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ORIGINAL PAPER

Sleep Problems, Circadian Rhythms, and Their Relation to Behavioral Difficulties in Children and Adolescents with Autism Spectrum Disorder

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Abstract

This was an exploratory cross-sectional study comparing 45 children with ASD to 24 typically developing drug-naïve controls, group-matched on age, sex, and body mass index. Objective data was obtained using the following: an ambulatory circadian monitoring device; saliva samples to determine dim light melatonin onset (DLMO): and three parent-completed measures: the Child Behavior Checklist (CBCL); the Repetitive Behavior Scale-Revised (RBS-R); and the General Health Questionnaire (GHQ28). The CBCL and RBS-R scales showed the highest scores amongst poor sleepers with ASD. Sleep fragmentation was associated with somatic complaints and self-injury, leading to a higher impact on family life. Sleep onset difficulties were associated with withdrawal, anxiety, and depression. Those with phase advanced DLMO had lower scores for "somatic complaints"; "anxious/depressed" state; and "social problems", suggesting that this phenomenon has a protective role.

Keywords Melatonin · Autism spectrum disorder · Sleep · Behavior · Actigraphy · Questionnaires

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Autism spectrum disorder (ASD) is a neurodevelopmental condition in which sleep problems (SPs) are highly prevalent, including difficulties with sleep onset latency, bedtime resistance, nighttime awakenings, and shortened sleep time (Baker & Richdale, 2015; Ballester et al., 2019; Goldman et al., 2017; Martinez-Cayuelas et al., 2021). Though the causes of SPs in ASD are multifactorial, it has been argued that misalignment of circadian rhythm, in which melatonin acts as a major regulator, plays a role in ASD etiology (Geoffray et al., 2016; Reynolds & Malow, 2011).

A significant proportion of individuals with ASD have been shown to have dysregulated cortisol and melatonin rhythm, circadian rhythm sleep–wake disorders, and an altered pattern of body temperature (Baker & Richdale, 2017; Ballester et al., 2019; Corbett et al., 2008; Glickman, 2010; Martinez-Cayuelas et al., 2021; Souders et al., 2009; Tordjman et al., 2005; Wiggs & Stores, 2004). Additionally, some reports have analyzed the association between sleep disturbances and behavioral outcomes in ASD, although most have drawn on subjective data when measuring sleep parameters. In both typically developing (TD) and ASD children, research has shown a link between SPs and behavioral difficulties (Allik et al., 2006b; Astill et al., 2012; Goldman

et al., 2009; Johnson et al., 2018; Malow et al., 2006; Mazurek & Sohl, 2016; Reynaud et al., 2018) -see Supplementary Material for detailed information about background literature-. It has been previously described that sleep disruption worsens the symptoms of ASD, causing greater impairment of social communication and higher rates of stereotypic behaviors; it also aggravates co-ocurring behaviors as aggression, self-injury, anxiety and inattention (Bangerter et al., 2020; Geoffray et al., 2016; Goldman et al., 2009; Hundley et al., 2016; Johnson & Zarrinnegar, 2021; Malow et al., 2006; Mazurek & Sohl, 2016; Williams Buckley et al., 2020; Yavuz-Kodat et al., 2020), thus increasing the burden on parents as well as overall familial distress (Abdullah et al., 2021; Johnson & Zarrinnegar, 2021). Moreover, some studies have pointed to a bidirectional relationship between sleep and behavior, and the prediction of SPs from the presence of aggressive behavior in ASD has also been described (Esposito et al., 2020; Lalanne et al., 2021; Rossignol & Frye, 2014; Shui et al., 2018; Sikora et al., 2012). To date, however, no direct relationship has been found between certain SPs and specific behavioral issues. Investigating the relationship between sleep and behavior continues to be complicated by a mix of internal and external factors that can affect both sleep and behavior in a bidirectional way (Anders et al., 2012; Martin et al., 2021).

Though most earlier research has relied on subjective data to measure sleep functioning, use of objective data has increased in recent years, with a number of papers including polysomnography or actigraphy to analyze the relationship between sleep and behavior difficulties in ASD (Aathira et al., 2017; Allik et al., 2006a; Elia et al., 2000; Elkhatib Smidt et al., 2021; Fletcher et al., 2017; Gagnon et al., 2021; Goldman et al., 2009; Leader et al., 2022; Malow et al., 2006; Richdale et al., 2014; Yavuz-Kodat et al., 2020). New generation actigraphs such as Ambulatory Circadian Monitoring (ACM) devices offer valuable information about light, temperature and movement, allowing both sleep parameters and other circadian data to be collected in studies in autistic populations (Ballester et al., 2019). Despite the existence of these new devices, data on circadian rhythm for this population remain scarce (Bangerter et al., 2020; Elkhatib Smidt et al., 2021; Yavuz-Kodat et al., 2020). Therefore, it would be recommended that studies including objective sleep parameters would expand their analysis with circadian data, enhancing in such a way, the knowledge about autism and the circadian system. In this regard, measures of melatonin, which are considered the most relevant peripheral index of human circadian rhythmicity (Burgess & Fogg, 2008; Mandrell et al., 2018; Pandi-Perumal et al., 2007) and have been applied to the study of individuals with ASD, can also add further knowledge about circadian functioning. Few, however, have explored the link between melatonin pattern anomalies and behavioral difficulties and their findings have been contradictory (Babinska et al., 2019; Tordjman et al., 2012). Specifically, the study of change to circulating melatonin levels measured by Dim Light Melatonin Onset (DLMO). The DLMO is a reliable marker of circadian phase, which is defined as the time at which a salivary concentration of melatonin of 3-5 pg/ml is reached, usually 2–3 h before sleep onset (Burgess & Fogg, 2008; Mandrell et al., 2018; Pandi-Perumal et al., 2007). Data on this process is more limited in autistic individuals (Baker et al., 2017; Goldman et al., 2014, 2017) and previous studies have not included information on concomitant behavior.

The coronavirus pandemic has provided evidence about the importance of sleep and effects of disruption to the circadian system. The COVID-19 lockdown determined important changes in the sleep and regularity of rhythms. Lockdown is reported to have significantly changed bedtime for nearly 58% of children with ASD and rise time for 69% (Bruni et al., 2022). Various researchers have reported reduced sleep duration, difficulties in falling sleep, night waking, sleep terrors and daytime sleepiness in autistic populations (Bruni et al., 2021, 2022; Reynaud et al., 2022; Türkoğlu et al., 2020); in addition, adults with autism displayed higher levels of sleep and circadian rhythm disturbances, with less favorable daily routines (Reynaud et al., 2022) and children with ASD reported higher presence of the 'eveningness' chronotype and higher autism symptom scores during the period of home confinement (Türkoğlu et al., 2020).

Due to the aforementioned gaps in the knowledge, this study was raised as an exploratory study without a priori hypotheses. Because of the particularly scant knowing of circadian objective data, we aimed to study the relationship between quantitative behavioral difficulties, sleep and circadian parameters—temperature, motor activity, time in movement and light exposure—measured by ambulatory circadian monitoring (ACM) and measures of dim light melatonin onset (DLMO).

Methods

Study Design and Procedure

This cross-sectional exploratory study was conducted in the pediatrics department of the Fundación Jiménez Díaz University Hospital between September 2018 and January 2021.

Participants

The sample described in this paper was analyzed in a previous publication, which contains a detailed account of the study design and procedures for patient recruitment (Martinez-Cayuelas et al., 2021, 2022). Briefly, children and

adolescents between 5 and 18 years of age who met the clinical criteria for ASD as confirmed by the Autism Diagnostic Observation Schedule—ADOS—(Lord et al., 2000) were recruited from the pediatric neurology clinic of the hospital. ADOS was applied by qualified and trained investigators; modules 2, 3 and 4 were administered. Those receiving any kind of medical treatment and those with attention deficit hyperactivity disorder, epilepsy and/or specific genetic syndromes, such as Fragile X, were excluded from the study. Children and adolescents with intellectual disability were included. The control group was composed of TD children and adolescents matched for sex, pubertal stage, and body mass index, with no mental or general disorder and under no pharmacological treatment. Both groups were requested to wear an ambulatory circadian monitoring (ACM) device and collect saliva samples for determination of DLMO. Caregivers were asked to complete the rating scales. Data are accessible via the Open Science Framework (https://osf.io/wq75u/).

Ethics

Parents of the participants provided written informed consent after receiving a full explanation of the nature of the procedures. The study was performed in accordance with the principles of the Helsinki Declaration as well as applicable Spanish legislation on clinical research in human subjects and was approved by the Clinical Research Ethics Committee of the IIS-Fundación Jiménez Díaz (PIC018_18FJD), approval date 3/13/2018.

Ambulatory Circadian Monitoring (ACM)

Each participant wore an ACM device (Kronowise®) on their non-dominant wrist for one week, including one complete weekend, and all were asked to follow their usual routine. Specific data on the device and parameters measured have been published elsewhere (Martinez-Cayuelas et al., 2021). Of all the raw data obtained from the devices, the specific variables described in this study included wrist temperature (WT, °C), motor activity (sum of accelerations from the 3 axes, expressed as G/h), time in movement (seconds), light exposure (including total light and blue light and measured as lux and $\log_{10}$lux), and sleep (converted into a binary code, with 1 signaling a period of resting and 0 an active period, and used to calculate nonparametric indices). An integrated variable, known as thermometry, actimetry, and body position (TAP), was then obtained by integrating wrist temperature (inverted), motor activity, and position variability. TAP expresses general activation through arbitrary units, where values near 1 indicate a high level of activation and values around 0 denote complete rest (Ortiz-Tudela et al., 2014). In addition, parents completed a 7-day sleep–wake diary used as a proxy for the ACM recordings if needed.

SPs were defined as the presence of one or more of the following ACM-derived criteria: sleep onset latency longer than 30 min, number of awakenings after sleep onset ≥ 4/h, low total sleep time for age (Paruthi et al., 2016), or circadian sleep disorders (American Academy of Sleep, 2014). Patients presenting any of the aforementioned conditions were categorized as poor sleepers.

Melatonin Sampling

To measure melatonin concentration, saliva samples were collected on one night during the week in which the children and adolescents wore the ACM device. Hourly samples were taken 3, 2, and 1 h before bedtime to record any continued rise in melatonin concentrations; additional measurements were taken at bedtime and 1 h after bedtime. This timing allowed determination of DLMO (Mandrell et al., 2018; Pandi-Perumal et al., 2007). Samples were kept frozen after collection, initially in the home at − 20 °C and subsequently in the laboratory at − 80 °C. Melatonin samples were analyzed using a salivary melatonin enzyme immunoassay kit (Salimetrics, PA, USA). The first non-zero standard of this assay was 0.78 pg/ml. Intra-assay coefficients of variation for low, medium, and high levels of salivary melatonin were 7.4%, 3.3%, and 3.9%, respectively. The respective inter-assay coefficients of variation for low, medium, and high levels were 15.6%, 5.6%, and 4.6%. The time of DLMO was defined as the time at which evening salivary melatonin concentrations increased and remained above a 4 pg/ml threshold, using linear interpolation between successive samples. DLMO pattern was also described as normal (concentration reaches the 4 pg/ml threshold during the recorded time and shows an upward trend), advanced (concentration reaches the 4 pg/ml threshold before the recorded time and shows an upward trend), delayed (concentration does not reach the 4 pg/ml threshold during the recorded time), or irregular (concentration does not follow an identifiable trend).

Caregiver-Reported Scales

Caregivers of individuals with ASD and TD controls completed the following rating scales on paper during the week in which their children and adolescents wore the ACM device.

Behavioral Difficulties

The *Child Behavior Checklist (CBCL)* (Achenbach, 2011) is a standardized questionnaire that aims to identify social, behavioral, and emotional problems. The questionnaire includes 8 subscales: Anxious/Depressed, Withdrawn/

Depressed, Somatic Complaints, Social Problems, Thought Problems, Attention Problems, Rule-Breaking Behavior, and Aggressive Behavior; these symptoms are categorized into one of 3 domains: Internalizing Problems, Externalizing Problems, and Other Problems. The CBCL has been validated in children by other authors (Mazefsky et al., 2011). By definition, T-scores of ≥ 70 (≥ 98th percentile) are in the clinical range, while values between 65 and 70 (93rd–98th percentile) are in the borderline clinical range, and measurements under 65 (< 93rd percentile) fall within the normal range.

The *Repetitive Behavior Scale-Revised (RBS-R)* (Lam & Aman, 2007; Yon, 2017) evaluates repetitive behaviors in people with ASD and/or disability through 43 items grouped into 6 different dimensions (stereotypic, self-injurious, compulsive, ritualistic, sameness, and restrictive behavior). The items are rated on a 4-point Likert scale (0 = behavior not occurring and 4 = very serious repetitive behavior). The RBS-R has been shown to have excellent psychometric properties and concurrent–divergent validity in a number of countries, including Spain.

Impact on Families

The *General Health Questionnaire (GHQ-28)* (Goldberg & Hillier, 1979; Goldberg et al., 1996) consists of 28 items related to symptoms experienced, which are divided into 4 subscales: A (somatic symptoms), B (anxiety and insomnia), C (social dysfunction), and D (depression). Each item has 4 possible answers, and the scores range from "less than usual" in columns 1 and 2, to "much more than usual", in columns 3 and 4. To identify chronic impact, columns 2, 3, and 4 score "1".

Statistical Analyses

The ACM parameter values for all nights measured were averaged for each participant. Descriptive statistics were obtained for all major variables. The Shapiro–Wilk normality test was used as the basis for selecting parametric or nonparametric statistical tests. Continuous variables are presented as mean and standard deviation (SD) or median and interquartile range (IQR, P25, P75). Categorical variables are expressed as percentages. A t-test for independent samples or a Mann–Whitney U test was used to assess differences between controls and ASD patients. Analysis of variance (ANOVA) or the Kruskall-Wallis test was used to assess differences across more than two groups. Correlations were determined to measure the association between quantitative variables; p-values of < 0.05 were considered statistically significant. A False discovery rate (FDR) correction was applied when multiple test were conducted, using the Benjamini–Hochberg procedure (Benjamini & Hochberg, 1995). Analyses were performed using IBM SPSS version 25 (New York, NY) and R v4.1.2.

Results

Fifty-two children and adolescents with ASD and 27 healthy children and adolescents were initially enrolled in the study; of these, 7 ASD children and 3 controls were not included in the final sample due to insufficient questionnaire completion. Characteristics of the study population are shown in Table 1. There were no significant differences between the ASD and control groups with respect to age, sex, or body mass index (BMI). Of the 69 children eligible for the study (45 ASD and 24 TD), 33 (73% of ASD individuals) and 10 (41.6% of

Table 1 Characteristics of the sample

		ASD (n = 45)	TD controls (n = 24)	P
Age (years) (mean ± SD, 95% CI)		10.02 ± 3.01 (9.12–10.93)	8.83 ± 2.46 (7.79–9.87)	0.15
Sex (%)		Male 88.9% Female 11.1%	Male 70.8% Female 29.1%	0.059
BMI (kg/m^2) (mean ± SD, 95% CI)		18.83 ± 4.08 (17.61–20.06)	17.46 ± 2.78 (16.29–18.64)	0.26
CBCL T-scores (N clinical range/N borderline/N normal range)	Anxious/depressed	4/5/36	0/0/24	0.05
	Withdrawn/depressed	10/7/28	0/0/24	< 0.01
	Somatic complaints	6/2/37	1/1/22	0.46
	Social problems	12/10/23	0/0/24	< 0.001
	Thought problems	12/10/23	0/0/24	< 0.001
	Attention problems	12/9/24	0/0/24	< 0.001
	Rule-breaking behavior	0/3/42	0/0/24	0.19
	Aggressive behavior	1/4/40	0/0/24	0.22
Good sleeper/poor sleeper		12/33	14/10	< 0.05

ASD autism spectrum disorder, *CI* confidence interval, *CBCL* child behavior checklist, *TD* typically developing

TD controls) were classified as poor sleepers (see Table 1). Seven autistic children had intellectual disability.

Of the 69 children included, melatonin in saliva was studied in 38 children with ASD and 20 TD controls (11 individuals were not included due to inadequate saliva collection).

Sleep and Circadian Parameters

Specific data on individuals included in this study with regard to sleep and circadian rhythm parameters can be found in Tables 2 and 3 in the Supplementary materials; these data were reported in our previous study (Martinez-Cayuelas et al., 2021). We observed significant differences between the ASD and control groups in sleep and circadian parameters. The ASD group showed significantly longer sleep latencies and lower total sleep time and sleep efficiency ($p < 0.01$, $p < 0.01$, and $p < 0.01$, respectively). Stability of rhythms concerning temperature, motor activity, sleep, and light intensity was also lower in the ASD group.

Melatonin in Saliva

DLMO was successfully determined in 20 individuals with ASD and 8 TD children and adolescents. This data were included in our previous study (Martinez-Cayuelas et al., 2022). For the sample included here, the average DLMO was later in ASD children than in the control group (ASD group: 21.68 ± 1.56 h, TD controls: 20.56 ± 0.81 h), peak melatonin levels in saliva were 34.51 ± 34.60 pg/ml in the ASD group and 41.53 ± 37.11 pg/ml among controls, and time of peak melatonin concentration was 22.96 ± 1.37 h in the ASD group and 23.0 ± 1.06 h in the control group. Neither showed statistically significant differences.

DLMO pattern was defined in 38 children with ASD (15 with a normal pattern, 10 with an advanced pattern, and 13 irregular) and 20 TD controls (8 with a normal pattern, 9 with an advanced pattern, and 3 irregular), without significant differences between groups. No patients in either group presented the delayed subtype.

Behavioral Difficulties and Their Relationship to Sleep/Circadian Parameters and Melatonin in Saliva

CBCL and RBS-R scores for each group are shown in Table 2.

Overall, scores from CBCL scales differed significantly among ASD patients and individuals in the TD control group (see Table 2). The highest CBCL scores for the categories "Withdrawn", "Somatic Complaints", "Anxious/Depressed", "Social Problems", "Thought Problems", and "Aggressive Behavior" were found among poor sleepers with ASD, intermediate scores were obtained for good sleepers with ASD (although without reaching statistical significance between these 2 subgroups), and the lowest scores were found in the TD control group. No differences were observed between the 2 subgroups of TD controls. Values for all CBCL items were significantly different between poor sleepers with ASD and TD participants with poor sleep, with the highest scores observed in the ASD group. All categories except for "Somatic Complaints" and "Aggressive Behavior" were statistically different between ASD and TD participants with good sleep.

With regard to *sleep parameters*, in the ASD group, the CBCL dimension of "Somatic Complaints" correlated with awakenings per hour ($r = 0.357$, $p = 0.01$) and wake after sleep onset (WASO) ($r = 0.417$, $p = 0.005$). "Withdrawn" correlated with sleep onset latency ($r = 0.347$, $p = 0.02$) and sleep efficiency ($r = -0.328$, $p = 0.03$). The "Anxious/Depressed" dimension correlated with sleep onset latency ($r = 0.381$, $p = 0.01$) and sleep efficiency ($r = -0.303$, $p = 0.04$). In contrast, no correlations were found in the control group.

Correlation between *circadian rhythm parameters* and CBCL dimensions in the ASD group can be found in Table 3. FDR was applied to these values given the amount of tests conducted.

No correlation was found between DLMO time, peak levels of melatonin and peak melatonin time, and CBCL subscale scores. Regarding DLMO patterns, within the ASD group those patients with an advanced pattern showed lower scores for "Somatic Complaints", "Anxious/Depressed", and "Social Problems" than those with a normal or irregular pattern ($p = 0.009$, $p = 0.028$, and $p = 0.036$, respectively; see Table 4). No differences were found in the TD control group.

Overall, the RBS-S subscales differed significantly between patients with ASD and the TD control group (see Table 2). Data from all subscales revealed the highest scores for the poor sleepers with ASD, with intermediate scores found in the good sleepers with ASD (although without reaching statistical significance between these 2 subgroups) and the lowest scores in the TD control group. No differences were found between the 2 subgroups of TD controls. All RBS-R items except for "Self-Injurious Behavior" were statistically different between poor sleepers with ASD and TD poor sleepers, with the highest scores found in the ASD group; the same results can be seen between good sleepers with ASD and TD good sleepers.

Regarding *sleep parameters*, in the ASD group "Self-Injurious behavior" correlated with awakenings ($r = 0.407$, $p = 0.006$), sleep efficiency ($r = -0.399$, $p = 0.007$), and WASO ($r = 0.419$, $0 = 0.004$). No correlations were found in the control group.

Correlations between *circadian rhythm parameters* and RBS-S subscales for the ASD group can be found in Table 5.

Table 2 Comparison of CBCL and RBS-S scores (mean ± SD) between ASD patients and TD controls with good and poor sleep

Questionnaires		ASD total (n = 45)	ASD good sleepers (n = 12)	ASD poor sleepers (n = 33)	TD controls total (n = 24)	TD controls good sleepers (n = 14)	TD controls poor sleepers (n = 10)	p-values				
								Overall (ASD vs TD controls)	ASD PS vs ASD GS	ASD GS vs TD GS	ASD PS vs TD PS	TD GS vs TD PS
CBCL	Withdrawn	5.43 ± 4.45	4.25 ± 3.57	5.88 ± 4.71	0.46 ± 0.93	0.36 ± 0.63	0.60 ± 1.26	< 0.001	NS	< 0.01	< 0.001	NS
	Somatic complaints	2.07 ± 2.28	1.75 ± 2.66	2.19 ± 2.16	1.04 ± 2.03	1.29 ± 2.52	0.70 ± 1.05	< 0.05	NS	NS	< 0.05	NS
	Anxious/depressed	5.41 ± 4.26	3.42 ± 2.87	6.16 ± 4.49	1.29 ± 1.98	1.71 ± 2.40	0.70 ± 1.05	< 0.001	NS	< 0.05	< 0.001	NS
	Social problems	5.75 ± 3.22	5.67 ± 3.62	5.78 ± 3.11	0.63 ± 1.05	0.71 ± 1.26	0.50 ± 0.70	< 0.001	NS	< 0.001	< 0.001	NS
	Thought problems	3.07 ± 2.26	2.25 ± 1.71	3.38 ± 2.39	0.04 ± 0.20	0.07 ± 0.26	0.00 ± 0.00	< 0.001	NS	< 0.01	< 0.001	NS
	Attention problems	8.77 ± 3.99	9.00 ± 3.88	8.69 ± 4.09	1.83 ± 2.22	2.00 ± 2.41	1.60 ± 2.01	< 0.001	NS	< 0.001	< 0.001	NS
	Rule-breaking behavior	1.89 ± 1.78	2.17 ± 2.48	1.78 ± 1.47	0.42 ± 1.01	0.50 ± 1.09	0.30 ± 0.94	< 0.001	NS	< 0.01	< 0.001	NS
	Aggressive behavior	7.55 ± 5.99	6.17 ± 4.21	8.06 ± 6.52	4.50 ± 5.54	5.21 ± 6.01	3.50 ± 4.95	< 0.05	NS	NS	< 0.05	NS
RBS-R (mean ± SD)	Stereotypic behavior	5.09 ± 4.78	4.25 ± 3.01	5.39 ± 5.29	0.21 ± 0.50	0.29 ± 0.61	0.10 ± 0.31	< 0.001	NS	< 0.001	< 0.001	NS
	Self-injurious behavior	1.11 ± 1.95	0.50 ± 1.16	1.33 ± 2.14	0.08 ± 0.282	0.14 ± 0.36	0.00 ± 0.00	< 0.05	NS	NS	NS	NS
	Compulsive behavior	3.50 ± 3.87	1.82 ± 1.83	4.06 ± 4.22	0.17 ± 0.482	0.21 ± 0.57	0.10 ± 0.31	< 0.001	NS	< 0.01	< 0.01	NS
	Ritualistic/sameness behavior	8.48 ± 7.87	5.91 ± 5.43	9.33 ± 8.43	0.46 ± 1.61	0.29 ± 1.06	0.70 ± 2.21	< 0.001	NS	< 0.001	< 0.001	NS
	Restrictive behavior	3.78 ± 2.30	3.08 ± 1.73	4.03 ± 2.45	0.38 ± 0.711	0.43 ± 0.75	0.30 ± 0.67	< 0.001	NS	< 0.001	< 0.001	NS

Table 3 Correlations between circadian parameters and CBCL subscales in the ASD group (only those parameters and subscales where significant correlations have been found are displayed)

CBCL	Wrist temperature			Time in movement					
	Mean	VM5	VL10	VL5	IV	NRA	VM10	IS	CFI
Withdrawn	NS	NS	NS	NS	r = 0.40, p = 0.006**	r = −0.38, p = 0.01**	r = −0.38, p = 0.01	r = −0.34, p = 0.02**	r = −0.34, p = 0.02 **
Somatic complaints	NS	NS	NS	r = 0.30, p = 0.04	NS	NS	NS	NS	NS
Thought problems	NS	r = 0.31, p = 0.03	NS	r = 0.30, p = 0.04	NS	NS	NS	NS	NS
Rule-breaking behavior	NS	NS	NS	NS	NS	NS	r = 0.31, p = 0.04	NS	NS
Aggressive behavior	r = 0.30, p = 0.04	NS	r = 0.35, p = 0.02**	NS	r = -0.31, p = 0.03	r = 0.33, p = 0.02	r = 0.37, p = 0.01	NS	NS
Anxious/ depressed	NS	NS	NS	NS	NS	NS	NS	NS	NS

CBCL	Motor activity				Light		Sleep		TAP		
	Mean	VM10	NRA	IS	CFI	VL5	Mean	IV	IV	NRA	VM10
Withdrawn	r = −0.31, p = 0.03**	r = −0.36, p = 0.01**	r = −0.36, p = 0.01**	r = −0.32, p = 0.03**	r = 0.33, p = 0.02**	NS	NS	NS	r = 0.41, p = 0.006**	r = −0.31, p = 0.03**	r = −0.33, p = 0.02**
Somatic complaints	NS	NS	NS	NS	NS	NS	NS	r = 0.38, p = 0.01	NS	NS	NS
Thought problems	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Rule-breaking behavior	NS	NS	NS	NS	NS	r = −0.32, p = 0.03	r = 0.34, p = 0.02	NS	NS	NS	NS
Aggressive behavior	r = 0.40, p = 0.007*	r = 0.42, p = 0.004*	r = 0.42, p = 0.004*	NS	NS	NS	NS	NS	NS	NS	NS
Anxious/ depressed	NS	NS	NS	NS	NS	NS	NS	NS	r = 0.34, p = 0.02	NS	NS

CFI circadian function index, *IS* inter-daily stability, *IV* intradaily variability, *NRA* normalized relative amplitude, *VM5* average measured for the 5 consecutive hours with the maximum values, *VL10* average measured for the 10 consecutive hours with the minimum values, *VL5* average measured for the 5 consecutive hours of minimum values, *VM10* averaged measured for the 10 consecutive hours of maximum values, *TAP* integrated variable known as thermometry, actimetry and body position

*Correlations that remained significant after FDR correction ($p < 0.05$)

**Correlations that remained significant after FDR correction ($p < 0.10$)

No correlation was found between DLMO, melatonin peak levels and melatonin peak time, and the RBS-R subscales. RBS-R did not show differences between the different patterns of DLMO for any group (Table 4).

Sleep and Circadian Parameters and Impact on Families (GHQ28)

The GHQ28 subscale, "Anxiety and insomnia", differed significantly between ASD families and the TD control group (Table 6). The subscales "Somatic Symptoms", "Anxiety and Insomnia", and total score were also different between families of ASD children with poor sleep and families of TD children with poor sleep, with higher values observed in the ASD group. No differences were found between the 2 subgroups of TD controls or between ASD subgroups.

Regarding *sleep parameters,* the GHQ28 subscale, "Somatic Symptoms", correlated with awakenings ($r = 0.336$, $p = 0.02$) and sleep efficiency ($r = -0.331$, $p = 0.03$). Total score and the "Anxiety and insomnia" subscales correlated with WASO ($r = 0.346$, $p = 0.02$ and $r = 0.367$, $p = 0.01$, respectively). In families of TD controls, total score correlated with sleep efficiency ($r = 0.440$, $p = 0.03$).

Data on the correlation between circadian parameters and GHQ28 subscales in the ASD group can be found in Table 7.

No correlation was found in any of the groups between DLMO time, melatonin peak levels and melatonin peak

Table 4 CBCL and RBS-S subscales (mean ± SD or median and interquartile range, IQR, P25, P75) distributed by patterns of DLMO

		Advanced pattern of DLMO (n = 10)	Normal pattern of DLMO (n = 15)	Irregular pattern of DLMO (n = 13)	p-values
CBCL	Withdrawn	5.0 (1.5–6.2)	5.0 (4.0–9.0)	2.0 (1.0–7.0)	NS
	Somatic complaints	0.0 (0.0–1.0)	2.0 (0.0- 4.0)	2.0 (0.5–4.0)	Kruskall-Wallis** Post-hoc: 1–2*/1–3*
	Anxious/depressed	1.5 (1.0–4.0)	8.0 (3.0–10.0)	5.0 (2.5–10.0)	Kruskall-Wallis* Post-hoc: 1–2*
	Social problems	4.77 ± 2.58	7.80 ± 3.48	4.90 ± 2.55	ANOVA* Post-hoc: 1–2*
	Thought problems	2.0 (1.0–3.2)	5.0 (2.0–6.0)	2.0 (1.0–3.5)	NS
	Attention problems	7.60 ± 3.50	10.60 ± 4.32	8.00 ± 3.34	NS
	Rule-breaking behavior	1.0 (1.0–1.2)	1.0 (1.0–4.0)	2.0 (0.5–3.0)	NS
	Aggressive behavior	4.0 (1.5–9.0)	8.0 (5.0–19.0)	6.0 (2.5–12.0)	NS
RBS-S	Stereotypic behavior	2.0 (0.7–5.5)	6.0 (1.0–7.0)	6.0 (2.5–10.0)	NS
	Self-injurious behavior	0.0 (0.0–0.0)	0.0 (0.0–2.0)	0.0 (0.0–4.0)	NS
	Compulsive behavior	2.0 (0.5–3.5)	2.0 (0.0–8.0)	4.0 (0.0–6.0)	NS
	Ritualistic/sameness behavior	3.0 (1.0–10.0)	10.0 (1.0–18.0)	9.0 (2.5–14.5)	NS
	Restrictive behavior	3.10 ± 2.23	3.80 ± 2.90	4.00 ± 2.12	NS

*p < 0.05; **p < 0.01

time, and the GHQ28 subscales. Regarding DLMO patterns, those ASD patients in the subgroup of patients with the advanced pattern showed the lowest scores in "Somatic Symptoms", "Anxiety and Insomnia", and total score (see Table 8). No differences were found in the TD control group.

Discussion

The primary aim of this study was to evaluate the relationship between sleep and circadian rhythm parameters and quantitative behavior difficulties in drug-naïve children and adolescents with ASD. To our knowledge, ours is the first study to include both sleep and circadian variables and caregiver-reported scales.

The main finding is that children and adolescents with poor sleep as detected by ACM differed from those with ASD and good sleep and TD controls on a variety of measures. The CBCL and RBS-R scales showed the highest scores among poor sleepers with ASD, followed by intermediate scores in the ASD good sleepers; individuals in the TD control group showed the lowest scores. Differences were found for all CBCL items between poor sleepers with ASD and TD poor sleepers, with higher scores observed in the ASD group. However, when comparing patients with good sleep (i.e., ASD and TD controls), no differences between groups were found for somatic complaints or aggressive behavior, which suggests that poor sleep could worsen these symptoms in ASD patients. Similar results were described in previous studies, although the study subjects in earlier research were classified as good or poor sleepers based on parental concerns (Aathira et al., 2017; Malow et al., 2006). Nevertheless, previous findings point to higher scores for irritability, hyperactivity, stereotypy, and social withdrawal in poor sleepers as compared to ASD patients who sleep well (Johnson et al., 2018; Malow et al., 2006), which appears to support our results. In contrast, no differences were found between the 2 subgroups of TD controls (good sleepers vs poor sleepers); this finding could be explained by the very low scores shown by the 2 groups and the caregiver-reported scales chosen.

Several dimensions on the CBCL and the RBS-S correlated with specific sleep parameters: sleep fragmentation was associated with somatic complaints and self-injury, while sleep onset difficulties were related to withdrawal, anxiety, and depression, while sleep efficiency was associated with self-injury. Given the nature of this study and its relatively small sample size, we cannot determine causality in these relationships. Our conclusions must be tentative as the findings need to be corroborated by larger studies; nevertheless, our findings may guide future interventional studies, as our results are in agreement with previous reports which concluded that increased awakenings are associated with greater severity of ASD symptoms. Furthermore, the presence of SPs (especially with sleep efficiency) could help to understand the severity of anxiety symptoms observed in ASD (Bangerter et al., 2020; Johnson et al., 2018; Mazurek & Sohl, 2016; Richdale et al., 2014). In addition, problems with sleep onset have been previously associated with worse daytime functioning in ASD (Richdale et al., 2014). However, when analyzing specific behavior difficulties, studies differ in their findings on the concrete relationship between

Table 5 Correlations between circadian parameters and RBS-S subscales in the ASD group (only those parameters and subscales where significant correlations have been found are displayed)

RBS-R	Wrist temperature			Time in movement				Motor activity	Light	Sleep				TAP	
	IS	NRA	CFI	Mean	VL5	NRA	VM10	Mean	L5	IV	IS	CFI	VM5	IS	CFI
Stereotypic behavior	NS	NS	NS	r = 0.35, p = 0.01	NS	r = 0.29, p = 0.04	r = 0.33, p = 0.02	r = 0.31, p = 0.03	NS	NS	NS	NS	NS	NS	NS
Self-injurious behavior	NS	NS	NS	NS	r = 0.40, p = 0.006	NS	NS	NS	NS	r = 0.37, p = 0.01	NS	NS	r = −0.35, p = 0.01	NS	NS
Compulsive behavior	NS	NS	NS	NS	r = 0.37, p = 0.01	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Restrictive behavior	r = −0.30, p = 0.04	r = −0.33, p = 0.02	r = −0.31, p = 0.03	NS	r = 0.33, p = 0.02	NS	NS	NS	r = 0.34, p = 0.02	NS	r = −0.33, p = 0.02	r = −0.34, p = 0.02	NS	r = −0,40, p = 0.006	r = −0.33, p = 0.02

CFI circadian function index, *IS* inter-daily stability, *IV* intradaily variability, *NRA* normalized relative amplitude, *VM5* average measured for the 5 consecutive hours with the maximum values, *VL10* average measured for the 10 consecutive hours with the minimum values, *VL5* average measured for the 5 consecutive hours of minimum values, *VM10* averaged measured for the 10 consecutive hours of maximum values, *TAP* integrated variable known as thermometry, actimetry and body position

Table 6 Comparison of GHQ28 scores between ASD and TD controls with good and poor sleep

		ASD total (n 43)	ASD good sleepers (n 12)	ASD poor sleepers (n 31)	TD controls total (n 24)	TD controls good sleepers (n 14)	TD controls poor sleepers (n 10)	p-values				
								Overall (ASD vs TD controls)	ASD PS vs ASD GS	ASD GS vs TD GS	ASD PS vs TD PS	TD GS vs TD PS
GHQ28	Total	14.09 ± 6.16	12.42 ± 5.14	14.74 ± 6.47	10.92 ± 2.94	11.50 ± 3.43	10.10 ± 1.96	NS	NS	NS	< 0.05	NS
	Somatic symptoms	3.12 ± 2.04	2.42 ± 1.67	3.39 ± 2.14	2.08 ± 1.50	2.43 ± 1.82	1.60 ± 0.69	NS	NS	NS	< 0.05	NS
	Anxiety and insomnia	3.86 ± 2.64	3.25 ± 3.27	4.10 ± 2.37	1.83 ± 1.97	2.21 ± 2.00	1.30 ± 1.88	< 0.05	NS	NS	< 0.01	NS
	Social dysfunction	6.05 ± 1.46	6.25 ± 1.48	5.97 ± 1.47	6.58 ± 0.77	6.43 ± 0.85	6.80 ± 0.63	NS	NS	NS	NS	NS
	Depression	1.09 ± 2.10	0.50 ± 0.67	1.32 ± 2.41	0.42 ± 0.58	0.43 ± 0.51	0.40 ± 0.69	NS	NS	NS	NS	NS

Table 7 Correlations between circadian parameters and GHQ28 subscales in the ASD group (only those parameters and subscales where significant correlations have been found are displayed)

GHQ28	Wrist temperature			Motor activity			Time in movement					
	IS	NRA	CFI	Mean	NRA	VM10	Mean	IS	NRA	VM10	CFI	VL5
Somatic symptoms	NS	NS	NS	$r = -0.31$, $p = 0.03$	$r = -0.34$, $p = 0.02$	$r = -0.35$, $p = 0.02$	$r = -0.32$, $p = 0.00$	$r = -0.35$, $p = 0.01$	$r = -0.36$, $p = 0.01$	$r = -0.34$, $p = 0.02$	$r = -0.36$, $p = 0.01$	NS
Anxiety and insomnia	$r = -0.38$, $p = 0.01$	$r = -0.36$, $p = 0.01$	$r = -0.39$, $p = 0.009$	NS	NS	NS	NS	NS	NS	NS	NS	NS
Social dysfunction	NS	NS	NS	NS	NS	$r = 0.31$, $p = 0.04$	$r = 0.36$, $p = 0.01$	$r = 0.40$, $p = 0.007$	$r = 0.39$, $p = 0.008$	NS	$r = 0.37$, $p = 0.01$	$r = -0.32$, $p = 0.03$

GHQ28	TAP	Sleep			
	IV	VM10	VM5	IV	VL10	CFI
Somatic symptoms	NS	NS	NS	$r = 0.31$, $p = 0.03$	NS	NS
Anxiety and insomnia	$r = 0.30$, $p = 0.04$	NS	$r = -0.34$, $p = 0.02$	NS	NS	NS
Social dysfunction	NS	$r = 0.45$, $p = 0.002$	NS	NS	$r = -0.33$, $p = 0.02$	$r = 0.32$, $p = 0.03$

CFI circadian function index, *IS* inter-daily stability, *IV* intradaily variability, *NRA* normalized relative amplitude, *VM5* average measured for the 5 consecutive hours with the maximum values, *VL10* average measured for the 10 consecutive hours with the minimum values, *VL5* average measured for the 5 consecutive hours of minimum values, *VM10* averaged measured for the 10 consecutive hours of maximum values, *TAP* integrated variable known as thermometry, actimetry and body position

sleep parameters and behavior. In some studies, sleep fragmentation has been associated with restricted/repetitive behaviors, irritability, and hyperactivity (Bangerter et al., 2020; Goldman et al., 2009; Mazurek & Sohl, 2016; Yavuz-Kodat et al., 2020). Also, an association has been found between short sleep duration and repetitive behavior, social skill deficits, and a desire for sameness (Gabriels et al., 2005; Schreck et al., 2004), and clinical improvement has been detected after treatment with prolonged-release melatonin, which led to longer sleep episodes (Schroder et al., 2019). On the other hand, some authors have not found any other correlation between sleep parameters and behavior, aside from the longest sleep episode (Anders et al., 2012; Yavuz-Kodat et al., 2020). Such discrepancies among studies may be attributed to several reasons. First, the age and characteristics of the study population in terms of inclusion criteria are not homogeneous. Second, different actigraphy-related methods were used with different definitions of sleep parameters, which complicates homogeneity. Finally, the design of the studies and the size of the sample do not allow causality or directionality to be established; as a result, larger studies in children with ASD on the relationship between objective sleep parameters and behavioral difficulties are required in order to better establish associations.

Regarding circadian parameters, it is interesting how temperature is related to different subscales of CBCL and RBS-S. Higher temperatures during sleep are related to higher presence of thought problems and higher temperature values during the day, the latter pointing to daytime somnolence, are associated with aggressive behavior. Moreover, these correlations are not limited to temperature values, as low stability of the temperature rhythm is also associated with behavioral difficulties, specifically with restrictive behavior. It is difficult to explain this relationship, as no other studies in ASD have focused on these 2 parameters. However, this finding may lead to new research evaluating circadian parameters such as temperature rhythm in different individuals with ASD classified by neuropsychological profile, as doing so may aid in distinguishing different patterns. As multiple measures were employed, a Benjamini–Hochberg correction was applied to control for false associations (Benjamini & Hochberg, 1995). Using this correction, an association between temperature and thought problems was no longer shown to be significant. In this study we have used 0.10 as the threshold after correction, for considering p-value statistically significant; the reason for this is that, given the exploratory nature of our study, in our opinion it is worth to be less restrictive and describe the maximum number of associational results so that it could be explored in future projects (Bender & Lange, 2001). This approach has been previously described in the scientific literature and it is a common method in studies that simultaneously test multiple hypotheses, such as those involving proteomics o

Table 8 GHQ28 score distributed by patterns of DLMO

		Advanced pattern of DLMO	Normal pattern of DLMO	Irregular pattern of DLMO	p-values
GHQ28	Total	9.60 ± 6.56	14.93 ± 5.7	16.50 ± 7.10	ANOVA* Post-hoc: 1–3*
	Somatic symptoms	1.0 (1.0–1.0)	3.0 (2.0–5.0)	3.5 (2.0–6.7)	Kruskall-Wallis** Post-hoc: 1–2*/1–3*
	Anxiety and insomnia	0.0 (0.0–3.0)	6.0 (3.0–7.0)	4.5 (3.0–7.0)	Kruskall-Wallis** Post-hoc: 1–3*/1–2*
	Social dysfunction	7.0 (5.0–7.0)	6.0 (6.0–7.0)	6.5 (5.0–7.0)	NS
	Depression	0.0 (0.0–0.0)	0.0 (0.0–1.0)	1.5 (0.0–3.7)	NS

*$p < 0.05$; **$p < 0.01$

genomics (Benjamini & Hochberg, 1995; Glickman et al., 2014; Korthauer et al., 2019). FDR allows control for type I error rates in those studies that suffer from reduced sensitivity and specificity due to a large number of analysis or database size. Although controlling for type I errors sounds ideal, by decreasing the false positives we can increase the number of false negatives, where there is a real effect but we fail to detect it. Therefore, when false negatives are important for future research it could be worth to be less restrictive. Applying strategies such as FDR can result in a better interpretation of data so that the reader can interpret the results in a more informed manner.

Motor activity showed multiple correlations with aspects of behavior. Higher time in movement during sleep, which points to sleep fragmentation, was associated with thought problems, restrictive behavior, somatic complaints, compulsive behavior, and sensorial and self-injurious behavior. Furthermore, increased daytime motor activity is associated with "aggressive", "stereotypic", and "rule-breaking behavior". In contrast, scores for the "withdrawn" scale were associated with low motor activity and low stability of its rhythm. After applying Benjamini and Hochberg procedure, the association between motor activity and "withdrawn" and "aggressive" behavior was still significant, especially with "aggressive" behavior. All this information suggests that ACM devices could be a useful tool for detecting or confirming certain behavioral concerns that involve increased or decreased motor activity. Furthermore, the association between sleep fragmentation, as indicated by higher time in movement during sleep, and behavior difficulties requires deeper study of the presence of restless sleep disorder in ASD individuals. Although the diagnosis of restless sleep disorder cannot be established in our sample given the requisite of polysomnography testing (DelRosso et al., 2020), we believe further research is necessary to better define the prevalence and consequences of this disorder in individuals with ASD, as data point to sleep fragmentation and movement during sleep as 2 of the main factors influencing behavior in ASD.

In this study, we also report that a lower percentage of hours of nighttime sleep and lower stability of motor activity, sleep, and light exposure are associated with restrictive behavior, self-injurious behavior, somatic complaints, and withdrawal. Though no causal relationships can be drawn from our data, these results are in agreement with previous studies that found higher variability and a decreased strength of the circadian rhythm in those with more pronounced core autism spectrum traits (Elkhatib Smidt et al., 2021; Yavuz-Kodat et al., 2020).

Regarding melatonin secretion and its relationship with behavior, an analysis that differentiated between DLMO patterns revealed that only those with an advanced pattern showed lower scores for "Somatic Complaints", "Anxious/Depressed" state, and "Social Problems". The few studies that have focused on this relationship produced contrasting results (Babinska et al., 2019; Tordjman et al., 2012). It has been described that nighttime melatonin excretion rate is negatively correlated with severity of autistic impairment, especially in imitative social play and repetitive use of objects (Tordjman et al., 2012); however, using a similar methodology, Babinska et al. did not observe these same correlations (Babinska et al., 2019). Some important differences make it difficult to compare our findings to this earlier research, as our method of melatonin sampling and the questionnaires chosen differ from both of the earlier studies. Previous studies of DLMO in ASD individuals (Baker et al., 2017; Goldman et al., 2014, 2017) were composed of adults and adolescents, with the exception of Goldman et al. (Goldman et al., 2014) who reported on nine prepubertal autistic children; none of these studies included data about behavior. In our previous study (Martinez-Cayuelas et al., 2022) we found that those patients in the ASD subgroup exhibiting the advanced pattern and those with later DLMO were more likely to have later bedtimes, prolonged sleep onset latencies, and increased number of awakenings. This appears to support the notion that the "advanced pattern" of DLMO plays a protective role in sleep and behavioral difficulties, although these results should be corroborated

in larger projects. Nevertheless, our results have to be taken with caution given the small sample size.

Concerning the impact on family life, "Somatic Symptoms", "Anxiety and Insomnia", and total score were higher in families of ASD children with poor sleep compared to families of TD children with poor sleep, and no differences were found between ASD subgroups. However, when analyzing sleep parameters, sleep fragmentation and low sleep efficiency were associated with these symptoms. Some authors have obtained similar results, finding an association between stress and depression rates with sleep fragmentation and sleep efficiency (Johnson et al., 2018; Leader et al., 2022; Schroder et al., 2019).

The main strength of our study is that it combines ACM findings and melatonin in saliva with parent-completed reports of autism symptoms. In our opinion, using ACM devices adds valuable and accurate information that would not be possible obtain using standard actigraphy, especially when analyzing circadian parameters such as temperature rhythm which is closely related to sleep physiology (Ortiz-Tudela et al., 2010; Sarabia et al., 2008). We limited our sample to children and adolescents with no medication or epileptic seizures, as these factors could contribute to heterogeneity and can also affect sleep. In addition, we isolated a subset of children with ASD with good sleep, while most investigators have combined all children with ASD into one group for comparison with TD controls. Furthermore, few studies have focused on circadian rhythm and its relation to daytime behavior.

Several limitations should be considered when interpreting this study. Firstly, our findings cannot determine causality, that is, whether good sleep positively influences behavior or vice versa. Future studies should examine pre- and post-intervention behavior in these patients. Secondly, though our study sample is larger than most studies focusing on objective data for circadian rhythm and sleep parameters, it may not possess sufficient statistical power to show differences between groups. This study should be viewed as exploratory and its results should be used as a guide for future projects with larger samples and more covariates (such as parental education, race/ethnicity, and residential area). In addition, our study included only non-medicated children and excluded specific genetic conditions such Fragile X and comorbid disorders such as ADHD. Given the high levels of comorbidity in the autistic population with other genetic neurodevelopmental disorders and psychopathologic, it is not possible to generalize our findings to the wider clinical ASD population. Other aspect of this study to bear in mind are: the fact that the MCA device registered only data for a single week and that saliva samples were obtained in a single night. This would fail to capture variation in sleep and other circadian parameters. Future projects should ideally gather information across a larger range of timepoints. The current study presented data on a wide age (5–18 years) and was unable to investigate or control for potential confounders such as diet and stress.

Conclusions

Behavioral difficulties are closely related to sleep and circadian rhythm disturbances in ASD. In particular, sleep fragmentation seems to be a meaningful parameter, as it was involved most of the behavioral traits studied. Thus, sleep fragmentation should be prioritized when examining sleep and behavioral phenotypes in ASD. Additionally, we found that the advanced pattern of melatonin secretion seems to play a protective role for sleep and behavioral difficulties. In sum, sleep and circadian rhythm disturbances should be assessed daily in these patients and included in behavioral and pharmacological management strategies in order to develop comprehensive intervention programs for children with ASD and their families.

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Declarations

Conflict of interest The author(s) declare no potential conflict of interest with respect to the research, authorship, and/or publication of this article.

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