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Day and nighttime excretion of 6-sulphatoxymelatonin in adolescents and young adults with autistic disorder

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Summary
Background: Several reports indicate that nocturnal production of melatonin is reduced in autism. Our objective was to examine whether melatonin production is decreased during the whole 24-h cycle, whether the melatonin circadian rhythm is inverted, and whether the reduction in melatonin production is related to the severity of autistic behavioral impairments.
Method: Day and nighttime urinary excretion of 6-sulphatoxymelatonin (6-SM) was examined during a 24-h period in post-pubertal individuals with autism ($N = 43$) and typically developing controls ($N = 26$) matched for age, sex and pubertal stage.
Results: Low 6-SM excretion (mean ± SEM) was observed in autism, both at daytime (0.16 ± 0.03 vs. 0.36 ± 0.05 μg/h, $p < 0.01$), nighttime (0.52 ± 0.07 vs. 1.14 ± 0.23 μg/h, $p < 0.05$), and during 24 h (8.26 ± 1.27 vs. 18.00 ± 3.43 μg/24-h collection, $p < 0.001$). Intra-individual nighttime—daytime differences (delta values) in 6-SM excretion were smaller in individuals with autism than in controls (0.36 ± 0.07 vs. 0.79 ± 0.23 μg/h, $p < 0.05$). Nocturnal excretion of 6-SM was negatively correlated with autism severity in the overall level of verbal language (Spearman $\rho = -0.30$, $p < 0.05$), imitative social play (Spearman $\rho = -0.42$, $p < 0.05$), and repetitive use of objects (Spearman $\rho = -0.36$, $p < 0.05$).

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Conclusion: A deficit in melatonin production is present both at daytime and at nighttime in individuals with autism, particularly in the most severely affected individuals. These results highlight interest in potential therapeutic uses of melatonin in autistic disorder, especially in individuals with severe autistic impairment and/or low urinary 6-SM excretion.

1. Introduction

Melatonin is a neurohormone produced mainly by the pineal gland and during the night. Pineal melatonin is important for the regulation of human circadian rhythms including the sleep–wake, neuroendocrine and body temperature cycles (Axelrod, 1974; Zhdanova et al., 1997). The measures of melatonin concentration in plasma and saliva, or of the urinary excretion of its predominant metabolite, 6-sulphatoxymelatonin (6-SM), are considered the best peripheral indices of human circadian timing (Arendt, 2006). There is also increasing evidence that melatonin is critically involved in early development through its direct effects on placenta, developing neurons and glia, and its role in the ontogenetic establishment of diurnal rhythms (Niles et al., 2004; Iwasaki et al., 2005).

The physiological increase in melatonin secretion during the night is well established with a peak around 2 AM and nighttime values usually at least three times greater than daytime values (Arendt, 1988). Pineal melatonin production is powerfully suppressed by light acting through the retino-hypothalamic tract (Revell and Skene, 2007). In addition to light and consequently seasons (Lindblom et al., 2002), pineal melatonin secretion can also be influenced by endogenous factors including sex, age and pubertal stage (Cavallo and Ritschel, 1996; Touitou, 2001). At daytime, it has been suggested that most of the melatonin production occurs outside the pineal gland, in the wall of the gut (Bubenik, 2002).

Melatonin is of interest in autism due to its apparent role in neurodevelopment (de Faria Poloni et al., 2011), reports of sleep–wake rhythm disturbances in individuals with autism (see Glickman, 2010 and Tordjman et al., 2005 for reviews), as well as beneficial effects of melatonin when administered to individuals with autism and sleep problems (Doyen et al., 2011; Rossignol and Frye, 2011; Guénolé et al., 2011). In addition, central and peripheral alterations in serotonin in autism have been widely reported and it is noteworthy that melatonin is synthesized in only two steps from serotonin in the pineal gland and the gut. (Richdale, 1999; Anderson, 2002; Nakamura et al., 2010). Prior studies of melatonin production in autistic disorder were often limited by small sample sizes and were not entirely consistent, but all reported abnormalities in the melatonin production (Ritvo et al., 1993; Nir et al., 1995; Kulman et al., 2000; Tordjman et al., 2005; Melke et al., 2008; Mulder et al., 2010). Our results (Tordjman et al., 2005), taken together with the other studies (except Ritvo et al.'s study, 1993), indicate that nocturnal secretion of melatonin is frequently reduced in autism. However, given the limitations of the available data, it has not been possible to conclude if there is a general decrease in melatonin secretion during the whole 24-h cycle, or if the melatonin circadian rhythm is altered or inverted in autistic disorder. In order to clarify these issues, we examined simultaneously the diurnal and nocturnal excretion of urinary 6-SM in a large, accessible and post-pubertal sample of adolescents and young adults with autistic disorder, and in a group of typically developing controls matched on age, sex and stage of puberty. Post-pubertal participants were recruited given the reported effect of puberty on melatonin secretion (Cavallo and Ritschel, 1996; Touitou, 2001). We also examined the relationship between 6-SM excretion and the severity of behavioral autistic impairments.

2. Methods

2.1. Participants

Adolescents and young adults with autistic disorder ($N = 43$), all post-pubertal, were matched with typically developing controls ($N = 26$) on age, sex and Tanner stage of puberty assessed by a pediatrician. Outpatients with autistic disorder were recruited from French day-care facilities and included 31 males and 12 females [mean age = 18.6 years, SEM (standard error of the mean) = 0.5]. Controls were recruited over a one-month period from a preventive medical center where they went for a regular check-up, and included 19 males and 7 females (mean age = 19.8 years, SEM = 0.8). In addition, controls were interviewed by a psychiatrist and had no sleep problems and no psychopathology based on the Mini International Neuropsychiatric Interview (MINI; Sheehan et al., 1998) and determined to be free of any. There was no family history of autistic disorder in the control group. All patients and controls were Caucasian, non-obese, had no history of encephalopathy or neuroendocrinological disease and were determined to be physically healthy based on a pediatrician's examination. All patients and controls were unmedicated for at least one month before urine collection. All subjects were sleeping in their parents' house and were attending high school or college (controls) or day-care facilities (individuals with autism) on a daily basis from 0900 h to 1600 h. The protocol was approved by the ethics committee of Bicêtre Hospital (Kremlin Bicêtre, France) and written informed consent was obtained from parents.

2.2. Cognitive and behavioral assessments

Cognitive functioning of patients with autistic disorder was assessed by two psychologists using French versions of the WAIS-III (the age-appropriate Wechsler intelligence scale; Wechsler, 2000) and the Kaufman K-ABC (Kaufman and Kaufman, 1993). All patients with autistic disorder were cognitively impaired (mean full scale IQ $\pm$ SEM: 42.1 $\pm$ 0.5, with a range of 40–58; mean verbal IQ $\pm$ SEM: 45.2 $\pm$ 0.3, with a range of 45–57; mean performance IQ $\pm$ SEM: 45.2 $\pm$ 0.7, with a range of 45–80).

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Behavioral assessments were performed using the French INSERM (Institut National de la Santé et de la Recherche Médicale, Third edition, 1993) version of the Autism Diagnostic Interview-Revised (ADI-R; see Lord, 1997). The ADI-R, an extensive semi-structured parental interview, was administered by two trained psychiatrists. The ADI-R scale assessed the three major domains of autistic impairments: reciprocal social interactions, verbal/non-verbal communication, and repetitive behaviors/restricted interests. Based on the direct clinical observation of the patient by two independent child psychiatrists, the diagnosis of autistic disorder was made according to the criteria of DSM-IV-TR, ICD-10 and CFTMEA and was confirmed by the ADI-R ratings (Misès and Quemada, 1993; American Psychiatric Association, 2000; Tordjman et al., 2001).

2.3. Urine collection

Urine samples were collected at home by parents during a whole 24-h cycle from 8 PM to 8 AM (nighttime collection) and from 0800 to 2000 h (daytime collection). Subjects were instructed to empty their bladder between 1945 and 2000 h before starting urine collection. Because of possible effects of season on the secretion of melatonin, urine was collected for all subjects in spring (March—May). Parents of autistic and control subjects completed a questionnaire reporting on the following information for the night of urine collection, and for what was typical over the month before the collection: time to bed, time to sleep, nighttime awakenings, if their child slept with the light on or got up during the night and turned on the light, and presence of other sleep disturbances/problems. On the night of urine collection, all subjects included in the analyses went to bed with lights out between 9 PM and 10 PM, and none was exposed to light until their morning wake-up between 0700 and 0745 h. Based on the brief parental sleep questionnaire, none of the study participants showed any significant sleep disturbance for the night of the urine collection or for the month before the collection. In addition, no bedwetting or accidental loss of urine was reported for any of the subjects. Collected urines were stored in a refrigerator until delivered to the laboratory within 24 h of the urine collection. The volume of the urine collection was measured and a portion was frozen until analyzed for creatinine and 6-SM. It should be noted that urine was originally collected from 41 controls; however, urine from 15 controls was rejected prior to biochemical analyses due to either parental report of late bedtimes or uncertainty regarding whether the daytime collection was placed in the nighttime collection jug (and vice versa).

2.4. Determination of urinary 6-SM

Melatonin production was assessed by measuring the urinary excretion of 6-SM which has been well established to give an accurate reflection of pineal melatonin secretion (Bojkowski et al., 1987). Blinded analysis of urine 6-SM levels was performed by radioimmunoassay using an assay kit from Stockgrand Ltd (Guildford, UK). The urine samples were diluted prior to assay (1/250). The intra-assay coefficient of variation was 6% ($N = 10$) for a 0.030 μg/ml control sample value. Excretion of 6-SM was expressed as μg excreted per hour (μg/h) over the collection period: the urine 6-SM concentration (μg/ml) was multiplied by the collection volume in ml and divided by the 12 h of collection.

2.5. Statistical analysis

Correlations between melatonin excretion rates and age, as well as correlations between nighttime and daytime melatonin excretion rates were assessed using Pearson correlation analyses. Group comparisons of urine collection volumes and of creatinine urinary excretions were performed using two-tailed Student's *t*-tests. Group and subgroup comparisons of urine 6-SM levels were performed using repeated-measures analyses of variance and two-tailed Student's *t*-tests. Relationships between melatonin excretion and autism severity within the major behavioral domains of impairment were studied using Spearman rank-order correlations. In order to balance type I and type II errors in the statistical analysis of behavioral domains, a hierarchical strategy was used (Cohen and Cohen, 1983). First, total behavioral domains and subdomains were examined. If there was a significant result for an overall domain or sub-domain, then a further level of analysis was performed on the subscores included in the domain. The severity of impairments in the behavioral domains of autism were scored following a previously described procedure that involved determining for each domain the median value of the subset of ADI-R items included in the ADI-R algorithm (Tordjman et al., 2001). Alpha risk was set at 0.05 for all analyses.

3. Results

3.1. Initial analyses

Mean ($\pm$ SEM) urine collection volumes for nighttime (2000—0800 h) and daytime (0800—2000 h) periods were not significantly different in the autism and control groups (nighttime: 467 ± 48 and 388 ± 47 ml, respectively; daytime: 542 ± 46 and 489 ± 71 ml, respectively). Similarly, creatinine urinary excretions were not significantly different in autism and control groups (nighttime: 431 ± 33 and 372 ± 74 mg/collection, respectively; daytime: 461 ± 33 and 606 ± 81 mg/collection, respectively). There were no significant effects of gender or age on the nighttime or daytime hourly 6-SM excretion rates in either the autism or control group.

3.2. Relationships between diagnosis and 6-SM excretion rates

Repeated-measures analysis of variance conducted on nighttime and daytime hourly 6-SM excretions indicated significant effects of group ($F(1,65) = 12.24$, $p < 0.001$), time ($F(1,65) = 35.00$, $p < 0.0001$), and group-by-time interaction ($F(1,65) = 4.88$, $p < 0.05$). Post hoc *t*-tests analyses showed significantly lower nighttime 6-SM excretion rates in individuals with autism compared to controls (0.52 ± 0.07 vs. 1.14 ± 0.23 μg/h, $p = 0.016$); daytime 6-SM excretion rates in individuals with autism were also lower compared to controls (0.16 ± 0.03 vs. 0.36 ± 0.05 μg/h, $p = 0.002$) (see Fig. 1). Furthermore, the total (nighttime + daytime) 6-SM excretion was significantly lower in individuals with autism

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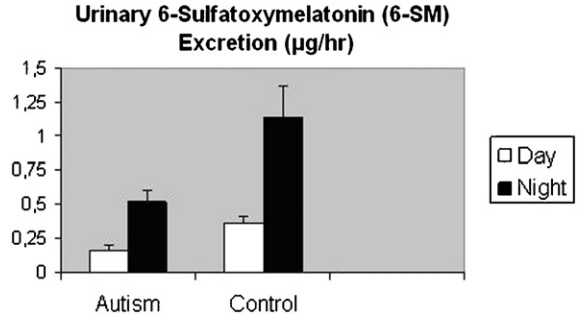

Figure 1 Daytime and nighttime urinary 6-sulphatoxymelatonin (6-SM) excretion rates (μg/h, mean ± SEM) in individuals with autism ($N = 43$) and typically developing controls ($N = 26$). Daytime and nighttime 6-SM excretion rates were significantly lower in individuals with autism compared to typically developing controls ($t = 3.15$, $df = 67$, $p = 0.002$ and $t = 2.53$, $df = 67$, $p = 0.016$, respectively). The total (nighttime + daytime) 6-SM excretion was significantly lower in individuals with autism compared to controls ($t = 3.00$, $df = 67$, $p = 0.005$). Finally, the mean (± SEM) intra-individual nighttime—daytime differences (delta values) in 6-SM excretion rate were significantly smaller in individuals with autism than in controls ($t = 2.21$, $df = 67$, $p = 0.031$).

compared to controls (8.26 ± 1.27 vs. 18.00 ± 3.43 μg/24 h collection, $p = 0.005$).

Box and whisker plots for daytime, nighttime, and total 24 h 6-SM excretion, in individuals with autism and controls are shown in Fig. 2A—C. The distributions of 6-SM excretion rates appear shifted downward in the autism group.

Concerning the significant group-by-time interaction, the mean (± SEM) intra-individual nighttime—daytime differences (delta values) in 6-SM excretion rate were significantly smaller in individuals with autism than in controls (0.36 ± 0.07 vs. 0.79 ± 0.23 μg/h, $p = 0.031$) (see Fig. 1). Among the 43 patients with autism, 30 (69.8%) displayed greater 6-SM excretion at night compared to the daytime (normal circadian rhythm), 10 (23.2%) displayed little or no nighttime—daytime variation in 6-SM excretion rates (nighttime—daytime differences in 6-SM excretion rate less than 0.1 μg/h, with nighttime values less than 3 times greater than daytime values), and 3 (7%) had higher daytime than nighttime excretion (''inverted'' circadian rhythm). Of the 26 controls, 23 (88.5%) showed greater nighttime 6-SM excretion, 1 (3.8%) displayed no nighttime—daytime variation in 6-SM excretion rates, and 2 (7.7%) displayed higher daytime excretion (see Fig. 3). The absence of circadian variation (nighttime—daytime differences in 6-SM excretion rate) was observed significantly more often than expected in individuals with autism compared to controls ($p = 0.043$, Fisher Exact Test). Nighttime and daytime 6-SM excretion rates were significantly correlated in both groups (individuals with autism: $r = 0.49$, $p < 0.001$; controls: $r = 0.44$, $p = 0.035$).

3.3. Relationships between autism severity and 6-SM excretion rates

Nighttime 6-SM excretion rates were significantly and negatively correlated with severity of autistic impairment in the overall level of verbal language (verbal language is defined in the ADI-R as the ''daily, functional and comprehensible use of spontaneous phrases of at least three words, including at least sometimes a verb'', Spearman $\rho = -0.30$, $p = 0.048$), in the non-verbal communication sub-domain C4 (''Lack of varied spontaneous make-believe or social imitative play'', Spearman $\rho = -0.45$, $p = 0.040$), and in the sub-domain D4 (''Preoccupations with part of objects or non-functional elements of materials'', Spearman $\rho = -0.47$, $p = 0.01$). A further level of analysis on the subscores indicated that the significant results in the C4 sub-domain were mainly due to the subscore ''Imitative social play'' (Spearman $\rho = -0.42$, $p = 0.043$), and in the D4 sub-domain to the subscore ''Repetitive use of objects'' (Spearman $\rho = -0.36$, $p = 0.045$). There was no significant correlation between nighttime 6-SM excretion rates and IQ scores. Furthermore, there was no significant correlation between daytime 6-SM excretion rates and autism severity. However, daytime 6-SM excretion rates were significantly and positively correlated with total IQ scores (Pearson $r = 0.43$, $p = 0.014$), with verbal IQ scores (Pearson $r = 0.38$, $p = 0.033$), and with performance IQ scores (Pearson $r = 0.43$, $p = 0.014$).

4. Discussion

A major finding of the study was that both daytime and nocturnal 6-SM excretion rates, as well as the total (nighttime + daytime) 6-SM excretion, were significantly lower in individuals with autism than in typically developing controls. The finding of low nighttime urinary excretion of 6-SM in autism is consistent with our previous study (Tordjman et al., 2005) and also with two other studies (Nir et al., 1995; Kulman et al., 2000), but contradicts a prior smaller study (Ritvo et al., 1993) that found unaltered levels of nighttime urinary melatonin in autism. However, in that study melatonin itself was measured, melatonin levels were expressed as a urine concentration (moles/liter), and a relatively small sample ($N = 10$) was studied. Our finding of low daytime excretion of melatonin in autism is consistent with Melke et al.'s study (2008), but contrasts with previous smaller studies of urinary melatonin (Ritvo et al., 1993) and plasma melatonin (Nir et al., 1995) that have reported higher daytime levels in autism.

Our results demonstrate that there is a deficit in melatonin production in a substantial proportion of individuals with autism and this deficit is present at night and during the day, indicating that pineal and, possibly, extra-pineal production of melatonin is lower in autism. Furthermore, the small intra-individual 6-SM nighttime—daytime differences and the significant absence of melatonin variation found in autism, might be a reflection of the lower day and nighttime levels, or an indication that there exists a subgroup of individuals with autism that have a dysregulation of their circadian rhythm, and more precisely an absent circadian rhythm. The hypothesis of an absent circadian rhythm in melatonin and other neuroendocrine functions is supported by Kulman et al.'s study (2000) in which 10 out of 14 children with autistic disorder showed no melatonin circadian variation, by Zapella (1993) who found a blunted circadian rhythm of melatonin secretion in a male adolescent with autism and hypomelanosis of Ito, and by reports in autism of

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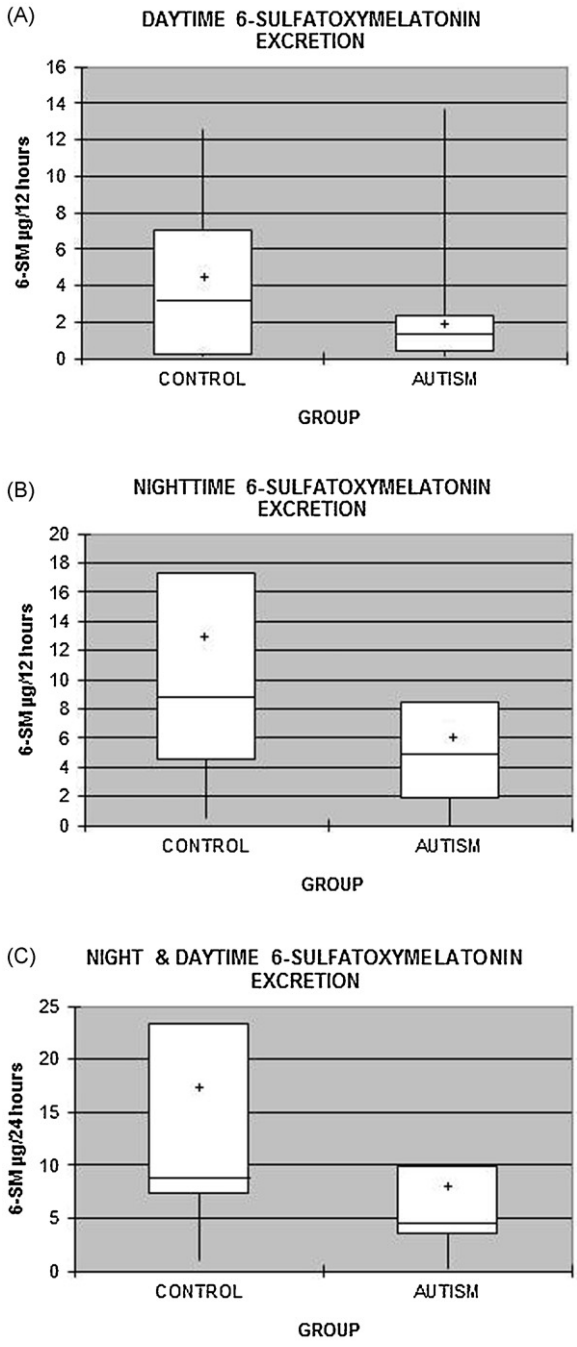

Figure 2 (A) Box and whisker plot of daytime urinary 6-sulphatoxymelatonin (6-SM) excretion (μg/12 h collection) in individuals with autism ($N = 43$) and typically developing controls ($N = 26$). Means are indicated by the cross, medians by the midline, second and third quartiles by the open box, and minimums and maximums by the lower and upper whiskers. The median values were 3.14 and 1.3 μg/12 h collection in the control and autism groups, respectively. (B) Box and whisker plot of nighttime urinary 6-sulphatoxymelatonin (6-SM) excretion (μg/12 h collection) in individuals with autism ($N = 43$) and typically developing controls ($N = 26$). Means are indicated by the cross, medians by the midline, second and third quartiles by the open box, and minimums by the lower whiskers. The median values were 8.6 and 4.8 μg/12 h collection in the control and autism groups, respectively. Maximum nighttime 6-SM excretion values were 60.6 and 29.0 μg/12 h collection in the control and autism groups, respectively (upper whiskers not presented). (C) Box and whisker plot of combined night and daytime urinary 6-sulphatoxymelatonin (6-SM) excretion (μg/24 h collection) in individuals with autism ($N = 43$) and typically developing controls ($N = 26$). Means are indicated by the cross, medians by the midline, second and third quartiles by the open box, and minimums by the lower whiskers. The median values were 8.6 and 4.8 μg/24 h collection in the control and autism groups, respectively. Maximum combined (night and daytime) 6-SM excretion values were 69.7 and 42.7 μg/24 h collection in the control and autism groups, respectively (upper whiskers not presented).

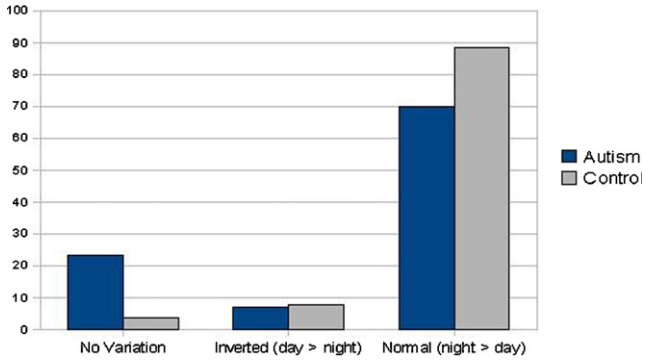

Figure 3 Distribution (%) of individuals with autism ($N = 43$) and typically developing controls ($N = 26$) with normal circadian rhythm (higher nighttime than daytime 6-SM excretion rates), inverted circadian rhythm (higher daytime than nighttime 6-SM excretion rates) and absent circadian rhythm (no nighttime—daytime variation in 6-SM excretion rates).

abnormalities in the circadian rhythm of cortisol secretion including an absence of cortisol variation (Tordjman et al., 1997). The possible existence and characteristics of a subgroup of patients with autism showing a deficit in melatonin production with no nighttime—daytime variations may be fruitfully examined in future larger studies. It is noteworthy that the circadian rhythm does not appear in the present study to be inverted (day > night) in more than a few of the individuals with autism (3/43) and that a similar small fraction of the controls (2/26) had inverted 6-SM excretion. This contrasts with the Kulman et al. Study (2000) where 4 of 14 children with autism were found to have inverted rhythms and is quite distinct from Smith Magenis syndrome, a genetic syndrome (del 17p11.2) displaying mental retardation, autistic symptoms, and an inverted pattern of melatonin secretion (Potocki et al., 2000).

It can be speculated that the present findings are related to peripheral or central abnormalities in serotonin physiology reported in autism possibly resulting from genetic factors, given that melatonin is synthesized directly from serotonin by N-acetylation and O-methylation (Tordjman et al., 2001; Anderson et al., 2002; Revell and Skene, 2007). The relationship of the melatonin deficit to the well-replicated platelet hyperserotonemia of autism is of special interest and there is some preliminary evidence that lower melatonin production may be more common in individuals with elevated platelet serotonin (Melke et al., 2008; Mulder et al., 2010). Certainly,

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the enzymes involved in the synthesis of melatonin from serotonin should be examined as potential contributors to the lower melatonin production. However, it appears that the individual melatonin deficit is usually one of degree, lessening the likelihood that loss-of-function mutations are frequently playing an important role. An initial report (Melke et al., 2008) suggesting a primary deficit in the enzyme catalyzing O-methylation (acetylserotonin O-methyltransferase, ASMT) and positing ASMT as a susceptibility gene for autism, has not been borne out by subsequent research (Toma et al., 2007; Jonsson et al., 2010). The report of an increased occurrence of a partial duplication in the ASMT gene in autism (6% vs. 2% in controls) has also increased interest in melatonin synthesis (Cai et al., 2008). The apparent lower production of both nighttime and daytime melatonin observed in the present study suggests that identification of aspects and regulatory factors held in common across the pineal and extrapineal tissues might lead to underlying mechanisms. Thus, the study of melatonin in other tissues, including gut, placenta, and skin, should be considered (Bubenik, 2002; Iwasaki et al., 2005; Slominski et al., 2008).

In addition to characterizing the nature of the alteration in melatonin production in autism, we aimed to identify potential associations between melatonin and behavioral expression of autism. We did observe that nocturnal 6-SM excretion rate was significantly negatively correlated with severity of impairment in the overall level of verbal language, imitative social play and repetitive use of objects. The observation of significant negative correlations between nocturnal 6-SM excretion and severity of autistic impairment in verbal communication and play replicates our previous finding (Tordjman et al., 2005). It is noteworthy that we replicated this result using the ADI-R (the ADI-R is based on a parental interview) which differs from the ADOS (the Autism Diagnostic Observation Schedule is based on a direct observation of the patient; Lord, 1997) used in our previous study. Nir et al. (1995) presented data suggestive of reduced melatonin production in individuals with autism and speech difficulties or with EEG abnormalities. More compelling is the agreement between our finding of a negative relationship of nocturnal 6-SM excretion with severity of language impairment and the study by Hu et al. (2009) in autism spectrum disorders (ASD) that reported substantially reduced expression of the gene encoding arylalkylamine N-acetyltransferase (AANAT, the rate limiting enzyme for melatonin synthesis) in ASD individuals with severe language impairment.

We did not observe sleep problems in the subjects studied, but the subjects were adolescents and young adults, all post-pubertal individuals, and the assessment was brief and not as detailed as a prior report linking lower melatonin with less nighttime N3 sleep and increased daytime sleepiness in children with autism (Leu et al., 2011).

We did not find significant correlations between nocturnal 6-SM excretion rates and IQ scores. However, the range of IQ scores in the patients was too narrow to test thoroughly the relationship between IQ scores and 6-SM levels. Previous studies of melatonin production in autism have provided little or no information regarding level of cognitive functioning of study subjects, and it is clear that IQ-matching would be desirable. Melatonin production in non-syndromic mental retardation appears unstudied, but it is of potential relevance that melatonin production in Down syndrome has been reported to be normal, while increased levels have been reported for Fragile X subjects (Reiter et al., 1996; Gould et al., 2000).

Finally, given the apparent role of melatonin in neural development (de Faria Poloni et al., 2011), it might be possible that a lack of melatonin occuring in utero or at birth could impair brain development. Furthermore, it is quite speculative but thought provoking to consider whether the lower mean melatonin production, the significantly smaller day—night differences and the significantly higher frequency of absence of circadian variation observed in individuals with autism compared to controls, might be playing a role in, or be a reflection of, the hypothesized alteration in time perception in autism (Boucher, 2001; Wimpory et al., 2002). Indeed, melatonin signals can drive daily rhythmicity and are also involved in the synchronization of peripheral oscillators (i.e., in the adjustment of the timing of existing oscillations) (Pevet and Challet, 2011). Thus, Boucher's model of autism relates the very poor intuitive sense of time observed in individuals with autism to abnormalities in circadian physiological measures and sleep-wake cycle (Boucher, 2001). Boucher suggests that timing problems in ''biological clocks'' would have physiological and psychological consequences that might be involved in autistic impairments. Furthermore, Wimpory et al. have theorized that anomalies in clock genes operating as timing genes in high frequency oscillator systems may underline timing deficits that could be important in the development of autistic disorder (Wimpory et al., 2002).

In summary, this is the first study showing clearly in a large sample of individuals with autism that a deficit in melatonin production is present both at night and during the day, and that the deficit is greater in more severely affected individuals. The biochemical, neuronal and genetic pathways governing melatonin production are well-characterized and offer promising avenues for investigation. The present study was limited by the low temporal resolution provided by the long 12-h urine collection periods (a consequence of inherent difficulties in obtaining urine collection at fixed time points in low functioning individuals with autism), by the narrow range of IQ scores in the autism group, and by the relatively brief assessment of sleep problems. Thus, further research is needed to determine whether the nocturnal melatonin acrophase is shifted, whether the melatonin deficit is influenced by the level of intellectual functioning, and whether the nocturnal deficit is important in the sleep problems often reported to be associated with autism. However, it is noteworthy that prior studies of the prevalence of sleep problems in autism typically have not used population-based samples and have rarely studied post-pubertal individuals. Additional studies are warranted to investigate the utility of urinary 6-SM in screening and subtyping, to identify underlying genetic and biological factors, and to elucidate the role of altered melatonin and associated factors in the pathophysiology and behavioral manifestations of autism. The results highlight interest in potential therapeutic uses of melatonin in autism, especially in individuals with severe behavioral autistic impairment and/or low in urinary 6-SM excretion, and suggest that urinary 6-SM could be examined as a potential predictor of response to melatonin.

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Conflict of interest

There are no conflicts of interest for any of the authors.

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