MA subscale, CSM and rMEQ-CA scores correlated moderately with earlier overall sleep onset times and earlier sleep offset times on weekends. Correlations of higher MESSi EV and PDSS scores with later sleep onset and offset times on weekends were weak to moderate. There was no significant correlation with sleep offset times on weekdays. Higher MESSi MA, rMEQ-CA and CSM scores were moderately to strongly linked to earlier midpoints of sleep, while higher MESSi EV and PDSS scores weakly to moderately corresponded to later midpoints of sleep. The DI subscale did not correlate significantly to the midpoints of sleep.

In correlation analyses with the activity parameters, the cosinor variable Acrophase showed more significant correlations to the questionnaires and sleep-wake parameters than the non-parametric M10- and L5-midpoints. The M10-

midpoint variables did not correlate significantly with the questionnaires or sleep-wake parameters.

Both chronotype classifications had a significant influence on the sleep onset and offset times and midpoints of sleep, except the sleep offset time on weekdays, in the ANOVA. Post-hoc analyses showed that MT had significantly earlier sleep onset times during the week than ET. Midpoints of sleep were also significantly earlier for MT than for ET. The number of significant differences between chronotype groups in post-hoc tests differed by chronotype classification. In the MANOVA analysis, age had a weak to moderate influence on all midpoints of sleep except the weekend MS of actigraphy. Older adolescents (16 years versus 13 years) had a later actigraphic MS on weekdays, as well as a later self-assessed MS and MSF-SC. Sex and the interaction between age and sex had no significant influence on questionnaire scores or the midpoints of sleep. We were able to determine a DLMO time point in 50 % of our subsample. Correlation analyses did not yield any significant results.

4 Discussion

Questionnaires are an efficient way to gather information on circadian preference in large samples. Although well established, few studies have provided objective validation data for the use of the rMEQ-CA, the CSM and the MESSi in adolescents.

Our study objective was to validate these three chronotype questionnaires against actigraphy and the DLMO.

Therefore, we examined correlations between the questionnaires, sleep-wake parameters and chronotype indices, such as the midpoints of sleep and the melatonin onset. In addition, we compared participants' rest-activity patterns and sleep-wake parameters with the questionnaire scores. We also evaluated the influence of age and sex on the questionnaire scores and MS.

This is, to our knowledge, the first comprehensive study to validate a set of chronotype questionnaires against sleep parameters determined via actigraphy.

Chronotype

The CSM and the rMEQ-CA showed significant differences in classifying the participants into the respective chronotype groups. The rMEQ-CA-classification classified a higher proportion of participants as ET, while the CSM classified more participants as MT. This could be due to the questionnaires' two different scoring approaches. The rMEQ-CA classifies by fixed cut-off points, while the CSM uses percentiles as relative cut-off scores.

In an adult study, the rMEQ also identified less participants as MT and more as ET as compared to the CSM (Randler, 2013). The two scoring approaches don't agree very well. It is debatable which type of scoring is a better reflection of the participants' circadian preference or if a fixed classification into different chronotype groups should be used at all. This point was already highlighted by other researchers (Caci et al., 2009).

Reliability

Overall, Cronbach's alpha showed a good internal consistency of the questionnaires. Alpha values regarding the MESSi subscales were very similar (MA 0.89, EV 0.82 and the DI 0.71) in a previous study (Weidenauer et al., 2019). Alpha values for the rMEQ were higher than previously stated values in an adolescent sample (0.54; Urbán et al., 2011) and closer to the 0.72 stated for an adult sample (Randler, 2013). In our sample, the CSM showed an alpha higher than 0.9, which can indicate a redundancy of the items in the questionnaire (Streiner, 2003). The CSM has already been criticized for this reason (Randler et al., 2016a). A 2015 review, however, found a comparatively lower alpha range of 0.61–0.86 for the CSM in literature (Tonetti et al., 2015a).

Correlations of the questionnaires

The results of our correlation analyses confirmed the good convergent validity of the chronotype questionnaires. In a previous validation study of the MESSi in adolescents (Weidenauer et al., 2019), correlations between the CSM and the MESSi MA and the MESSi EV subscale were at 0.88 and -0.57 (N = 46), respectively, and therefore in a comparable range. The same study showed

correlations between the PDSS and the MA and EV subscale at -0.72 and 0.37 (N = 118), respectively.

A higher PDSS score correlated with higher EV and lower rMEQ, CSM and MA scores in our study. This indicates that evening-oriented participants in our sample experienced more daytime sleepiness. In a US-American study on high school students, eveningness (lower chronotype questionnaire score) and sleepiness were moderately associated (-0.44; Owens et al., 2016). Of note, chronotype and sleepiness were measured using different questionnaires as in our study.

In our correlation analysis of the MESSi subscales, the DI subscale correlated moderately with the MA subscale and did not correlate with the EV subscale. This fits the hypothesis that morning-oriented adolescents show lower amplitude in mood and performance and, therefore, have a higher stability (Weidenauer et al., 2019). We did not find a positive correlation between DI and EV, as seen in the previous validation study.

Comparative data for correlations between the rMEQ and the MESSI subscales could only be found in the adult age range. In this validation study (Faßl et al., 2019), correlations with both the MA (0.91) and the EV subscale (-0.87) were stronger than in our analyses.

Similar correlations between the rMEQ and the CSM have been reported in German (0.89; Randler, 2013) and French university students (0.90; Caci et al., 2009).

Correlations with sleep-wake parameters

The questionnaire scores correlated well with sleep onset and offset times, except for sleep offset times on weekdays. This was not surprising, as for most participants, sleep offset times on weekdays followed a fixed school schedule and not personal preference. Otherwise, a later sleep timing correlated with lower rMEQ-CA, CSM and MESSi MA scores. This shows that higher eveningness was associated with a later sleep-wake timing. Sleep onset and offset times were earlier in morning-oriented participants. The MESSi EV score correlated with

sleep onset and offset times on weekends, but, to our surprise, not with sleep onset times on weekdays.

A study in German adolescents, which compared self-reported sleep onset and offset times to the CSM, also found that sleep offset timing on weekdays did not significantly correlate with the questionnaire score. Pearson correlations of the other self-reported sleep onset- and offset times yielded similar results (-0.40 sleep onset on weekdays, -0.45 sleep onset on weekends and -0.56 sleep offset on weekends; N = 491; Randler, 2009). In a comprehensive validation study of seven chronotype questionnaires including the MEQ, rMEQ, and CSM against actigraphy in university students, Pearson correlations between sleep onset and offset times and the CSM were also significant (-0.55 and -0.57, respectively; N = 166; Thun et al., 2012). Unfortunately, this study did not differentiate between weekday and weekend sleep onset and offset times. University students often follow a less fixed schedule on weekdays, in contrast to school children, where differentiating between work and non-work days could be more important.

Later actigraphically measured midpoints of sleep correlated, as expected, with lower rMEQ-CA, CSM and MESSi MA scores. Surprisingly, the EV subscale did not correlate with sleep onset time on weekdays. However, it did show the expected correlation with MS on weekdays. This correlation was very weak (0.29). Our study had a rather small ET group and we might have been underpowered in this regard. A higher score on the EV score should reflect a stronger tendency towards eveningness. Our participants' sleep timing on school days might have been influenced by their parents' and not just their personal preference.

Midpoints of sleep calculated from self-reported sleep onset and offset times correlated moderately to strongly with the midpoints of sleep calculated from objective data (actigraphy). This shows that the adolescents in our study were able to accurately estimate and report their sleep onset- and offset times when filling out the questionnaire.

Correlations with activity parameters

We compared the chronotype questionnaires to rest-activity patterns derived from two different approaches, both the cosinor and the non-parametric approach. As chronotypes have different timings of peak performance (earlier in MT, later in ET) (Adan et al., 2012), we hypothesized that earlier peak activity would correlate with higher morningness (indicated by higher MA, rMEQ-CA and CSM and lower EV scores). Similarly, we expected evening-oriented adolescents to have later peak activity (Acrophase, M10 midpoint), as well as a later lowest activity (L5-midpoint).

Rest-activity patterns' descriptive results were of similar range as reported before in a normative study in children (N = 58, age 11.6 ± 3.8) (Mitchell et al., 2017).

An earlier full-week Acrophase correlated moderately with higher MESSi MA, rMEQ-CA and CSM scores and with less daytime sleepiness (PDSS score). M10- and L5-midpoint did not correlate with the questionnaire scores. This is in line with the validation study of the MESSi in a university student sample (N = 97) (Faßl et al., 2019), in which M10 and L5 midpoint also had no significant correlations with the MESSi subscales. In the cited study, however, correlations of the full-week Acrophase with the MA and EV subscales were high (-0.71 and 0.69, respectively), and of similar strength when separated into a weekday and weekend variable. This is in contrast to our results, where correlations of the full-week variable and the MA subscale were only moderate (-0.31) and where correlations with the EV subscale were not significant. Furthermore, the weekday and weekend variables did not show significant correlations with the subscales at all. These differences might be explained by the larger study sample in the university student population.

Overall, the cosinor variable correlated better with our questionnaires and sleep-wake variables than the non-parametric variables indicating that in our study, the cosinor approach was the better fit. The cosinor model should work best in subjects with a circadian rhythm resembling a cosinor curve (Cornelissen, 2014).

<u>Correlations with DLMO</u>

The correlations of the DLMO with questionnaires, sleep-wake and activity parameters did not yield any significant results. A previous study found significant correlations between the DLMO and the MEQ (-0.70), as well as with the MSF-SC derived from another chronotype questionnaire (0.68) (Kantermann et al., 2015). In a study in adults (N = 13), moderate to strong correlations of the DLMO with M10- and L5-midpoints were reported (Bonmati-Carrion et al., 2015). We had a very limited sample size for the correlation analyses and were probably underpowered.

The average DLMO in our sample was at 20:48 ± 0:12. This is close to the averages stated in previous adolescent studies, which are displayed in a timeline (Figure 9). In the study of Crowley et al., DLMOs were stated for a summer (vacation) group and a school group (age 13-16, summer group N=29 and school group N=54) (Crowley et al., 2006). The mean DLMO of both groups is shown in the timeline. However, as our study took place during the school year, our DLMO is more comparable to the school group's average DLMO (20:33 ± 0:48).

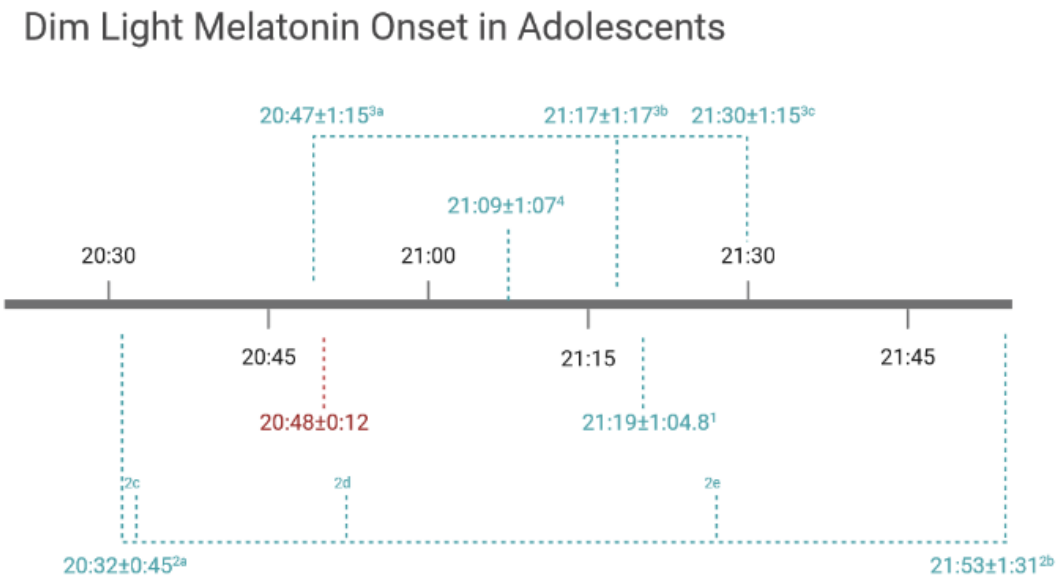

Figure 9. Literature values for dim light melatonin onsets in adolescents

(created with www.biorender.com).

1 (Dolsen and Harvey, 2018), 10-18 yo.

2 (Crowley et al., 2014), ranging from 2a-2b in 11-18 yo. 2c 20:34±0:57 (15 yo), 2d 20:50 ±0:59 (16 yo), 2e 21:27±0:49 (13 yo)
3 (Crowley et al., 2016), 14-17 yo, 3a 2D threshold, 3b 3 pg/ml threshold, 3c 4 pg/ml threshold
4 (Crowley et al., 2006), 9-17 yo.
Red: Mean DLMO in our participants
yo = year-olds

Univariate analyses

The influence of chronotype classification on sleep onset and offset times and midpoints of sleep was significant in every case except for sleep offset time on weekdays. This showed again that wake time on weekdays rather reflected social obligations than personal circadian timing in our participants.

In the post-hoc analyses, the MT group had significantly earlier midpoints of sleep and weekday sleep onset times than the ET group in both chronotype classifications, as well as an earlier weekend sleep onset time in the rMEQ-classification.

In a study on Finnish adolescents (N=183), the MT group also had significantly earlier midpoints of sleep than the ET group (Merikanto et al., 2017). Thun et al. observed that the MT group had significantly earlier sleep onset and offset times than the ET group in pairwise t-tests, but did not differentiate, as described above, between weekdays and weekends (Thun et al., 2012).

Multivariate analysis

Evening preference and a delayed sleep timing seemed to be more prevalent in boys (*i.e.,*Adan and Natale, 2002, Randler, 2011), as summarized in a comprehensive review from 2012 (Adan et al., 2012). Several studies also found a shift towards eveningness during early adolescence (Roenneberg et al., 2004, Roenneberg et al., 2007, Randler et al., 2017). For these reasons, we examined the effect of age and sex on the questionnaires and the midpoints of sleep.

In our multivariate analyses, age had a weak to moderate effect on the midpoints of sleep (except for the actigraphic weekend MS), but not on the questionnaires.

Sex did not have an effect. In post-hoc tests, the youngest age group (13 years) had a significantly earlier MS than the oldest age group (16 years) in all four examined midpoints of sleep. 16-year-olds had a later MS than every other age group on weekdays.

General strengths and limitations

A strength of our study cohort was that it had an almost even sex distribution. We were able to obtain data on each participant, since we had no drop outs. A limitation of our study were the relatively small sample sizes (N=55 and N=24 [DLMO subgroup]). Also, self-selection bias might have played a role, as interested adolescents had to contact our group in order to participate in the study.

Actigraphy

We used an objective method to measure activity and sleep-wake rhythms (actigraphy). A potential weakness of actigraphy is that it is not able to differentiate between real sleep and inactive phases (Sadeh, 2011). For this reason, we used the sleep diary to countercheck the information.

Our study might have been limited by the short actigraphy recording duration. Seven recording days are the common duration in actigraphy studies (Thun et al., 2012, Quante et al., 2019, Tonetti, 2007). The seven recording days could have been indicative of habitual sleep-wake patterns in our participants. They could, however, also have been externally influenced by sickness or unusual obligations. An example for this is that we had to discard one weekend night in 13 participants because their sleep onset timing was influenced by the participation in our movie night. We tried to pay attention to potentially conflicting factors by including the question “Was today a normal day?” in the sleep diary. However, a longer study duration might have reflected habitual sleep onset- and offset times more accurately. In a review on actigraphy measurements, seven days were stated as the minimum duration of data collection. The authors recommended a two-week measurement if differences between weekdays and weekends are of interest (Meltzer et al., 2012).

Dim Light Melatonin Onset

Sample collection procedures took place in a rather controlled setting, as all participants were seated in a dimly lit room. Our study team was present throughout the whole collection period and thus able to control for possible confounders of evening melatonin secretion, such as light exposure, posture and consumption of certain foods and beverages during collection (Kennaway, 2020).

Our sample collection possibly started too late for the early sleep onset time of some adolescents. We were not able to determine a baseline melatonin concentration in our participants, as we didn't have enough values to calculate a baseline average before melatonin concentration started to increase. In 8 participants, first values were already above the threshold. According to the literature, the melatonin onset takes place approximately two or three hours before habitual bedtime (Benloucif et al., 2008). An exploratory data analysis showed that early sleepers in our subsample went to bed around 10:30 p.m. This would have resulted in an expected melatonin onset at (approximately) 7:30 to 8:30 pm for adolescents who are habitual early sleepers. A better starting time for sample collection would have been 5 pm or earlier. It would have provided us with a baseline value of melatonin levels and would have ensured that we didn't miss the early rise in melatonin concentration in some participants. Other DLMO studies in adolescents started sample collection at 5 hours (Crowley et al., 2006, Crowley et al., 2016) or 5.5 hours (Dolsen and Harvey, 2018) before habitual bedtime. We were not able to compare different methods of calculating the DLMO, as the relative methods require a baseline value.

In our first internal laboratory analyses, concentration levels were implausibly high in some subjects. Nighttime melatonin concentration is highly variable in individuals and concentration in saliva can range from low (around 20 pg/ml) to very high concentration levels around 200 pg/ml (Middleton, 2013). This shows that the possible range for melatonin concentration is very broad. However, we obtained results > 300 pg/ml. Assuming that melatonin concentration in saliva is approximately 30 % of plasma melatonin levels (Middleton, 2013), this would imply a plasma melatonin concentration of 1000 pg/ml. This seemed implausibly

high. There are several reasons for too high results. Contamination of the saliva sample through traces of food or beverages in the mouth can lead to cross-reactivities. This is also observable after the consumption of certain foods or medication in the days prior to sample collection (Kennaway, 2020). Participants were instructed not to consume certain products for a defined period of time in advance, but breaches in compliance to these instructions are possible. The additional results from an external laboratory (IBL, Hamburg, Germany) were in a more plausible range, while the CVs in our own analyses were too high. It was, therefore, more convincing that our results were due to either human technical error or equipment failure (*i.e.,* pipette calibration, plate washer or photometer programming errors).

The final analysis which yielded the results examined in this study took place externally (Bayer laboratory in Leinfelden-Echterdingen, Germany). Due to communication errors, samples were measured in duplicate but not on the same plate. This complicated using the average of these double determinations in subsequent calculations. This and other factors, such as the small sample size of calculatable DLMOs, mean that results obtained from our statistical analysis using the DLMOs should be regarded with caution.

Conclusion and Outlook

Overall, we were able to successfully validate three chronotype questionnaires against an objective method of sleep assessment, namely actigraphy, in a group of healthy adolescents. We could show that stronger evening preference corresponded to later sleep-wake timing and midpoints of sleep, while morning preference was associated with earlier sleep-wake rhythms. To our knowledge, this was the first comprehensive study that validated multiple questionnaires against actigraphy in this age group. Actigraphy was a suitable tool for different chronotype dimensions of the questionnaires (rMEQ-CA, CSM, MA and EV subscale). We did not, however, use the right tools to validate the DI subscale of the MESSi. The DI subscale is an indicator of the (in)stability of mood and performance during the day. Therefore, this subscale is independent of the dimensions measured in the MA and EV subscales (Randler et al., 2016a). This

is why, in contrast to the other two subscales, the DI dimension cannot be validated by being correlated with sleep-wake patterns. Morning-oriented people possibly show higher stability (Weidenauer et al., 2019). The DI subscale should be examined with more adequate tools in future adolescent studies, for example by testing its convergent validity against the distinctness/amplitude dimensions in other chronotype questionnaires.

Overall, the questionnaires showed good internal consistency and good convergent validity.

We were not able to validate the questionnaires against the DLMO and discussed several possible reasons why this has failed. It would have been interesting to further investigate how much the results of different means of DLMO estimation differ from each other and how this variation impacts accurate phase estimation in adolescents, where samples sizes are often rather small due to the required parental agreement. DLMO is considered the gold standard for measuring circadian rhythms (Reid, 2019). This makes improving the accuracy and comparability of the results an important objective for further research.

5 Summary (English and German)

5.1 Summary

Chronotype and diurnal preferences have consistently been shown to influence academic performance and many health-related issues and behaviors. A fundamental understanding of the impact of circadian timing is crucial for acting adequately on these findings. Large epidemiologic studies are required to obtain the necessary information. In order to conduct these studies, valid and reliable instruments are needed. Unfortunately, there are few known validation studies against objective measures in the adolescent age group even for widely used and well-known questionnaires such as the rMEQ-CA and the CSM. Our study was the first validation study of the MESSi in adolescents against an objective instrument.

At total of 55 healthy 13- to 16-year-olds completed the MESSi, rMEQ-CA and CSM and provided information about their sleep-wake rhythm through a 7-day actigraphy monitoring and a sleep diary. Participants also completed the pediatric daytime sleepiness scale (PDSS). We examined correlations between sleep-wake and activity parameters and the questionnaires and analyzed the influence of chronotype classification on sleep-wake parameters, age and sex using uni- and multivariate analyses. We measured the evening rise in melatonin concentration in 24 adolescents and examined correlations with the questionnaires.

The questionnaires had good internal consistency and convergent validity. Spearman correlations revealed earlier sleep onset and offset times and midpoints of sleep in more morning-oriented participants and later respective timings in participants with a stronger evening orientation. Due to technical problems, we were underpowered and could not examine correlations of the DLMO with the questionnaires. The cosinor activity parameter Acrophase showed stronger correlations with questionnaire scores and sleep onset and offset times than comparative non-parametric parameters. Chronotype classification differed significantly between questionnaires. Age and sex had no significant influence on questionnaire scores.

5.2 Zusammenfassung

In der aktuellen Literatur wurde bereits mehrfach ein Einfluss des Chronotyps und der Tagespräferenzen auf akademische Leistungen, gesundheitliche Probleme und gesundheitsspezifische Verhaltensweisen bei Jugendlichen nachgewiesen. Um auf diese Ergebnisse adäquat reagieren zu können, ist ein grundlegendes Verständnis des Einflusses des individuellen zirkadianen Timings essenziell. Es sind valide und reliable Messinstrumente erforderlich, um die nötigen Daten im Rahmen von epidemiologischen Studien erheben zu können. Leider sind selbst für sehr häufig verwendete Fragebögen wie den CSM und den rMEQ-CA nur wenige Validierungsstudien bei Jugendlichen bekannt. Die hier vorliegende Dissertation ist die erste Validierungsstudie des MESSi gegen andere objektive Messinstrumente im Jugendalter.

Insgesamt füllten 55 gesunde Jugendliche im Alter von 13 bis 16 Jahren den MESSi, den CSM und den rMEQ-CA aus. Eine siebentägige Aktigraphiemessung und das Ausfüllen eines Schlaftagebuchs gaben Aufschluss über den Schlaf-Wach-Rhythmus der Probanden. Ebenso füllten die Jugendlichen den PDSS, einen Tagesschläfrigkeits-Fragebogen, aus. Wir untersuchten die Korrelationen zwischen den Fragebögen und Schlaf-Wach- und Aktivitätsparametern und analysierten den Einfluss des Chronotyps auf Schlaf-Wach-Rhythmen, Alter und Geschlecht in uni- und multivariaten Analysen. Wir versuchten den abendlichen Anstieg der Melatoninkonzentration bei 24 Jugendlichen zu bestimmen, um dessen Korrelation mit den Fragebögen zu analysieren. Aufgrund technischer Probleme war diese Analyse leider underpowert.

Die Fragebögen zeigten eine gute interne Konsistenz und konvergente Validität. In den Spearman-Korrelationen zeigten sich frühere Schlaf- und Wachzeiten und Schlafmittelpunkte bei morgenorientierten Teilnehmern und jeweilig spätere Zeitpunkte bei abendorientierten Jugendlichen. Der Kosinor-Aktivitätsparameter Acrophase zeigte stärkere Korrelationen mit den Fragebögen und Schlaf-Wach-Parametern als die entsprechenden non-parametrischen Variablen. Die Chronotypklassifizierung unterschied sich zwischen den Fragenbögen. Alter und Geschlecht hatten keinen signifikanten Einfluss auf die erreichte Punktzahl in den Fragebögen.

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7 Declaration (Erklärung zum Eigenanteil)

Diese Arbeit wurde in der Abteilung für Neonatologie der Universitätskinderklinik Tübingen unter Betreuung von Frau PD Dr. Mirja Quante durchgeführt.

Die Konzeption der Studie erfolgte in Zusammenarbeit mit Frau PD Dr. Mirja Quante und Herrn Prof. Christoph Randler.

Die Studienplanung wurde bereits im Ethikantrag dargelegt, welchen ich mit der Unterstützung von Frau Quante selbst formuliert habe.

Die Probandenrekrutierung erfolgte eigenständig durch mich.

In der ersten Rekrutierungsrunde unterstützte mich Frau Dr. Corina Weidenauer beim Austeilen der Aktigraphen an zwei Tagen. Die Speichelprobensammlung am 16.03.2019 erfolgte gemeinsam mit Frau Quante, Herrn Randler und mit Hilfe von dessen Arbeitsgruppe. Die erste Verarbeitung der Proben erfolgte durch mich, nachdem ich durch Mara Thomas (PhD-Kandidatin von Frau Prof. Vanessa Nieratschker) eingearbeitet wurde. Die Vergleichsanalyse der Proben wurde auf unserer Seite durch mich, auf der Gegenseite durch das IBL-Labor in Hamburg durchgeführt. Die endgültige Probenanalyse wurde durch das Labor Dr. Bayer des SYNLAB Labors in Leinfelden-Echterdingen von Frau Silke Lehner durchgeführt.

Die Auswertung der Aktigraphie erfolgte nach Anleitung durch Frau Dr. Corina Weidenauer durch mich. Die Berechnung der Aktivitätsparameter in MATLAB führte ich nach Vorarbeit durch Herrn Michael Rueschman vom Brigham Women's Hospital in Boston, USA (Erstellung des Sleep-wake reports) eigenständig durch.

Die statistische Auswertung erfolgte nach Beratung durch Frau Dr. Annette Stauch vom Center for Pediatric Clinical Studies (CPCS) und nach Anleitung durch Herrn Randler und Frau Quante durch mich.

Ich versichere, das Manuskript selbständig (unter Anleitung und Korrektur durch Frau Quante und Herrn Prof. Christian Poets) verfasst zu haben und keine weiteren als die von mir angegebenen Quellen verwendet zu haben.

Tübingen, den

8 Publication

(Online version of record before inclusion in an issue)

Paciello, L. M., Quante, M. (equal contribution), Weidenauer, C., Rueschman, M., Nieratschker, V., Poets, C. F., & Randler, C. (2022). Validity of chronotype questionnaires in adolescents: Correlations with actigraphy. Journal of Sleep Research, 00, e13576. https://doi.org/10.1111/jsr.13576.

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10 Appendix:

10.1 Tables of non-significant results

Table S1. Spearman correlations of M10 midpoint with the questionnaires and sleep-wake parameters.

	M10 Whole Week hrs	M10 Weekdays hrs	M10 Weekends hrs
MESSi MA	-0.13[a]	-0.08 [a]	-0.02 [a]
MESSi EV	-0.04 [a]	-0.07 [a]	0.04 [a]
MESSi DI	0.09 [a]	-0.16 [a]	0.17 [a]
rMEQ-CA	-0.06 [d]	-0.01 [d]	-0.10 [d]
CSM	-0.14 [d]	-0.08 [d]	-0.10 [d]
PDSS	0.17 [a]	0.14 [a]	0.14 [a]
Sleep onset time Weekdays	0.20 [a]	0.19 [a]	0.04 [a]
Sleep offset time Weekdays	0.02 [a]	0.04 [a]	0.06 [a]
Sleep onset time Weekends	0.23 [b]	0.19 [b]	0.17 [b]
Sleep offset time Weekends	0.11 [b]	0.10 [b]	0.03 [b]
MS Weekdays Actigraphy	0.19 [a]	0.19 [a]	0.02 [a]
MS Weekends Actigraphy	0.24 [b]	0.21 [b]	0.18 [b]

MS Weekdays SA	0.16 [c]	0.13 [c]	0.04 [c]
MS Weekends SA	0.07 [b]	0.08 [b]	0.02 [b]
MSF-SC SA	0.17 [c]	0.14 [c]	0.01 [c]

a N = 52, b N = 51, c N = 50, d N = 49, * $p < 0.05$.

CSM=Composite Scale of Morningness, DI = Distinctness/Amplitude subscale, EV = Evening affect subscale, Hrs = hours, MA = Morrning affect subscale, MESSi=Morningness-Eveningness-Stability-Scale, MS = midpoint of sleep, MSF-SC = midpoint of sleep on free days, PDSS = Pediatric Daytime Sleepiness Scale, rMEQ-CA= Reduced Morningness-Eveningness Questionnaire for Children and Adolescents, SA = self-assessed sleep onset and offset times.

Table S2. Spearman correlations of the Dim Light Melatonin Onset with questionnaire scores and sleep-wake parameters

	DLMO
MESSi MA	-0.14
MESSi EV	0.15
MESSi DI	0.32
rMEQ-CA	-0.08
CSM	-0.23
PDSS	0.05
Sleep onset time Weekdays	-0.01
Sleep offset time Weekdays	0.21
Sleep onset time Weekends	-0.38
Sleep offset time Weekends	0.22

MSF-SC SA	-0.46

N = 12, * $p < 0.05$.

CSM=Composite Scale of Morningness, DI = Distinctness/Amplitude subscale, DLMO = Dim light melatonin onset, EV = Evening affect subscale, Hrs = hours, MA = Morrning affect subscale, MESSi=Morningness-Eveningness-Stability-Scale, MS = midpoint of sleep, MSF-SC = midpoint of sleep on free days, PDSS = Pediatric Daytime Sleepiness Scale, rMEQ-CA= Reduced Morningness-Eveningness Questionnaire for Children and Adolescents, SA = self-assessed sleep onset and offset times

10.2 Declarations of consent / Recruitment

Declarations of consent: Parents

Universitätsklinikum Tübingen
KLINIK FÜR KINDER- UND JUGENDMEDIZIN

Geschäftsleitung
Prof. Dr. med. R. Handgretinger
S. Rich

www.medizin.uni-tuebingen.de/kinderklinik

Titel der Studie: Validierung von Fragebögen zum Chronotyp im Jugendalter mittels Aktigraphie und Dim Light Melatonin onset-Test

Projektleitung
Dr. med. Mirja Quante
Pädiatrische Schlafmedizin
Abteilung Neonatologie
Klinik für Kinder- und Jugendmedizin
Calwer Str. 7, 72076 Tübingen
Tel.: +49 7071 29-81283; Fax: +49 7071 294356
E-Mail: mirja.quante@med.uni-tuebigen.de

Liebe Eltern,

Haben Sie Interesse, mit Ihrem Kind an einer Studie zur Validierung von Fragebögen zum Chronotyp (Eule oder Lerchentyp) teilzunehmen? Bitte nehmen Sie sich etwas Zeit und lesen Sie sich die nachfolgende Information genau durch, bevor Sie sich für oder gegen eine Studienteilnahme entscheiden. Bitte stellen Sie uns zuvor alle Fragen, die Sie zu der Studie haben.

Wie wird der Chronotyp bestimmt?
Die Chronobiologie befasst sich damit, die individuellen Unterschiede in den Rhythmen der Menschen zu untersuchen und deren Auswirkungen auf unseren Alltag zu ermitteln. Der Chronotyp wird für gewöhnlich mit einem Selbstbeurteilungs-Fragebogen bestimmt. Die Personen sollen sich selbst einschätzen und über ihr Schlafverhalten, sowie andere Tagesabläufe Auskunft geben.

Warum führen wir diese Studie durch?
Wir möchten wissen, wie gut vier aktuell im Jugendalter verwendete Fragebögen zu Bestimmung des Chronotyps im Vergleich zum „Dim light Melatonin onset-Test" (s.u.) und zur objektiven Überwachung von Aktiv- und Ruhephasen mittels Aktigraphie funktionieren. Schlaf-Wach-Zyklen werden u.a. durch das Schlafhormon Melatonin bestimmt, welches am Abend ausgeschüttet wird und im Speichel bestimmt werden kann (Dim light Melatonin onset-Test). Aktigraphen sind Bewegungsmesser, die ähnlich wie eine Armbanduhr für eine Woche am Handgelenk getragen werden. In der Auswertung ist es anschließend möglich, den Ruhe- und Aktivitäts-Rhythmus der untersuchten Person zu erkennen, da die geringere Häufigkeit von Bewegungen während des Schlafes im Vergleich zum Wachzustand Rückschlüsse auf Einschlafzeit und Schlafdauer zulassen.

Wie lange wird die Studie für Ihr Kind dauern? Was ist alles involviert?
An einem ersten Termin werden wir Ihrem Kind ein Aktigraphie-Gerät und ein Schlaftagebuch sowie vier Fragebögen zur Bestimmung des Chronotyps aushändigen. Das Aktigraphie-Gerät soll anschließend für 7 Tage kontinuierlich, außer beim Baden oder Duschen, getragen werden. Zeiten, zu denen das Gerät abgelegt wurde, werden im Schlaftagebuch dokumentiert. Zusätzlich werden wir das Alter, das Geschlecht, das Gewicht und die Länge Ihres Kindes erfragen. Am 16. März 2019 möchten wir dann die Ausschüttung des Schlafhormons Melatonin bestimmen. Hierzu laden wir alle Studienteilnehmer um 19.30 Uhr in den Raum 220 der Crona-Klinik ein. Dort werden wir dann während der Vorführung eines altersentsprechenden Filmes („Jumanji" oder „Forest Gump") von 20.00 Uhr bis 00.00 Uhr halbstündlich Speichelproben zur anschließenden Melatonin-Messung sammeln. Während der Filmvorführung werden die Kinder von Studienassistenten betreut. Für die

Abholung um 00.10 Uhr können Sammel-Taxis zur Verfügung gestellt werden. Zudem werden eventuelle Parkgebühren auf dem Klinikgelände werden erstattet. Der Gesamtaufwand der Studie beinhaltet das Tragen eines Aktigraphie-Gerätes für eine Woche, das Ausfüllen von vier Fragebögen (Ausfüllzeit ca. 15 bis 20 Minuten) und die Abgabe von Speichelproben während eines Kino-Abends.

Wie viele Kinder werden insgesamt an der Studie teilnehmen?
Insgesamt werden 40 Jugendliche an dem Projekt teilnehmen.

Nutzen und Risiken
Die geplante Untersuchung dient der Gewinnung neuer wissenschaftlicher Erkenntnisse und hat daher keine unmittelbare Konsequenz für Sie und Ihr Kind. Als Aufwandsentschädigung erhält jeder Studienteilnehmer einen Buchgutschein im Wert von 20 Euro. Bei jeder Erhebung, Speicherung und Übermittlung von Daten bestehen sehr geringe Vertraulichkeitsrisiken (z.B. die Möglichkeit der Namens-Identifizierung). Wir versichern Ihnen, dieses Risiko so klein wie möglich zu halten, indem wir den Namen Ihres Kindes für die Datenarchivierung und -analyse durch einen Code ersetzen werden. Die Datenschutzrechte gemäß der DSGVO werden gewährleistet. Beim Tragen des Aktigraphie-Gerätes kann es in seltenen Fällen zu einer Hautreizung durch das Armband kommen. In diesem Fall sollte das Gerät unverzüglich abgelegt werden. Zudem bitten wir Sie um eine sofortige Kontaktaufnahme zu unserer Studienleitung.

Kann ich von der Teilnahme an der Studie zurücktreten?
Die Teilnahme an der Untersuchung ist vollkommen freiwillig und Sie können Ihr Einverständnis jederzeit ohne Angabe von Gründen und ohne Nachteile für Sie oder Ihr Kind widerrufen. Beim Widerruf haben Sie das Recht zu verlangen, dass die Daten Ihres Kindes anonymisiert oder gelöscht werden und dass die Speichelproben vernichtet werden.

Titel der Studie: **Validierung von Fragebögen zum Chronotyp im Jugendalter mittels Aktigraphie und Dim Light Melatonin onset-Test**

Projektleitung
Dr. med. Mirja Quante
Pädiatrische Schlafmedizin
Abteilung Neonatologie
Klinik für Kinder- und Jugendmedizin
Calwer Str. 7, 72076 Tübingen
Tel.: +49 7071 29-81283; Fax: +49 7071 294356
E-Mail: mirja.quante@med.uni-tuebigen.de

Prof. Dr. med. Christian F. Poets
Abteilung für Neonatologie, Klinik für Kinder- und Jugendmedizin
Calwer Str. 7, 72076 Tübingen, Tel.: +49 7071 29-81283; Fax: +49 7071 294356
E-Mail: Christian-F.Poets@med.uni-tuebingen.de

Prof. Dr. Christoph Randler
Didaktik der Biologie
Morgenstelle 28, 72076 Tübingen, Tel.: +49 7071 29-74685
E-Mail: christoph.randler@uni-tuebingen.de

Einverständniserklärung zur Studienteilnahme

Ich wurde über die Ziele, die Dauer, den Ablauf und den Nutzen der Untersuchung sowie über Risiken und Belastungen der Teilnahme mündlich und schriftlich aufgeklärt und erkläre mich als Sorgeberechtigter damit einverstanden, dass mein Kind (Name in Blockschrift) _____ an dieser Studie teilnimmt.

Ich erkläre mich damit einverstanden, dass die im Rahmen dieser Untersuchung erhobenen Daten auf Fragebögen und elektronischen Datenträgern aufgezeichnet und ausgewertet werden dürfen. Der Umgang mit personenbezogenen Daten wurde mir in der separaten Aufklärung zum Datenschutz dargelegt.

Ich bin auch darüber informiert, dass die Teilnahme an der Untersuchung vollkommen freiwillig ist und dass ich ohne Angabe von Gründen und ohne Nachteile jederzeit meine Einwilligung widerrufen kann.

_____ _____
Datum Unterschrift der Eltern des Probanden

_____ _____
Datum Unterschrift des aufklärenden Arztes

Declarations of consent: Adolescents

Universitätsklinikum Tübingen
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Projektleitung
Dr. med. Mirja Quante
Pädiatrische Schlafmedizin, Abteilung Neonatologie, Klinik für Kinder- und Jugendmedizin
Calwer Str. 7, 72076 Tübingen, Tel.: +49 7071 29-81283; Fax: +49 7071 294356
E-Mail: mirja.quante@med.uni-tuebigen.de

Titel der Studie: Validierung von Fragebögen zum Chronotyp im Jugendalter mittels Aktigraphie und Dim Light Melatonin onset-Test

Studieninformationsblatt für Jugendliche

Liebe/r Jugendliche,

hast Du Interesse, an einer Studie zur „inneren Uhr" teilzunehmen? Bitte lies Dir dieses Info-Blatt zusammen mit Deinen Eltern durch und stelle uns Deine Fragen.

Wie wird die „innere Uhr" gemessen?
Die innere Uhr bestimmt, ob wir eher Morgenmenschen oder Nachteulen sind. Dies lässt sich mit einem Fragebogen herausfinden, in dem Personen zu ihrem Tagesablauf und ihren Schlafzeiten befragt werden.

Warum führen wir diese Studie durch?
Wir wollen herausfinden, wie gut Fragebögen zur inneren Uhr im Vergleich zur Bestimmung des Schlafhormons und zur Bewegungsmessung funktionieren. Das Schlafhormon kann am Abend im Speichel gemessen werden. Der Bewegungsmesser wird wie eine Armbanduhr am Handgelenk getragen wird. Da man sich im Schlaf weniger bewegt, kann das Gerät erkennen, ob jemand wach war oder geschlafen hat.

Wie lange wird die Studie dauern? Was muss ich dafür machen?
Wenn Du mitmachst, erhältst Du an einem ersten Termin einen Bewegungsmesser und ein Schlaftagebuch. Wir bitten Dich zudem vier Fragebögen zur Bestimmung der „inneren Uhr" auszufüllen. Der Bewegungsmesser soll anschließend für 7 Tage/24 Stunden, außer beim Baden oder Duschen, getragen werden. Im Schlaftagebuch schreibst Du auf, wenn Du das Gerät abgelegt hast. Wir erfragen zudem Dein Alter, Dein Geschlecht, Dein Gewicht und Deine Länge. Am 16. März 2019 möchten wir dann Dein Schlafhormon messen. Hierzu laden wir alle Studienteilnehmer um 19.30 Uhr in den Raum 220 der Crona-Klinik ein. Dort werden wir dann während der Vorführung eines Jugendfilmes („Jumanji" oder „Forest Gump") von 20.00 Uhr bis 00.00 Uhr halbstündlich Speichelproben zur Messung des Schlafhormons sammeln.

Wie viele Jugendliche werden teilnehmen? Kann ich von der Teilnahme an der Studie zurücktreten? Insgesamt werden 40 Jugendliche an dem Projekt teilnehmen. Die Teilnahme an der Untersuchung ist vollkommen freiwillig und Du kannst Dein Einverständnis jederzeit ohne Angabe von Gründen und ohne Nachteile zurückziehen.

Ist es für mich von Vorteil, wenn ich an der Studie teilnehme? Gibt es auch Nachteile?
Die Studie wird nicht direkt Dir selbst helfen, jedoch möglicherweise anderen Jugendlichen in der Zukunft. Ganz selten kann es zu Hautreizungen durch den Bewegungsmesser kommen. Die Abgabe der Speichelproben ist ohne Risiko und wir behandeln die erhobenen Daten sehr sorgfältig, damit sie nicht in falsche Hände geraten.

Titel der Studie: **Validierung von Fragebögen zum Chronotyp im Jugendalter mittels Aktigraphie und Dim Light Melatonin onset-Test**

Projektleitung

Dr. med. Mirja Quante
Pädiatrische Schlafmedizin, Abteilung Neonatologie, Klinik für Kinder- und Jugendmedizin
Calwer Str. 7, 72076 Tübingen, Tel.: +49 7071 29-81283; Fax: +49 7071 294356
E-Mail: mirja.quante@med.uni-tuebigen.de

Prof. Dr. med. Christian F. Poets
Abteilung für Neonatologie, Klinik für Kinder- und Jugendmedizin
Calwer Str. 7, 72076 Tübingen, Tel.: +49 7071 29-81283; Fax: +49 7071 294356
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Morgenstelle 28, 72076 Tübingen, Tel.: +49 7071 29-74685
E-Mail: christoph.randler@uni-tuebingen.de

Ich habe diese Information gelesen und meine Fragen wurden beantwortet.

☐ Ja, ich möchte an der Studie teilnehmen.

☐ Nein, ich möchte nicht an der Studie teilnehmen.

Name des Jugendlichen in Blockschrift	Unterschrift des Jugendlichen

Data consent

EBERHARD KARLS
UNIVERSITÄT
TÜBINGEN

UNIVERSITÄTS
KLINIKUM
TÜBINGEN

PROJEKTLEITUNG
Dr. med. Mirja Quante
Pädiatrische Schlafmedizin
Abteilung Neonatologie
Klinik für Kinder- und Jugendmedizin
Calwer Str. 7, 72076 Tübingen
Tel.: +49 7071 29-81283; Fax: +49 7071 294356
E-Mail: mirja.quante@med.uni-tuebigen.de

Information und Einwilligungserklärung zum Datenschutz

Studientitel: Validierung von Fragebögen zum Chronotyp im Jugendalter mittels Aktigraphie und Dim Light Melatonin onset-Test

Information zum Umgang mit in einer Studie erhobenen Daten:

Im Rahmen der Studie werden personenbezogene Daten (Namen, Geburtstag, Adresse)erhoben und verarbeitet.

Die Dokumentation der Daten zu Ihrem Kind und deren Archivierung erfolgt pseudonymisiert in einer geschützten elektronischen Datenbank, zu der nur befugte Mitarbeiterinnen und Mitarbeiter einschließlich auf das Berufs- und Datengeheimnis verpflichteter Doktorandinnen und Doktoranden Zutritt haben. Alle beteiligten Mitarbeiter unterliegen der Schweigepflicht.

Die im Rahmen der Studie erhobenen Daten können auch für künftige Forschungsvorhaben der Klinik bzw. des Instituts genutzt und weiterverarbeitet werden.

Die Verarbeitung und Nutzung der pseudonymisierten Daten erfolgt auf Erhebungsbögen und elektronischen Datenträgern im Regelfall für die Dauer von 10 Jahren, soweit der Zweck der Studie, z. B. bei Einbringung in eine Datenbank und bei Langzeitstudien keine längere Speicherdauer erfordert.

Die im Verlauf dieser Studie gewonnenen Informationen können für wissenschaftliche Zwecke auch an Kooperationspartner im Geltungsbereich der Europäischen Datenschutz- Grundverordnung und an Kooperationspartner außerhalb des Europäischen Wirtschaftsraumes, d.h. in Länder mit geringerem Datenschutzniveau (dies gilt auch für die USA) übermittelt werden. Soweit die Daten Ihres Kindes in Länder mit geringerem Datenschutzniveau übermittelt werden, wird der Verantwortliche alle erforderlichen Maßnahmen treffen, um das Datenschutzniveau zu gewährleisten. Sollte dies nicht möglich sein, werden die Daten Ihres Kindes lediglich dann übermittelt, wenn Sie in die vorgeschlagene Datenübermittlung ausdrücklich einwilligen, nachdem Sie über die für Ihr Kind bestehenden möglichen Risiken einer derartigen Datenübermittlung unterrichtet wurden.

Die Forschungsergebnisse aus der Studie werden in anonymisierter Form in Fachzeitschriften oder in wissenschaftlichen Datenbanken veröffentlicht. Bei der Veröffentlichung der Forschungsergebnisse wird die Identität Ihres Kindes nicht bekannt. Die Prüfärzte vor Ort können jedoch mit Hilfe einer Patientenliste bei Rückfragen die Daten zu Ihrer Person zurückführen.

Sie können jederzeit Auskunft über die gespeicherten Daten von Ihrem Kind anfordern sowie die Überlassung einer kostenlosen Kopie verlangen und haben das Recht, fehlerhafte Daten berichtigen zu lassen. Sie können auch jederzeit verlangen, dass die Daten Ihres Kindes gelöscht oder anonymisiert werden, so dass ein Bezug zu Ihrem Kind nicht mehr hergestellt werden kann. Diese Rechte sind nach § 13 des Landesdatenschutzanpassungsgesetzes bzw. § 27 des Bundesdatenschutzanpassungsgesetzes insoweit beschränkt, als diese Rechte voraussichtlich die Verwirklichung der jeweiligen Forschungszwecke unmöglich machen oder ernsthaft beeinträchtigen und die Beschränkung für die Erfüllung der jeweiligen Forschungszwecke notwendig ist. Das Recht auf Auskunft besteht darüber hinaus nicht, wenn die Daten für Zwecke der wissenschaftlichen Forschung erforderlich sind und die Auskunftserteilung einen unverhältnismäßigen Aufwand erfordern würde.

Die Studienleiterin (*Dr. med. Mirja Quante, mirja.quante@med.uni-tuebingen.de*) ist für die Datenverarbeitung und die Einhaltung der gesetzlichen Datenschutzbestimmungen verantwortlich.

Bei Beschwerden können Sie sich an den Datenschutzbeauftragten des Universitätsklinikums Tübingen oder den Landesdatenschutzbeauftragten des Landes Baden-Württemberg wenden.
Für die Erhebung, Speicherung, Nutzung und Weitergabe der Daten Ihres Kindes ist Ihre ausdrückliche Zustimmung durch Unterzeichnung der Einwilligungserklärung zum Datenschutz erforderlich.

Rechtsgrundlage für die Verarbeitung der Daten Ihres Kindes sind Art. 6, 7, 9, 89 der Datenschutz-Grundverordnung in Verbindung mit §§ 4, 5, 6, 8, 9, 12, 13 des Landesdatenschutzgesetzes Baden-Württemberg in der ab 25. Mai 2018 geltenden Fassung.

Einwilligungserklärung zum Umgang mit den in einer Studie erhobenen Daten:

(sofern die Einwilligungserklärung zum Datenschutz in die allgemeine Einwilligungserklärung zur Studienteilnahme integriert wird, muss dieser Abschnitt graphisch hervorgehoben werden)

Ich erkläre, dass ich mit der im Rahmen der Studie erfolgenden Erhebung und Verarbeitung von Daten und ihrer verschlüsselten (pseudonymisierten) Weitergabe einverstanden bin.
Mir ist bewusst, dass die Ergebnisse dieser Studie in medizinischen Fachzeitschriften veröffentlicht werden, allerdings in anonymisierter Form, so dass ein direkter Bezug zu meinem Kind nicht hergestellt werden kann.
Ich wurde darüber informiert, dass ich jederzeit Auskunft über die gespeicherten Daten meines Kindes und die Berichtigung von fehlerhaften Daten verlangen kann.
Ich weiß, dass ich jederzeit, beispielsweise beim Widerruf der Studienteilnahme, verlangen kann, dass die bis dahin erhobenen Daten meines Kindes gelöscht oder unverzüglich anonymisiert werden.
Ich erkläre, dass ich über die Erhebung und Verarbeitung in dieser Studie erhobenen Daten meines Kindes und meine Rechte angemessen informiert wurde.
Ich stimme der Verwendung der im Rahmen dieser Studie erhobenen Daten in der oben beschriebenen Form zu.

Kontaktinformationen:
Datenschutzbeauftragter des Universitätsklinikums Tübingen
Calwerstraße 7/4, 72076 Tübingen,
Tel. 07071 29-87667, E-Mail: dsb@med.uni-tuebingen.de

Landesbeauftragter für den Datenschutz und die Informationsfreiheit in Baden-Württemberg
Postanschrift: Postfach 10 29 32, 70025 Stuttgart
Tel.: 0711/615541-0, FAX: 0711/615541-15, E-Mail: poststelle@lfdi.bwl.de

Tübingen, den	Unterschrift der Eltern	Name des Patienten/der Patientin in Blockschrift
Tübingen, den	Unterschrift	Name des aufklärenden Arztes/ Wissenschaftlers in Blockschrift

Recruitment Flyer

Warum führen wir diese Studie durch?

Die innere Uhr bestimmt, ob wir eher Morgenmenschen oder Nachteulen sind. Wir wollen herausfinden, wie gut Fragebögen die innere Uhr im Vergleich zur Bestimmung des Schlafhormons und zur Aktigraphie erfassen. Das Schlafhormon kann am Abend im Speichel gemessen werden. Bei der Aktigraphie handelt es sich um einen Bewegungsmesser zur Registrierung von Ruhe- und Aktivitätsphasen, der wie eine Armbanduhr am Handgelenk getragen wird. Da man sich im Schlaf weniger bewegt, kann das Gerät erkennen, ob jemand wach war oder geschlafen hat.

Wie kann man sich anmelden?

Ihr habt Interesse an unserer Studie teilzunehmen? Dann bittet eure Eltern, uns zu kontaktieren:

Abteilung für Neonatologie
Universitätskinderklinik Tübingen

Leonie Paciello, Doktorandin
Mobil: 0162 - 6710064
Email: leonie-marie.paciello@student.uni-tuebingen.de

Studienleitung:
Dr. med. Mirja Quante
Abteilung für Neonatologie
Universitätskinderklinik Tübingen

Prof. Dr. med. Christian F. Poets
Abteilung für Neonatologie
Universitätskinderklinik Tübingen

Prof. Dr. C. Randler
Didaktik der Biologie
Universität Tübingen

Teilnehmer für „Kino-Studie gesucht!“

Wissenschaftliche Untersuchung zur Testung von Fragebögen zum Chronotyp

Kinderklinik Tübingen

Wie lange wird die Studie dauern? Was muss ich dafür machen?

An einem ersten Termin erhalten die Studienteilnehmer ein Aktigraphie-Gerät und ein Schlaftagebuch. Wir bitten zudem alle Studienteilnehmer vier Fragebögen zur Bestimmung des Chronotyps auszufüllen. Das Aktigraphie-Gerät soll anschließend für 7 Tage/24 Stunden, außer beim Baden oder Duschen, getragen werden. Im Schlaftagebuch wird notiert, wenn das Gerät abgelegt wurde. Wir erfragen zudem Informationen zum Alter, zum Geschlecht, zum Gewicht und zur Länge. Am 16. März 2019 möchten wir dann das Schlafhormon messen. Hierzu laden wir alle Studienteilnehmer um 19.30 Uhr in den Raum 220 der CRONA-Klinik ein. Dort werden wir dann während der Vorführung eines Jugendfilmes („Jumanji“ und/oder „Forest Gump“) von 20.00 Uhr bis 00.00 Uhr halbstündlich Speichelproben zur Messung des Schlafhormons sammeln.

Wer kann teilnehmen?

Teilnehmen können Jugendliche im Alter von 13 bis 16 Jahren, die keine diagnostizierte Schlafstörung, keine neurologische Erkrankung oder psychiatrische Erkrankung haben. Die reguläre Einnahme von Medikamenten (außer Kontrazeptiva), sowie eine Reise über mehr als 2 Zeitzonen im letzten Monat stellen schließen ebenfalls von der Studienteilnahme aus.

Ist es für mich von Vorteil, wenn ich an der Studie teilnehme? Gibt es auch Nachteile?

Die Studie wird nicht direkt den Studienteilnehmern selbst helfen, jedoch möglicherweise anderen Kindern in der Zukunft. Ganz selten kann es zu Hautreizungen durch das Aktigraphie-Gerät kommen. Die Abgabe der Speichelproben ist ohne Risiko und wir behandeln die erhobenen Daten sehr sorgfältig, damit sie nicht in falsche Hände geraten.

10.3 Questionnaires

Morningness-Eveningness Stability Scale (improved; MESSi) (Randler et al., 2016a) and self-assessed habitual sleep onset and offset times

XX.XX.2018 Codewort: _____

Bitte beantworte alle Fragen. Bitte kreuze jeweils nur eine Antwort an.
Bitte überlege nur kurz und antworte spontan.

Wie leicht fällt es dir normalerweise morgens aufzustehen?
1 [ ] überhaupt nicht leicht
2 [ ] nicht so leicht
3 [ ] teils/teils
4 [ ] ziemlich leicht
5 [ ] sehr leicht

Wie lange dauert es bei dir morgens nach dem Aufstehen, bis du einen klaren Kopf hast und klar denken kannst?
5 [ ] 0 bis 10 Minuten
4 [ ] 11 bis 20 Minuten
3 [ ] 21 bis 40 Minuten
2 [ ] 41 bis 60 Minuten
1 [ ] mehr als 60 Minuten

Wie wach fühlst du dich morgens in der ersten halben Stunde nach dem Aufwachen?
1 [ ] überhaupt nicht wach
2 [ ] etwas wach
3 [ ] mittel
4 [ ] ziemlich wach
5 [ ] sehr wach

Bitte gib dein Geschlecht an.
0 [ ] männlich 1 [ ] weiblich
2 [ ] keine Angabe

Alter: _____Jahre

Im Allgemeinen, wie hoch ist deine Energie

1) ***morgens***: 1 [ ] sehr niedrig 2 [ ] niedrig 3 [ ] mittel 4 [ ] hoch 5 [ ] sehr hoch

2) ***abends:*** 1 [ ] sehr niedrig 2 [ ] niedrig 3 [ ] mittel 4 [ ] hoch 5 [ ] sehr hoch

Bitte gib an, inwieweit die folgenden Aussagen auf dich zutreffen. *Bitte mache in jeder Zeile ein Kreuz.*	**Trifft völlig zu**		**Teils/ teils**		**Trifft überhaupt nicht zu**
	5	4	3	2	1
Nach dem Aufwachen fühle ich mich noch längere Zeit schläfrig.	[ ]	[ ]	[ ]	[ ]	[ ]
Mein bevorzugter Zeitpunkt zum Lernen ist abends.	[ ]	[ ]	[ ]	[ ]	[ ]
Meine Stimmung ist den ganzen Tag über gleich.	[ ]	[ ]	[ ]	[ ]	[ ]
Ich kann mich zu jeder Tageszeit konzentrieren.	[ ]	[ ]	[ ]	[ ]	[ ]
Meine Motivation ist zu jeder Tageszeit gleich.	[ ]	[ ]	[ ]	[ ]	[ ]
Es gibt Zeiten am Tag, an denen ich mich zu nichts in der Lage fühle.	[ ]	[ ]	[ ]	[ ]	[ ]
Es gibt Tageszeiten, an denen es mir schwer fällt zu denken.	[ ]	[ ]	[ ]	[ ]	[ ]
Ich bin eher abends als morgens aktiv.	[ ]	[ ]	[ ]	[ ]	[ ]
Abends bin ich am leistungsfähigsten.	[ ]	[ ]	[ ]	[ ]	[ ]
Meistens bin ich abends bester Laune.	[ ]	[ ]	[ ]	[ ]	[ ]

Zu welcher Uhrzeit stehst du an Wochentagen auf?
[ ] : [ ] Uhr

Zu welcher Uhrzeit stehst du am Wochenende auf?
[ ] : [ ] Uhr

Wann gehst du vor Wochentagen ins Bett?
[ ] : [ ] Uhr

Wann gehst du am Wochenende ins Bett?
[ ] : [ ] Uhr

Reduced Morningness-Eveningness Questionnaire for Children and Adolescents (rMEQ-CA) (Randler, 2013), adapted for adolescents in this study (2018)

rMEQ-CA (Reduced Morning-Eveningness-Questionnaire for Children and Adolescents / German Version)

Nach Randler (2013) in der reduzierten Version, angepasst für Kinder und Jugendliche (2018).

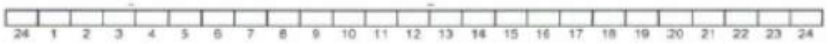
Wenn es nur nach Deinem eigenen Wohlbefinden ginge und Du Deinen Tag völlig frei einteilen könntest (schulfrei), wann würdest Du dann aufstehen? Bitte markiere die Uhrzeit auf der Zeitleiste.

Wie müde fühlst Du Dich morgens in der ersten halben Stunde nach dem Aufwachen?

- sehr müde
- ziemlich müde
- ziemlich frisch
- sehr frisch

Um wie viel Uhr wirst Du abends müde und hast das Bedürfnis schlafen zu gehen? Bitte markiere die Uhrzeit auf der Zeitleiste.

Zu welcher Tageszeit fühlst Du Dich Deiner Meinung nach am besten?



Man spricht bei Menschen von „Morgen-“ und „Abendtypen“. Morgentypen stehe eher früh auf und gehen früh zu Bett, Abendtypen stehen eher später auf und gehen auch später ins Bett. Zu welchem der folgenden Typen zählst Du Dich am ehesten?

- eindeutig „Morgentyp“
- eher „Morgen-“ als „Abendtyp“
- eher „Abend-“ als „Morgentyp“
- eindeutig „Abendtyp“

Composite Scale of Morningness (CSM), German version (Randler, 2007)

CSM-Fragebogen **Codewort:_____**

Bitte beantworte die Fragen zum Thema „Schlaf" ohne lange nachzudenken.
Bitte kreuze jeweils nur eine Antwort an.

Stell dir vor, die Schule fällt aus. Du darfst aufstehen, wann du möchtest. Wann stehst du morgens auf?
5[ ] vor 6:30 Uhr
4[ ] zwischen 6:30 Uhr und 7:45 Uhr
3[ ] zwischen 7:45 Uhr und 9:45 Uhr
2[ ] zwischen 9:45 Uhr und 11 Uhr
1[ ] nach11Uhr

Du hast morgen keine Schule und du darfst ins Bett gehen wann du möchtest. Wann gehst du abends ins Bett?
5[ ] vor21Uhr
4[ ] zwischen 21 Uhr und 22:15 Uhr
3[ ] zwischen 22:15 Uhr und 0:30 Uhr
2[ ] zwischen 0:30 Uhr und 1:45 Uhr
1[ ] nach 1:45 Uhr

Wie leicht fällt es dir morgens aufzustehen?
1[ ] überhaupt nicht leicht
2[ ] nicht so leicht
3[ ] ziemlich leicht
4[ ] sehr leicht

Wie wach fühlst du dich morgens in der ersten halben Stunde nach dem Aufwachen?
1[ ] überhaupt nicht wach
2[ ] etwas wach
3[ ] ziemlich wach
4[ ] sehr wach

Wie müde fühlst du dich morgens in der ersten halben Stunde nach dem Aufwachen?
1[ ] sehr müde
2[ ] ziemlich müde
3[ ] ziemlich fit
4[ ] sehr fit

Der Sportunterricht beginnt um 7 Uhr. Wie wäre das für dich?
4[ ] Ich wäre gut in Form.
3[ ] Ich wäre ziemlich in Form.
2[ ] Es wäre ziemlich schwierig für mich.
1[ ] Es wäre sehr schwierig für mich.

Wann wirst du abends müde und möchtest deshalb schlafen gehen?
5[] vor 21 Uhr
4[ ] zwischen 21 Uhr und 22:15 Uhr
3[ ] zwischen 22:15 Uhr und 0:30 Uhr
2[ ] zwischen 0:30 Uhr und 1:45 Uhr
1[ ] nach 1:45 Uhr

Für eine Klassenarbeit, die sehr anstrengend ist, möchtest du in Bestform sein. Du kannst dir deinen Tag völlig frei einteilen. Wann würdest du diese schreiben?
4[ ]von 8 bis 10 Uhr
3[ ]von 11 bis 13 Uhr
2[ ]von 15 bis 17 Uhr
1[ ]von 19 bis 21 Uhr

Manche Menschen sind Morgentypen, andere dagegen Abendtypen. Zu welchem Typ würdest du dich zählen?
4[ ] eindeutig „Morgentyp"
3[ ] eher „Morgentyp" als „Abendtyp"
2[ ] eher „Abendtyp" als „Morgentyp"
1[ ] eindeutig „Abendtyp"

Wann würdest du am liebsten morgens aufstehen, um zur Schule zu gehen?
4[ ] vor 6:30 Uhr
3[ ] zwischen 6:30 Uhr und 7:30 Uhr
2[ ] zwischen 7:30 Uhr und 8:30 Uhr
1[ ] nach 8:30 Uhr

Stell dir vor, du müsstest jeden Morgen um 6:00 Uhr aufstehen. Wie wäre das für dich?
1[ ] sehr schwierig und unangenehm
2[ ] ziemlich schwierig und unangenehm
3[ ] etwas unangenehm, aber kein größeres Problem
4[ ] einfach und nicht unangenehm

Wie lange dauert es bei dir morgens nach dem Aufstehen, bis du richtig wach bist und klar denken kannst?
4[ ] 0 bis 10Minuten
3[ ] 11 bis 20 Minuten
2[ ] 21 bis 40 Minuten
1[ ] mehr als 40 Minuten

Bist du eher morgens oder abends aktiv?
4[ ] ausgesprochen morgens aktiv
(morgens wach, abends müde)
3[ ] eher morgens aktiv
2[ ] eher abends aktiv
1[ ] ausgesprochen abends aktiv
(morgens müde, abends wach)

Pediatric Daytime Sleepiness Scale (PDSS) (Drake et al., 2003), German version (Schneider and Randler, 2009)

Bitte denke an die letzten vier Schulwochen. Bitte gib wie häufig dies in letzten vier Wochen geschah.

		immer	häufig	manch-mal	selten	nie
1	Wie oft wirst du während des Unterrichts müde oder schläfst ein?	○	○	○	○	○
2	Wie oft wirst du müde, während du deine Hausaufgaben erledigst?	○	○	○	○	○
3	Wie häufig fühlst du dich tagsüber hellwach?	○	○	○	○	○
4	Wie oft bist du tagsüber müde und genervt?	○	○	○	○	○
5	Wie oft hast du Probleme morgens aufzustehen?	○	○	○	○	○
6	Wie häufig passiert es dir, dass du morgens noch einmal einschläfst, nachdem du geweckt wurdest?	○	○	○	○	○
7	Wie häufig muss dich morgens jemand wecken?	○	○	○	○	○
8	Wie oft fühlst du dich unausgeschlafen?	○	○	○	○	○

Sleep diary (Paciello et al., 2019), adapted for this study (2018)