HHS Public Access

Author manuscript

J Autism Dev Disord. Author manuscript; available in PMC 2018 June 01.

Published in final edited form as:

J Autism Dev Disord. 2017 June ; 47(6): 1682–1695. doi:10.1007/s10803-017-3089-1.

Characterizing Sleep in Adolescents and Adults with Autism Spectrum Disorders

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Abstract

We studied 28 adolescents/young adults with autism spectrum disorders (ASD) and 13 age/sex matched individuals of typical development (TD). Structured sleep histories, validated questionnaires, actigraphy (four weeks), and salivary cortisol and melatonin (four days each) were collected. Compared to those with TD, adolescents/young adults with ASD had longer sleep latencies and more difficulty going to bed and falling asleep. Morning cortisol, evening cortisol, and the morning-evening difference in cortisol did not differ by diagnosis (ASD vs. TD). Dim light melatonin onsets (DLMOs) averaged across participants were not different for the ASD and TD participants. Average participant scores indicated aspects of poor sleep hygiene in both groups. Insomnia in ASD is multifactorial and not solely related to physiological factors.

Keywords

melatonin; cortisol; actigraphy; Adolescent Sleep Wake Scale; Adolescent Sleep Hygiene Scale

Autism spectrum disorder (ASD) is one of the most common neurodevelopmental disorders with a prevalence of up to 1 in 68 children, characterized by core deficits in social

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Authors have no conflict of interest.

Compliance with Ethical Standards:

Ethical approval:

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent: Informed consent was obtained from all individual participants included in the study.

communication and restricted interests with repetitive behaviors (Christensen et al. 2016). Sleep difficulties are common in individuals with ASD. The causes of sleep problems in ASD are multifactorial, involving factors intrinsic to ASD (neurotransmitter abnormalities) as well as medical (gastrointestinal disorders, epilepsy) and behavioral (poor sleep habits) etiologies (Reynolds and Malow, 2011). Consequences of sleep difficulties include challenging daytime behavior such as physical aggression, irritability, inattention, and hyperactivity (Mazurek and Sohl, 2016). Sleep difficulties in children with ASD have been studied extensively, with 50–80% of children affected. Difficulty falling asleep (sleep onset insomnia) is the most common sleep concern parents express (Krakowiak et al. 2008; Mayes & Calhoun 2009; Williams et al. 2004). These previous publications, as well as the majority of literature in the field, has included children only. There is a paucity of literature on sleep patterns in adolescents and young adults with ASD.

A few studies have shown that sleep difficulties are a lifelong condition in this population, continuing to persist into adolescence (Goldman et al. 2012) and adulthood (Tani et al. 2003; Matson et al. 2008; Limoges et al. 2005). One of the first studies to examine sleep disturbances in adolescents and young adults with ASD documented low sleep efficiency and prolonged sleep latency by actigraphy in 80% of individuals (Oyane et al. 2005). In a large registry study (n = 1859), adolescents with ASD had more problems with delayed sleep onset, shorter sleep duration, and daytime sleepiness on the Children's Sleep Habits Questionnaire compared to younger children with ASD (Goldman et al. 2012).

Only a few studies have compared sleep patterns in adolescents and adults with ASD and individuals of typical development (TD). In a study using actigraphy to compare sleep patterns in adolescents with ASD to an existing database of age and sex-matched typically developing controls, Baker et al. (2013) documented longer sleep latencies and reduced sleep efficiency in adolescents with high-functioning ASD. They also found that the ASD group was three times more likely to report a sleep problem than the TD group. Both groups displayed a later bedtime on weekends compared to school days, although the ASD and TD groups did not differ significantly in bedtimes.

Two studies of young adults found similar results to the adolescent work. Baker and Richdale (2015) found that adults with high functioning ASD (ages 21–44 years) had higher scores on the Pittsburgh Sleep Quality Index, longer sleep latencies by actigraphy, and poorer sleep efficiency by diary compared to typically developing adults. In one of the few studies to include polysomnography in adolescents/adults with ASD (ages 16–27 years), compared with typically developing adolescents/adults, those with ASD had a longer sleep latency, more frequent nocturnal awakenings, lower sleep efficiency, and increased N1 sleep (Limoges et al. 2005).

The physiological correlates of sleep and circadian rhythms in adolescents and adults with ASD have also received sparse attention. Cortisol, a marker of the hypothalamic-pituitary-adrenal (HPA) axis, demonstrates a circadian rhythm, with levels highest in the morning and falling in the evening as bedtime approaches. Dysregulation of the cortisol rhythm, with diminished reduction of the expected fall in evening cortisol, has been observed in insomnia (Buckley & Schatzberg 2005) and also in ASD (Corbett et al. 2008; Tomarken et al., 2015)

in association with daytime stressors such as fears or a change in routine (Corbett et al. 2009). Tomarken et al. (2015) identified a subset of children with ASD showing an attenuated linear decline in evening cortisol, and encouraged follow-up studies to investigate the functional significance of their findings, including effects on sleep disturbance.

Melatonin, synthesized in the pineal gland from serotonin, regulates the sleep-wake cycle in humans (Ackermann & Stehle 2006; Brzezinski 1997; Masana & Dubocovich 2001) with the dim light melatonin onset (DLMO) occurring approximately 2–3 hours before sleep onset (Burgess & Fogg 2008). Dysregulation of biological pathways maintaining adequate levels of salivary melatonin have been proposed to contribute to the expression of sleep disturbances in ASD (Glickman 2010). Lower levels of nocturnal (Nir et al. 1995; Kulman et al. 2000) and daytime (Melke et al. 2008) blood melatonin levels and lower levels of its primary metabolite 6-sulfoxymelatonin (Tordjman et al. 2005) have been observed in individuals with ASD when compared to typically developing controls. In contrast, a study conducted in a small subset of children with ASD and well-defined comorbid sleep onset insomnia observed *normal* overnight blood melatonin profiles. These children subsequently exhibited a therapeutic response to supplemental melatonin (Goldman et al. 2014). Maximal melatonin concentration and time to peak concentration were comparable to those previously published in the literature for typically developing children. Dim light melatonin onsets (DLMO), measured by blood sampling, were captured in the majority of children. Adding further complexity to the melatonin literature in ASD, Braam and colleagues (2013) documented *elevated* salivary melatonin levels (>50 pg/ml) or prolonged half-lives (>5 hours) in 15 intellectually-disabled children and adolescents (seven with ASD) with sleep onset insomnia and evidence of disappearing effectiveness of exogenous melatonin. However, salivary melatonin was sampled during the day, at noon and at 4 pm. To our knowledge, no studies of evening salivary melatonin in children, adolescents, or young adults with ASD have been published.

A better understanding of sleep patterns in this population, in combination with physiological measures, is important for the development of targeted interventions to improve sleep in this population. Age-matched individuals with typical development were included as a comparison group. The aim of this study was to define sleep patterns in a carefully phenotyped cohort of adolescents and young adults with ASD using a combination of parent and participant report, sleep questionnaires, and actigraphy measurements, as well as two physiological markers of sleep and circadian rhythms-- salivary melatonin and cortisol levels. Our hypotheses were that in comparison to those of typical development, individuals with ASD would have prolonged sleep latencies (e.g., increased time to fall asleep), along with abnormalities in salivary cortisol and melatonin that explained the prolonged sleep latencies. In contrast to prior work, we emphasized adolescents and adults, an understudied population, and physiological measures.

Methods

Participants

This study received approval from our Institutional Review Board (IRB). Participants included adolescents and young adults with ASD, and TD participants with an age and sex

match ratio of 2:1 (for every two individuals with ASD, we planned enrollment of an age and sex-matched control). The 2:1 ratio was used as it was challenging to recruit individuals who were TD. Participants from a variety of settings (rural, urban, and suburban) were recruited via flyers distributed in our clinics, letters, websites, emails, and through Subject Locator (an IRB-approved tool available through our electronic medical record which identifies potential participants for specific studies). Potential participants were screened by telephone interview and review of their electronic medical record (including ensuring that the TD participants did not have a diagnosis of ASD). Study inclusion criteria (for both the ASD and TD participants) included: (a) age 11–30 years; (b) having entered puberty based on a parent-completed rating scale (Petersen et al. 1998); and (c) not taking medications or on a stable dose of medication for at least 30 days without medication changes anticipated during the study period.

Adolescents/young adults taking supplemental melatonin were allowed to participate in the study as long as they were on a stable dose for at least 30 days; however, these individuals did not participate in melatonin sampling and instead participated in salivary cortisol sampling. Participants with ASD had a clinical diagnosis (autistic disorder, pervasive developmental disorder not otherwise specified, or Asperger syndrome) made on the basis of the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV-TR) (4th ed., American Psychiatric Association 1994) criteria and confirmed by the Autism Diagnostic Observation Schedule (Lord et al. 2000), which was done by a psychologist who was research-reliable on the measure during the screening process. Individuals (both the ASD and TD participants) were excluded if they were taking stimulant medications (given their adverse effects on sleep), or if they had untreated medical conditions impacting sleep (e.g., epileptic seizures, gastrointestinal reflux disease, mood disorders, untreated sleep apnea, or frequent parasomnias (greater than once a week), based on a structured sleep history conducted by two of the authors with expertise in sleep and autism). To be able to generalize our results to the larger ASD population, we included subjects taking psychotropic medications other than stimulants. Medications taken are listed below in the results section. Adult participants provided consent. Adolescent participants provided assent with their parents providing consent. Participants were compensated for their time upon completion of study procedures.

Design and Study Procedures

This study was a cross-sectional, observational study. Subjects were enrolled between April 2012 and May 2015, and each participant was in the study for a total of 28 days.

Sleep Histories and Survey Forms

Participants (parent and adolescent/independent adult) had an in-person or telephone structured sleep interview with one of the two author clinicians who specialize in sleep concerns in adolescents and adults with autism. The interview reviewed the individual’s sleep and medical history. The Adolescent Sleep-Wake Scale and the Adolescent Sleep-Hygiene Scale were also completed to provide standardized data on the participant's sleep. Participants and their parents completed these surveys together.

Adolescent Sleep Wake Scale (ASWS)—The ASWS (LeBourgeois et al. 2005) consists of 28 items in five dimensions focused on the adolescent's sleep in the past month: going to bed, falling asleep, maintaining sleep, reinitiating sleep, and returning to wakefulness. Response choices are on a six-point Likert-type scale ranging from 1 ("always") to 6 ("never") (Shahid et al. 2012). Internal consistency of this measure ranges from 0.80 to 0.86 for subscales (LeBourgeois et al. 2005). Higher scores indicate better sleep quality. The Going to Bed subscale consists of five questions related to going to bed -- wanting to stay up and do other things, having trouble going to bed, ready to go to bed (reverse scored), enjoying bedtime (reverse scored), and trying to delay going to bed. The Falling Asleep subscale includes six questions related to going to sleep -- having trouble settling down, feeling sleepy (reverse scored), lying down but then getting up and coming out of the bedroom, having trouble going to sleep, needing help getting to sleep, and falling asleep quickly (reverse scored).

Adolescent Sleep Hygiene Scale (ASHS)—The ASHS (LeBourgeois et al. 2005) consists of 28 items in nine dimensions focused on sleep hygiene in the past month: physiological (5-items), cognitive (6-items), emotional (3-items), sleep environment (4-items), daytime sleep (1-item), substance use (2-items), bedtime routine (1-item), sleep stability (4-items), and bed/bedroom sharing (2-items). Cronbach's alpha for the full scale is 0.8 (LeBourgeois et al. 2005). Response choices are on a six-point Likert-type scale ranging from 1 ("always") to 6 ("never"). Higher scores indicate better sleep hygiene.

Actigraphy

Each participant wore an AW Spectrum Actiwatch® (Philips Respironics, Bend, OR) on the non-dominant wrist. Implementing a procedure used in a previous study of sleep education for children (Malow et al. 2014), the actigraphy watches were configured to a 1-min epoch, with a sleep interval of 10 epochs for onset of sleep and an awake threshold setting of 40 (medium), based on previous work (Malow et al., 2014). The Philips Respironics Actiware software algorithm was used to extract sleep variables from the actigraphy data. Based on bedtimes and wake times reported on parent/participant completed sleep diaries and event markers, rest intervals were set, which then calculated the sleep variables. Both parent and participant were trained to use the actigraphy watch through a structured session during the initial sleep screening visit according to standardized procedures (Fawkes et al. 2014). The training included demonstration of watch use by study staff, a return demonstration by parent and participant, and discussion of wearing the watch in a series of scenarios. Parent and participant also were required to achieve 80% mastery on a brief quiz covering essential instructions of the actigraphy watch. They were given written instructions, including a simple watch diagram to take home for reference. Four consecutive weeks of actigraphy was obtained to assess sleep/wake patterns in the home environment. The initial 8 days was obtained with the salivary sampling period, followed by an additional 20 days.

Melatonin Sampling

Melatonin salivary collection was collected over four nights, starting at 6:00 pm and repeated every 30 minutes until bedtime. This timing allowed for the capture of the dim light melatonin onset (DLMO), which usually occurs two to three hours before the individual's

habitual sleep onset time (Burgess & Fogg 2008). Sampling Thursday through Sunday provided weekday and weekend samples; in all participants, at least one weekday and one weekend sample was successfully collected. Participants used Salivette© devices to collect saliva. During the DLMO collection, we followed specific procedures, as outlined below, recommended in the literature (Crowley 2013 and personal communication, Dr. Helen Burgess). Each participant was in dim lighting (<30 lux) and was instructed to be at least six feet away from televisions, computers and other light-emitting devices. The lux level was confirmed by parents who used light meters (Extech Instruments, Nashua, NH) near the participants' eyes. The parents measured light at the beginning of the collection period and the participants remained in that light level or lower during the collection process. The light sensors present on the actigraphy devices allowed for additional confirmation that salivary melatonin samples were collected in the proper light level; participants were instructed to wear short sleeve shirts or pull up sleeves to allow for proper detection of light. Any participants taking supplemental melatonin were excluded from the study due to the interference of the melatonin salivary samples. Caffeine, alcohol, and smoking were not permitted for 24 hours prior to collection or during the collection process; snack restrictions included Gatorade®, bananas, and chocolate based on their possible interference with melatonin sampling. Participants were instructed to rinse their mouths with water after eating a snack or having a drink and to refrain from brushing teeth with toothpaste, or taking non-steroidal anti-inflammatory medications such as ibuprofen, during sampling. The melatonin samples were kept frozen and shielded from light directly after collection.

In a subset of nine ASD and seven TD participants, melatonin salivary samples were centrally analyzed by Solid Phase, Inc (Portland, Maine) by using the Bühlmann Direct Saliva Melatonin RIA kit (ALPCO Diagnostics, Windham, NH). The first non-zero standard of this assay was 0.5 pg/mL. Intraassay coefficients of variation for low, medium, and high levels of salivary melatonin are 20.1%, 4.1%, and 4.8%, respectively. The interassay coefficients of variation for low, medium, and high levels of salivary melatonin are 16.7%, 6.6%, and 8.4%, respectively. We initially used a different laboratory that used enzyme-linked immunosorbent assay for analysis of samples; however, results were highly variable and difficult to interpret. Thus samples reported are limited to those analyzed by Solid Phase.

Cortisol Sampling

Salivary cortisol was collected immediately before bedtime and immediately upon awakening for four days starting on the morning after the last night of melatonin sample collection was completed (total collection of eight samples/participant). Participants were asked to passively drool in a small test tube (at least 0.5 ml) each morning and evening before brushing teeth and during the evening sample the participant rinsed their mouth at least 30 minutes before collecting the salivary sample. The cortisol samples were frozen immediately after collection. For cortisol sampling, we followed similar procedures to that of melatonin collection, as outlined above.

The cortisol samples were analyzed using established protocols (Arch 2014; Gozansky 2005). Samples were stored in a 70°C freezer until all samples from the same subject could

be processed in the same assay. The salivary cortisol concentration was determined using a commercial expanded range high sensitivity enzyme immunoassay kit (Salimetrics; State College, PA) that detects cortisol levels in the range of 0.083–82.77 nmol/L Samples with duplicate CVs greater than 10% were rerun in triplicate and the median value of the rerun was reported. Inter- and intra-assay coefficients of variability were 2.6 and 9.6%, respectively.

Other Procedures

Families completed forms providing information about their education backgrounds and brief descriptions of their occupations. These data allowed us to provide estimates of socioeconomic status (SES) based on the Hollingshead Four Factor Index of Social Status (Hollingshead 1975). The Wechsler Abbreviated Scale of Intelligence-Second Edition (WASI-II; Wechsler 2011) was given as a measure of cognitive ability and the Vineland-II (Sparrow and Cicchetti, 1985) as a measure of adaptive behavior. The Pubertal Developmental Scale was completed by the adolescent's parent or young adult as a measure of pubertal development (categories 1 through 5 with higher categories indicating greater pubertal development). Participants who had entered puberty (category 2 or higher) were included, and those who had not entered puberty (category below 2) were excluded.

Data Analysis

Preliminary analysis showed that the actigraphy parameters in the sampling period for cortisol and melatonin (week 1) were similar to the rest of the actigraphy sampling period and that saliva sampling did not further disrupt sleep. Therefore, to increase precision, sleep parameters for the entire actigraphy sampling period were averaged across nights for each participant to compare sleep latency, total sleep time, sleep efficiency, and wake time after sleep onset as well as bedtime (derived from event markers in the actigraphy watches indicating bedtime and wake time, and sleep diaries that accompanied actigraphy), time the participant fell asleep (derived from the sum of bedtime and sleep latency) and morning wakeup time (from actigraphy). For the cortisol and melatonin analyses, only week 1 data were used given that cortisol and melatonin sampling occurred during week 1. Cortisol values were averaged across the four sample collection days to determine a morning and evening mean value for each participant. To measure the change in cortisol from morning to evening, for each participant, we calculated the difference between morning mean cortisol values and evening mean cortisol values. Cortisol measurements were log (10) transformed to achieve approximate normality and to be consistent with the literature in ASD (Corbett, 2009). The time of the dim light melatonin onset (DLMO) was configured using linear interpolation by one of the authors with expertise in melatonin interpretation (HJB), using a 3K threshold for the sampling nights, averaged across nights for participants (Voultsios et al. 1997). Data on bedtime (based on sleep diaries and event markers) and sleeptime (based on actigraphy) from the cortisol and melatonin sampling period (week 1) were analyzed in relation to evening cortisol and DLMO. We also examined the relationship of sleep latency to evening cortisol, morning cortisol, and the difference between morning and evening cortisol. The relationship of DLMO to evening cortisol and the difference between morning and evening cortisol were also examined. To accomplish this, the timing of DLMO (hours)

relative to 6 pm was calculated and its correlation with the cortisol levels was then estimated. A partial correlation was also performed to adjust for diagnosis (ASD vs. TD).

Weekday and weekend DLMO (Saturday and Sunday) values were analyzed separately and were found to be comparable with overall values so only the overall values are reported.

Descriptive statistics were conducted on all major variables. Two sample t-tests were used to compare continuous variables between the ASD and TD groups. Pearson correlations were used to assess the association between age and cortisol, sleep latency and cortisol, and DLMO (relative to 6 pm) and cortisol. A Spearman correlation was used to examine the association between cortisol and pubertal development (as the pubertal development score is an ordinal variable). Linear regression models were used to examine the contributions of (1) diagnosis (ASD vs. TD), age and bedtime to sleep latency and (2) evening cortisol, age, and diagnosis to bedtime. An analysis of variance compared medicated individuals with ASD, medication-free individuals with ASD, and those with TD. A p-value of less than .05 was considered statistically significant. Given the pilot nature of this study, we presented means, standard deviations, and uncorrected p-values for all parameters analyzed. Analyses were performed using IBM SPSS version 23 (New York, NY) and SAS version 9.3 (SAS Institute, Cary, NC).

Results

Participants

Of 43 participants with a clinical diagnosis of ASD who were consented, 15 screen-failed or withdrew from the study and 28 completed the study. Reasons for screen failure included: not having a clear diagnosis of ASD, confirmed by history and study measures (six participants), taking medication affecting sleep (dextroamphetamine; one participant), or did not meet criteria for entry into puberty based on the pubertal developmental scale (one participant). Reasons for study withdrawal included: could not follow study procedures (two participants), had behavioral concerns precluding participation in study (one participant), and chose not to continue (four participants).

Of 18 participants who were TD, 5 screen-failed or withdrew from the study and 13 completed the study. Reasons for screen failure included: working nights which confounded sleep and circadian rhythm assessment in one participant, and atypical behaviors (e.g., poor eye contact, very anxious, repetitive language) in one participant. Reasons for study withdrawal included: chose not to continue the study after filling out the surveys (two participants), and could not follow study procedures (one participant).

See Table 1 for demographics. For 26 of the 28 participants with ASD who completed study procedures, there was an age and sex-matched TD individual. The age of the participants ranged from 11 to 26 years (four ASD participants and two TD participants were 18 years or older) and all participants were either white or African-American. All participants were non-Hispanic. Psychotropic medications were taken by 12 participants with ASD and none with TD. Medications included: aripiprazole, guanfacine, valproic acid, hydroxyzine, fluoxetine, sertraline, escitalopram, melatonin, diphenhydramine, lisdexamfetamine, clonidine, and

bupropion. To minimize the effects of seasonal sampling on melatonin or cortisol, only three participants, all with ASD, had sampling of melatonin and cortisol during the summer months and their results were similar to those sampled in winter months. All of the participants with ASD were regularly attending school and living at home with their parents, except for one participant who was living independently and working in a hardware store. The participants with TD were all regularly attending school and living at home with their parents except for one living independently.

Sleep Parameters

On the structured sleep histories, eight participants with ASD reported sleep onset delay of 30 minutes or greater. Parent and participant report were comparable with the exception of only one parent-participant pair where the participant denied sleep problems and the parent reported sleep onset delay. One adolescent with ASD and sleep onset delay said he went to bed because it was when his mother said to go to bed, but that he was not necessarily sleepy. He stated that his “brain wouldn’t shut down” and he would think of random things. None of the participants had symptoms suggestive of sleep disordered breathing, sleepwalking, or dream-enacting behavior.

The ASWS Total Score and the Going to Bed and Falling Asleep subscales were significantly lower for participants with ASD (Table 2), indicating that they had more difficulty going to bed and falling asleep than participants with TD. Scores for maintaining sleep, reinitializing sleep, and returning to wakefulness did not differ between the two groups. The ASHS did not significantly differ for participants with ASD and TD (Table 3); a trend toward significance was noted for the emotional subscale and total scale (p values < 0.1). However, as compared to other reports using the ASHS (LeBourgeois 2005; Tan 2012), average participant scores on the ASHS for both the ASD and TD groups were relatively low, consistent with poor sleep hygiene. Both the ASD and TD groups had scores of 4 or less in the cognitive factor, sleep stability factor, and in the bed/bedroom sharing factor.

Actigraphy parameters are presented in Table 4. Sleep latency (SL) was longer and sleep efficiency (SE) was lower for the participants with ASD. Wake time after sleep onset (WASO) and total sleep time (TST) did not differ between the groups.

Referring to sleep diaries and event markers (indicating bedtime) that accompanied the actigraphy data, average bedtimes were approximately 30 minutes (overall), 34 minutes (weekdays) and 19 minutes (weekends) earlier for the participants with ASD compared to the participants with TD (Table 5), although statistical significance was not achieved. In the ASD group, three participants had a weekday bedtime earlier than 9 p.m. and six participants had weekday bedtimes earlier than 10 p.m. In the TD group, no participants went to bed before 9 p.m and three participants went to bed earlier than 10 p.m. On weekends, six ASD participants went to bed before 10 p.m. None of the TD participants went to bed before 10 p.m on weekends. Sleep times and wake times did not differ between ASD and TD participants.

In a regression model incorporating sleep latency as the dependent variable and diagnosis and bedtime as independent variables, diagnosis (B = 18.598; p = 0.014) was significant

with the ASD group having longer sleep latency, and bedtime was not significant. A second model also included age, along with diagnosis and bedtime, as independent variables (sleep latency was the dependent variable). Bedtime and age were not significant and diagnosis remained significant (B = 18.871; p = 0.013).

Cortisol

Cortisol was collected in 26 participants with ASD and 13 participants who were TD. In all of the TD individuals, and all but two of the individuals with ASD, evening cortisol was lower than morning cortisol. In one of these individuals (ASD 9), the average bedtime was very late (1:59 am). In the second individual (ASD 4), the participant reported a variable sleep schedule (including staying up all night and then going to bed at 4 pm).

In the complete dataset, morning cortisol, evening cortisol, and the morning-evening difference in cortisol did not significantly differ by diagnosis (ASD vs. TD) and were not related to age or pubertal development. After excluding the two outliers with ASD noted above (who had evening cortisol values lower than morning cortisol values) the ASD and TD groups still did not significantly differ in morning, evening, or the morning-evening difference in cortisol. In the groups combined, there was no significant correlation between sleep latency and morning (r = 0.019; p =0.908), evening (r =−0.153; p =0.353), and the morning-evening difference (r =0.150; p =0.361), in cortisol. As medications can affect cortisol, we performed an analysis of variance of medicated individuals with ASD compared to medication-free individuals with ASD and those with TD (who were all medication-free). There were no significant differences in morning (F = 0.881; p = 0.354), evening (F = 0.004; p = 0.949), or the morning-evening difference in cortisol among groups (F = 0.111; p = 0.741).

Dim Light Melatonin Onset (DLMO) profiles

Profiles of DLMO patterns were available for 7 ASD and 7 TD participants. Figure 1 shows that all of the ASD and TD participants exhibited DLMOs with the exception of ASD 4 and 5. ASD 4 had an erratic bedtime routine and did not sleep several nights around salivary sampling nights. ASD 5 had sensory aversion to placing cotton swabs in her mouth so challenges with sample collection may have played a role in the absent DLMO.

Table 7 presents the mean DLMO time, the mean bedtime and sleep time, and the differences between DLMO and the mean bedtime and sleep time. Each of these variables was compared across diagnosis (ASD vs. TD) and significance was not achieved (p > 0.10). It is notable, however, that individuals with ASD went to bed, on average, 49 minutes earlier than those with TD. This difference was even more pronounced when comparing earliest (58 minute difference) and latest (1 hour and 13 minute difference) bedtimes. In contrast to bedtime, the mean sleep time was only 30 minutes different between the ASD and TD participants.

The DLMOs averaged across participants were not different for the ASD and TD participants and were within 11 minutes of each other, occurring around 21:00. While there was individual variability, the majority of ASD and TD participants showed the expected 2–3 hour window between DLMO and sleep time (Burgess & Fogg 2008). Participant ASD 6

had a bedtime which preceded the DLMO; all other participants had DLMOs which preceded bedtimes.

Evening cortisol was not correlated with DLMO ($r = 0.268$; $p = 0.354$). The morning to evening difference in cortisol was negatively correlated with DLMO ($r = -0.626$; $p = 0.017$) even after adjusting for diagnosis (partial correlation of -0.627; $p = 0.022$). This finding suggests that a lower drop in cortisol is related to a later DLMO across participants, and is not limited to those with ASD or TD.

Discussion

To our knowledge, this study is the first to combine parent and child report of sleep with validated questionnaires, actigraphy, and measurements of salivary cortisol and melatonin, and examine the relationships between these variables, along with the inclusion of a TD age and sex-matched control group. We had hypothesized that individuals with ASD would have longer sleep latencies and have abnormalities in salivary melatonin and cortisol.

Our findings support our first hypothesis. Sleep latency, as measured by actigraphy, was longer in adolescents/adults with ASD. Those with ASD also had more difficulty going to bed and falling asleep on a validated questionnaire compared to their TD counterparts. Our findings in adolescents/adults are consistent with those previously reported in children (Allik, 2006; Krakowiak, 2008).

Review of sleep diaries and event markers from actigraphy showed that those with ASD also went to bed earlier, particularly on weekdays than those with TD, although the difference did not reach statistical significance, likely due to our small sample size and variability among participants. In models adjusting for diagnosis and bedtime, and for diagnosis, bedtime and age, only diagnosis was significantly associated with sleep latency. Our findings do not support our second hypothesis, that individuals with ASD have abnormalities in salivary melatonin and cortisol. While lower levels of nocturnal (Nir et al. 1995; Kulman et al. 2000) blood melatonin levels have been observed in individuals with ASD when compared to typically developing controls, methodological differences in these studies compared to ours may explain the different results. For example, we sampled salivary melatonin over more frequent time intervals. All of the participants with TD and most of the participants with ASD had higher cortisol in the morning compared with evening levels. The diurnal decline in cortisol throughout the day, with lowest levels in the evening, has been well established (Anders 1982; Weitzman 1971).In ASD, an attenuated linear decline in cortisol has been observed in many children with ASD (Tomarken et al. 2015), with this subset postulated to have associated sleep disturbance or dysregulation in arousal. Two adolescents with ASD had higher cortisol at night than in the morning. Of note, one participant had an erratic sleep/wake schedule, and one had a very late bedtime. While these bedtime schedules may have biologically contributed to our findings of a higher cortisol at bedtime than in the morning, it is also possible that technical factors were at play; their erratic bedtime schedules may have been associated with their not being meticulous collectors of cortisol. It is also important to note that previous studies reporting differences in cortisol regulation, including elevated evening cortisol, have been conducted in children

(not adolescents or young adults) with ASD emphasizing the need to carefully examine age and pubertal effect over the course of development (Corbett et al., 2006; 2008; Tomarken et al., 2015). Additionally, psychotropic medications taken by participants with ASD may have affected cortisol results (Granger et al. 2009). Future studies, with meticulous salivary cortisol sampling, are needed to confirm whether erratic sleep schedules and late bedtimes interfere with the diurnal pattern of cortisol secretion.

In the majority of participants, in both the ASD and TD groups, a physiological dim light melatonin onset was recorded. This response was not delayed or decreased in amplitude in the ASD group. Furthermore, although variability among individual participants was present, the relationship of DLMO to sleep onset was consistent across ASD and TD participants (2–3 hours before sleep onset on average) and the average DLMO occurred at similar clock times for ASD and TD participants (~ 21:00). A lower drop in morning to evening cortisol was correlated with a later DLMO across participants, supporting prior research that endocrine and circadian systems are interrelated (reviewed in Tsang et al., 2013). However, adjusting for diagnosis (ASD vs TD) did not affect the strength of the cortisol-DLMO correlation. These findings, taken together, suggest that individuals with ASD do not exhibit group differences in cortisol or DLMO and that sleep onset delay in ASD should not be attributed solely to circadian dysfunction or arousal dysregulation. The causes and contributors to sleep onset delay in ASD are likely multifactorial (e.g., related to biological, medical/pharmacological, and behavioral contributors). The role of behavioral contributors in relation to biological and medical/pharmacological factors will require further study. Of note, Loring et al. (2016) implemented behavioral sleep interventions in adolescents with ASD, which significantly declined the sleep onset latency from 78.8 to 45.3 minutes.

The reasons for adolescents/young adults with ASD having more difficulty going to bed and falling asleep compared to their TD counterparts is unclear. Some adolescents/young adults with ASD had a relatively early bedtime on weekdays (before 9 pm). This may have been due to several factors. Medications may have contributed to an earlier bedtime due to their side effect of drowsiness, although it is notable that despite this earlier bedtime, the adolescents/young adults with ASD had more difficulty falling asleep, perhaps secondary to overarousal. Behavioral factors may also have played a role in contributing to an earlier bedtime-- adolescents/young adults with ASD may have less social pressure to interact with friends. They may be more rule-bound about respecting a parental curfew. Due to their child's disability, their parents may be more involved in enforcing a bedtime, or think that their child's daytime behavior may be better if they go to bed earlier. The parents may understandably need time to themselves in the evening, with an earlier end to caring for their child. A too early bedtime, however, can have unintended sleep consequences. The onset of sleep is gated by circadian processes, which require an individual to be in the appropriate circadian phase for sleep to occur (Borbely 1982; Daan, Beersman, & Borbely 1984).

Apart from a too early bedtime, other sleep habits may have contributed to prolonged sleep latency in individuals with ASD. While sleep hygiene subscales on a standardized questionnaire did not differ between participants with ASD and TD, both groups did exhibit habits known to interfere with sleep (e.g., bedtime routine, sleep environment, cognitive and

emotional factors). Tan et al. (2012) implemented sleep hygiene interventions which significantly improved the total ASHS scores in an adolescent population with self-reported sleep problems. Loring et al. (2016) also reported that ASHS subscales (cognitive, behavioral, sleep stability, and sleep environment) showed statistically significant improvement with behavioral sleep education and sleep hygiene interventions. In both our TD and ASD groups, there were sleep hygiene difficulties resulting in relatively low ASHS scores. By implementing sleep hygiene treatment modalities, the participants' overall sleep disturbance may improve.

Our study has several limitations. First, the sample size was relatively small and emphasized higher functioning adolescents and adults with ASD who could cooperate with study procedures, including wearing an actigraphy watch for four weeks and using cotton swabs to collect salivary samples. Some of our participants had erratic sleep schedules. We also included participants with a broad age range and taking medications. Including participants with erratic sleep schedules, a broad age range, and taking medications is consistent with the heterogeneity reflective of the broader ASD population. Second, the subset of individuals with DLMO sampling was also small. Third, we did not define circadian preference using a morningness/eveningness questionnaire or a similar instrument. Fourth, our participants were enrolled during the school year as well as during break times, which could contribute to variable sleep schedules. Fifth, our SES scores in the family, and IQ scores in the child, could have been broader. Sixth, given that our enrollment spanned several years, we included participants diagnosed by both DSM-IV and DSM-5 criteria, which limited our ability to evaluate results in relation to ASD severity or specific ASD subtypes., Seventh, psychiatric comorbidities may have confounded our results. In particular, affective disorders are common in the ASD population and contribute to sleep disturbance (Lugnegård et al. 2011). Finally, while excluding patients with untreated comorbidities, such as untreated epilepsy or gastrointestinal issues, is a relative strength, it can also be viewed as a limitation given that these conditions are present in the larger ASD population.

Despite these limitations, our results suggest that despite prolonged sleep latency, some individuals with ASD show similar patterns to those who are TD with respect to profiles of melatonin and cortisol, two measures of circadian regulation. Our findings will need to be confirmed in larger samples. These preliminary findings emphasize that a multifactorial approach, which includes elements focused on behavioral sleep education (Loring et al. 2016), an evidence-based approach to improve sleep quality (e.g., avoiding too early of a bedtime, limiting electronic devices, limiting, caffeine intake, increasing exercise, and implementing a calming bedtime routine) is important in treating sleep disturbance in this population.

Acknowledgments

Funding:

This research was supported in part by a grant from Autism Speaks, as well as CTSA award No. UL1TR000445 from the National Center for Advancing Translational Sciences.

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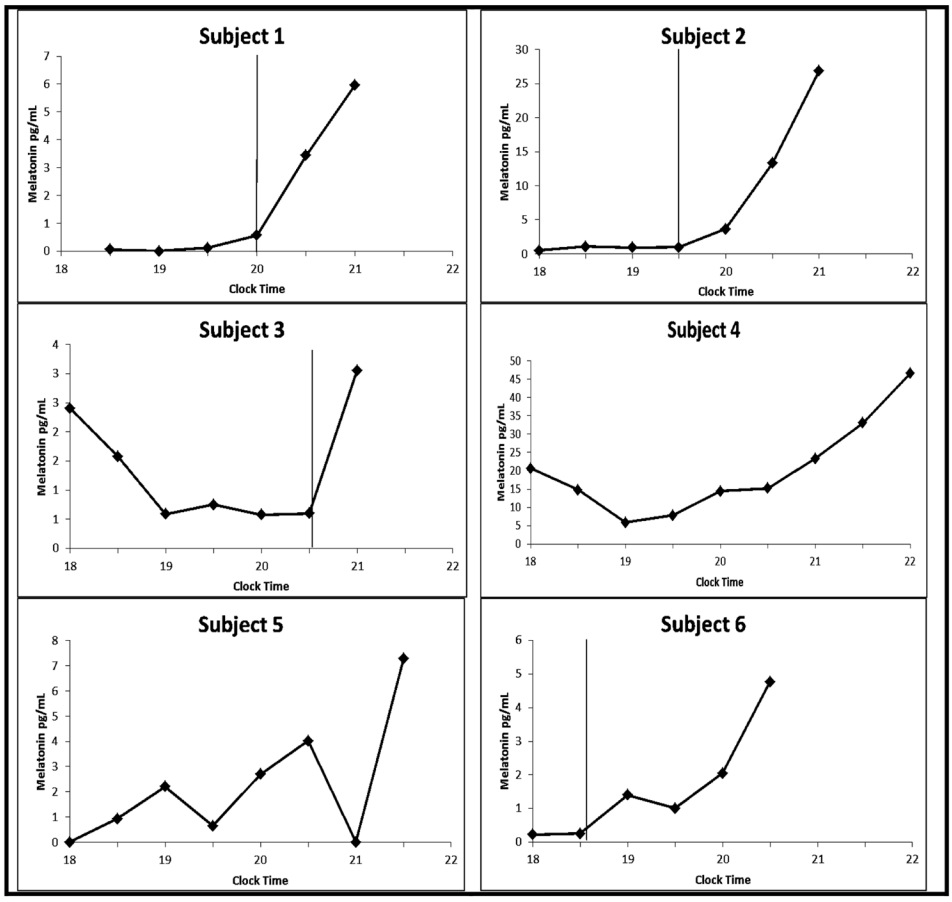
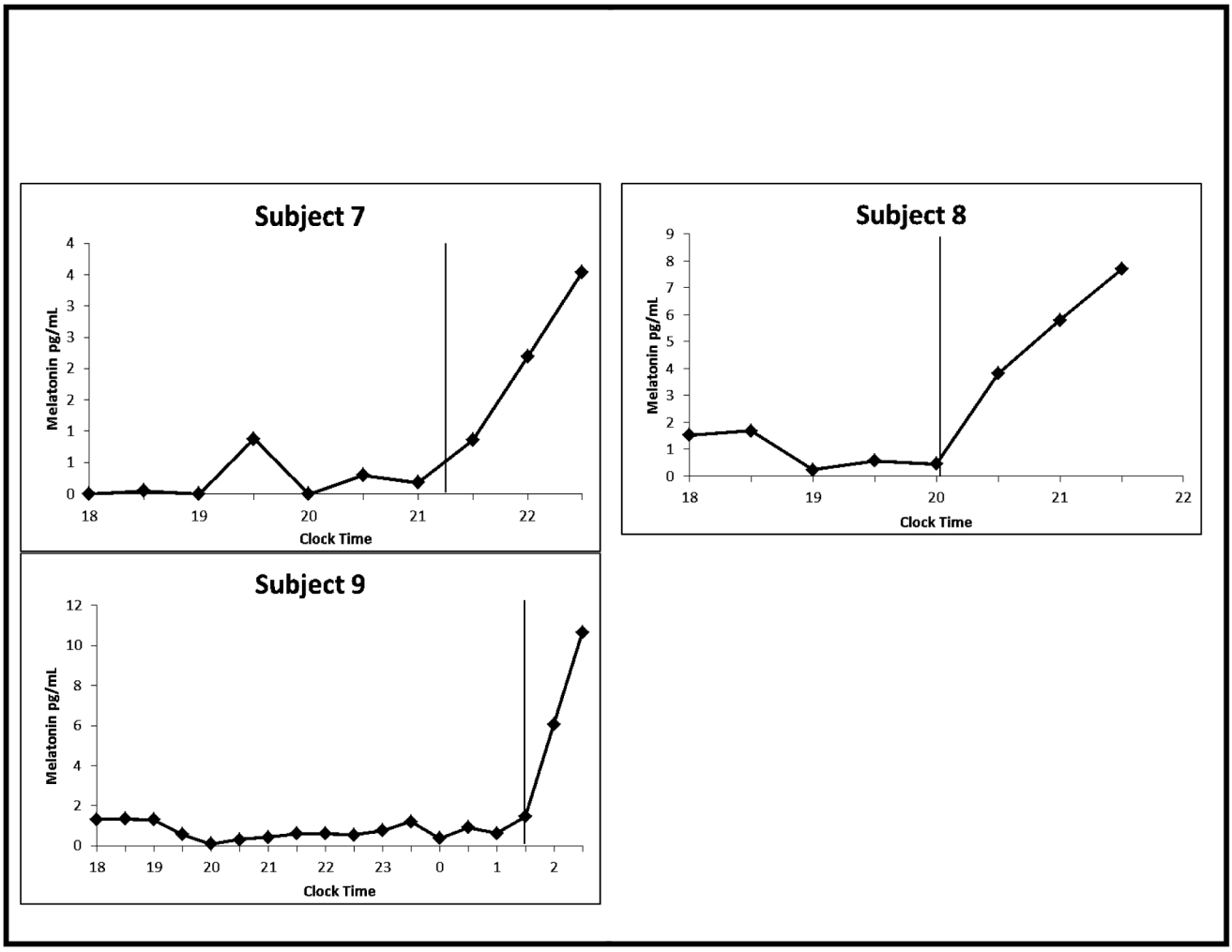
ASD Weekday DLMOs:





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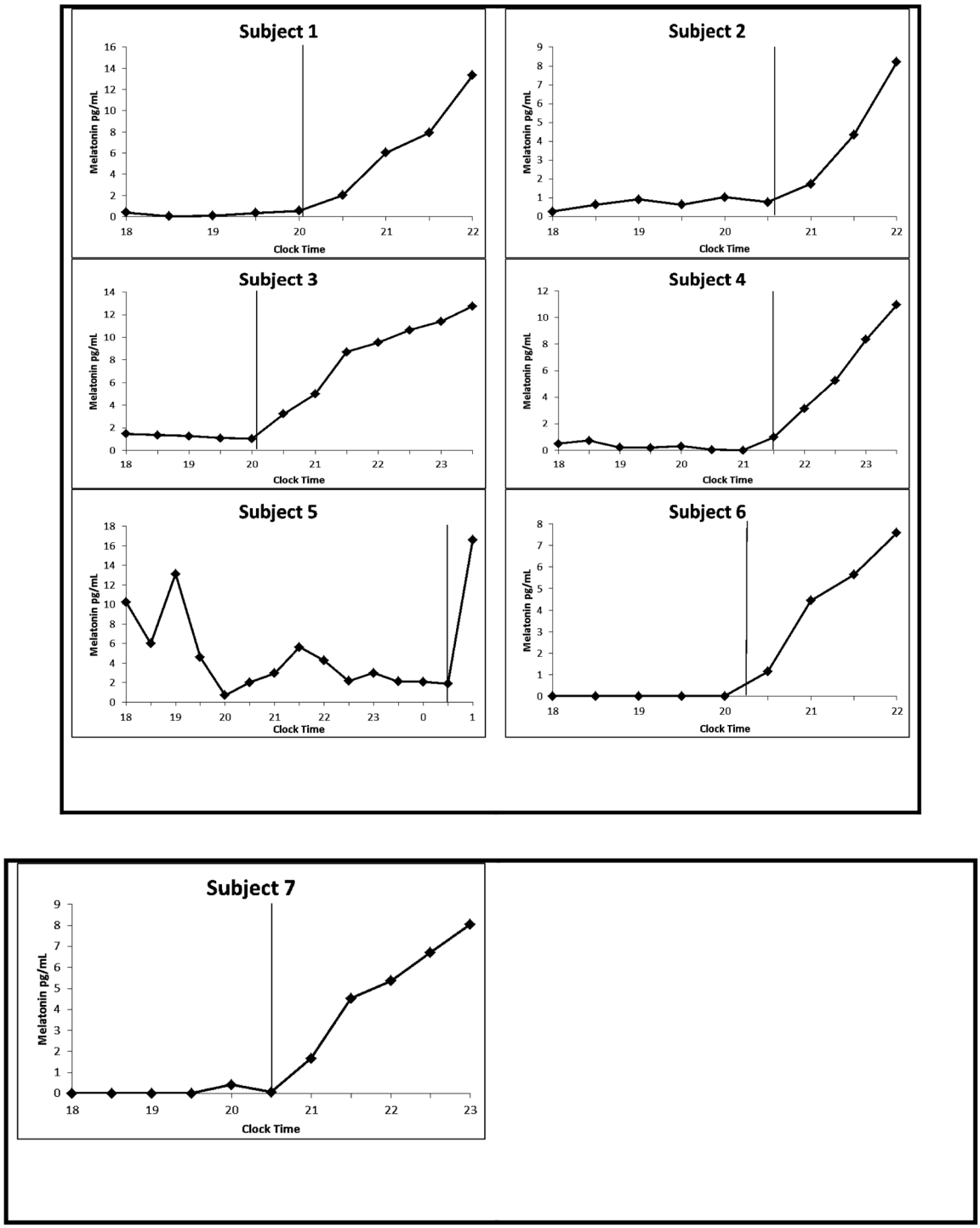

Figure 1.

The weekday melatonin & dim light melatonin onset (DLMO) salivary samples (pg/ml) collected from 18:00 until bedtime with the corresponding DLMO 3K threshold represented as a line. ASD 4 & ASD 5 did not produce a 3K DLMO threshold value (see text for further details).

Table 1

Demographics. Mean (percent or standard deviation).

	ASD (n =28)	TD (n = 13)	p-value
Age in years (SD, range)	15.6 (2.8; 11–26)	15.6 (2.1; −13–20)	0.961
Sex (male) (%)	20 (71%)	6 (50%)	0.118
Race (White) (%)	25 (89%)	12 (92%)	0.762
SES (SD)	46.3 (12.2)	44.8 (10.7)	0.717
Full Scale IQ (SD, range)	100.1 (22.8; 41–134)	107.5 (10.9; 86–122)	0.272
Vineland-II (SD, range)	76.0 (28.0; 53–209)	103.4 (10.4; 83–118)	**0.002**

SES = socioeconomic status based on Hollingshead Four Factor Index of Social Status

SD = standard deviation

Vineland-II = Adaptive Behavior Composite Standard Score

Table 2

Adolescent Sleep-Wake Scale (ASWS). Mean (standard deviation).

	ASD (n=28)	TD (n=13)	p-value
Going to Bed	3.21 (1.21)	4.22 (0.86)	**0.011**
Falling Asleep	3.49 (1.16)	4.42 (0.96)	**0.015**
Maintaining Sleep	4.14 (0.69)	4.23 (0.58)	0.689
Reinitializing Sleep	4.67 (0.90)	4.97 (0.66)	0.276
Returning to Wakefulness	2.82 (1.06)	3.03 (1.09)	0.563
Total Score	3.67 (0.73)	4.18 (0.62)	**0.036**

Table 3

Adolescent Sleep Hygiene Scale (ASHS). Mean (standard deviation).

	ASD (n=28)	TD (n=13)	p-value
Physiological Factor	5.05 (0.61)	4.97 (0.39)	0.663
Cognitive Factor	3.43 (1.15)	4.00 (1.06)	0.130
Emotional Factor	4.48 (0.85)	5.00 (0.77)	0.061
Sleep Environment Factor	5.04 (0.76)	5.27 (0.56)	0.349
Daytime Sleep Factor	4.93 (1.27)	5.23 (0.73)	0.342
Substances Factor	5.96 (0.19)	6.00 (0.00)	0.326
Bedtime Routine Factor	4.04 (1.75)	4.69 (1.11)	0.155
Sleep Stability Factor	3.60 (0.79)	3.58 (0.46)	0.929
Bed/Bedroom Sharing Factor	3.48 (0.67)	3.50 (0.20)	0.899
Total Score	4.47 (0.38)	4.69 (0.35)	0.083

Table 4

Sleep Parameters Derived from Wrist Actigraphy. Mean (standard deviation).

Sleep Parameter	ASD (n=28)	TD (n=13)	p-value
Sleep Latency (minutes)	36.7 (24.5)	16.2 (9.6)	< **0.0001**
Total Sleep Time (minutes)	424.7 (53.0)	419.7 (26.2)	0.692
Wake Time after Sleep Onset	55.0 (18.0)	46.9 (13.2)	0.152
Sleep Efficiency	79.1 (7.5)	84.3 (3.9)	**0.023**

Table 5

Sleep Diary/Event Marker Parameters Weekdays & Weekends. Mean (standard deviation).

Sleep Parameter	ASD (n=28)			TD (n=13)			p-values comparing ASD and TD		
	Weekday	Weekend	Overall	Weekday	Weekend	Overall	Weekday	Weekend	Overall
Bedtime	22:37 (1:14)	23:13 (1:30)	22:47 (1:13)	23:11 (1:20)	23:32 (1:07)	23:17 (1:15)	0.196	0.505	0.240
Sleeptime	23:09 (1:20)	23:51 (1:32)	23:21 (1:14)	23:28 (1:14)	23:51 (1:02)	23:35 (1:09)	0.463	0.988	0.566
Waketime	7:38 (1:12)	8:05 (1:23)	7:45 (1:12)	7:30 (1:11)	8:04 (0:55)	7:40 (1:02)	0.744	0.947	0.805

Table 6

Log 10 Cortisol Measures (nmol/L). Mean (standard deviation).

Parameter	ASD (n=26)	TD (n=13)	p-value
Am Cortisol	0.757 (0.198)	0.848 (0.211)	0.198
Pm Cortisol	−0.075 (0.494)	0.064 (0.346)	0.371
Am/Pm Cortisol Difference	0.832 (0.544)	0.784 (0.352)	0.772

Table 7

DLMO in relation to Bedtime & Sleep Time

Participant*	Mean DLMO	Mean bedtime (earliest-latest)	Mean Sleep Time	Diff bw DLMO & bedtime	Diff bw DLMO & Sleep Time
ASD 1	19:27	21:32 (21:09–21:45)	21:53	2:04	2:25
ASD 2	19:10	21:37 (20:14–22:22)	21:55	2:27	2:45
ASD 3	20:33	22:17 (21:19–23:34)	00:01	1:44	3:28
ASD 6	22:45	22:05 (20:57–22:42)	22:45	−0:40	0:00
ASD 7	21:04	23:08 (20:35–0:55)	23:29	2:03	2:25
ASD 8	19:51	22:24 (19:32–0:08)	22:35	2:33	2:44
ASD 9	1:27	1:59 (22:39–5:10)	2:04	0:32	0:37
TD 1	20:29	22:26 (21:57–23:11)	22:54	1:57	2:25
TD 2	20:46	22:35 (21:29–23:52)	22:58	1:48	2:12
TD 3	19:31	22:45 (21:25–0:07)	22:49	3:13	3:17
TD 4	21:00	1:08 (23:46–2:14)	1:12	4:08	4:12
TD 5	0:33	1:39 (20:35–5:32)	1:48	1:06	1:15
TD 6	20:15	22:57 (21:57–0:11)	23:03	2:42	2:48
TD 7	20:29	23:18 (22:04–2:03)	23:28	2:49	2:59
ASD mean	**21:11**	**22:43 (20:55–0:05)**	**23:15**	**1:32**	**2:03**
TD mean	**21:00**	**23:32 (21:53–1:18)**	**23:45**	**2:32**	**2:44**

*ASD 4 & ASD 5 did not produce a 3K DLMO threshold value (see text for further details).