A sad day's night

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CHAPTER 2

Intra- and interindividual variability of longitudinal daytime melatonin secretion patterns in depressed and non-depressed individuals

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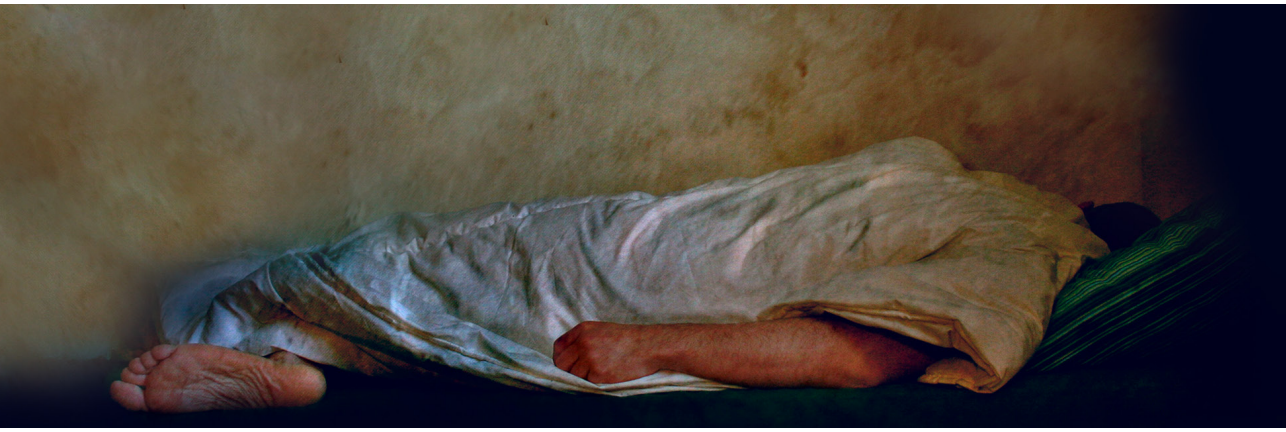


Short abstract

Disrupted melatonin secretion is regarded as a link between circadian rhythm and major depression, but results have been contradictory. We hypothesize that this might be due to averaging across individuals and too short measurements periods. In this study, pair-matched depressed and non-depressed individuals sampled saliva three times a day, 30 days, in their natural environment. The depressed group showed significantly more variance and higher melatonin levels ($p < 0.05$). Substantial interindividual heterogeneity and day-to-day variability was found. The individual time-series approach allowed us to reveal this variability. Important information remains unnoticed when analyzing melatonin only at the group level.

Introduction

The impact of biological rhythm disruptions on mood disorders has received a lot of attention over the past years. A key hormone in the synchronization of the biological rhythm is melatonin[1–3]. Depressive disorders have been associated with markedly lower melatonin levels compared with healthy controls[4] but the literature is not entirely consistent: both decreased and increased levels of melatonin have been reported in patients with depressive disorders[5]. Treatments targeting the biological rhythm, such as sleep deprivation and light therapy, have been proven effective in some, but not all, depressed patients[6,7]. The exact nature of the relationship between melatonin levels and depressive symptoms has remained largely unclear.

One limitation of the studies to date is that melatonin levels are usually aggregated over individuals and presented as group averages – an approach referred to as the nomothetic approach[8]. This approach implicitly assumes that the same model holds for all individuals, resulting in loss of information on individual secretion patterns when this assumption does not hold. As an alternative, time-series designs with multiple repeated measurements have been suggested to investigate dynamic patterns within individuals (idiographic approach)[9]. This approach has not yet been applied on melatonin data.

A second shortcoming of previous studies is the short time period during which melatonin is assessed. Up to now, most studies on melatonin have been performed over a period of 24 hours or less, in which only one secretion cycle of melatonin can be covered at the most[10]. The implicit assumption here is that secretion patterns are the same for every 24 h. In case of large day-to-day variability, single-day assessments will yield unreliable information. Multiple-day assessments of 24-h melatonin levels have been performed before, for example, to study the consistency of plasma melatonin levels between days of different menstruation phases[11]. In such a design, however, measurement days are not sequential but interrupted. Consecutive measurements during a more prolonged time period may provide different information[12]. To the best of our knowledge, no naturalistic studies have measured melatonin for a prolonged time period yet.

We documented the temporal characteristics of longer-term daytime melatonin secretion patterns in pair-matched depressed and non-depressed individuals, using a replicated single-subject time-series design. We examined mean levels, variability and stability of individual melatonin secretion and explored potential differences between depressed and non-depressed individuals.

Materials and Methods

This study is part of the Mood and Movement in Daily Life (MOOVD) study. The MOOVD study investigates the dynamic relationship between physical activity and mood, and its underlying physiological processes, in depressed and non-depressed individuals. Using detailed repeated assessments, this study allows us to investigate the temporal patterns in

physical activity, hormone secretion and depressed mood, individual differences therein, and clues for tailor-made interventions. A replicated single-subject time-series design was used, in which pair-matched depressed and non-depressed individuals were monitored for 30 days within their natural environment, three times a day. Participants filled out electronic diaries, wore an accelerometer, and sampled saliva at each assessment point, resulting in time series of up to 90 repeated measurements per individual.

Subjects

We selected the first 10 depressed individuals and 10 matched non-depressed controls of the MOOVD study for the present analyses. This number suffices to provide a valid reflection of between-subject variations and detect relevant effect sizes (ES > 0.5) with adequate power, while still allowing a detailed description of individual time series. The participants were included in the period January 2012 until June 2013 and pair-matched based on gender, age, smoking status, and body mass index to facilitate pair-wise comparison. Depressed individuals were recruited from a patient population of the University Center of Psychiatry (UCP), University Medical Center Groningen and from the Center for Integrative Psychiatry. This resulted in the inclusion of eight outpatients and two inpatients. Non-depressed individuals were recruited from the general population. Inclusion criteria for the non-depressed group were Beck Depression Inventory (BDI[13]) score <9 and absence of DSM-IV major depressive disorder (MDD), and for the depressed group BDI score >14 and MDD (current or recent; <2 months). The presence of MDD was established in those who met inclusion criteria on the BDI by means of the Composite International Diagnostic Interview (CIDI[14]). All participants had regular sleep-wake schedules (i.e. no night-shift workers) and were capable of following the research procedure for 30 days, i.e. keeping an electronic diary three times a day, sampling saliva while filling out the electronic diary, abstaining from eating or drinking (except water) during 30 min before sampling and wearing an accelerometer 24 h a day. Exclusion criteria were: current or recent (<2 years) episode of a psychotic or bipolar disorder, visual or hearing impairments, and pregnancy. The study protocol was approved by the local medical ethics committee and performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. All participants gave written informed consent prior to inclusion in the study. The protocol is conform to international ethical standards[15].

Study design

Saliva was sampled at three fixed time points a day (every six h) during a 30-day period in the participants' natural environment resulting in a maximum of 90 melatonin measurements per individual. Saliva sampling was chosen because the long measurement period combined with the naturalistic design did not allow for repeated blood sampling. The time of the evening saliva assessments was set at 30 min prior to the regular bedtime (as assessed by the Munich Chronotype Questionnaire[16]), to avoid interference with the natural sleep pattern of the participants. For most participants, this resulted in assessments at the end of the morning (mean ≈ 1000h), afternoon (mean ≈ 1600h), and evening (mean ≈ 2200h). Nocturnal melatonin measurements were not collected because of the high burden of interrupting the participants' sleep patterns. The individuals were warned by an alert

30 min before every saliva sampling moment, and instructed not to eat, drink (apart from water), smoke, or brush their teeth until saliva was collected. An electronic diary was completed containing check-up questions to control whether the individual complied with the food and beverage restrictions over the previous 30 min. Individuals were instructed to collect saliva by keeping a Salivette® in their mouth for approximately 3 min while filling out the electronic diary. After saliva collection, the individuals were instructed to store the Salivette® in their refrigerator immediately if possible, and otherwise at least within 4 h after sampling. A logbook was provided to note abnormalities and protocol violations in sampling, for example changes in sampling time, extra medications taken that day, and being ill.

Electronic diaries were collected using the PsyMate®, an electronic device that was developed to facilitate the monitoring of daily life behavior (PsyMate BV, Maastricht, The Netherlands). The PsyMate® generated an alert every time the diary had to be completed. Questionnaires about mood, sleep, activities and cognitions influencing physical activity and mood such as social interactions, important events, rumination and self-esteem, were monitored with the PsyMate®.

Accelerometers, the ActiCal®, were used throughout the total study period for objective measurement of physical activity by means of registering the participant's energy expenditure (Respironics, Bend, OR). The ActiCal® was continuously worn on the wrist of the non-dominant arm.

Melatonin assays

The saliva samples were centrifuged weekly and stored at -80°C until analysis. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) was used to analyze all samples. LC-MS/MS has broad analytic compatibility and high analytical performance[17]. Melatonin was analyzed by means of online-solid phase extraction in combination with isotope dilution LC-MS/MS. In short, 250uL of saliva was used for the analysis and deuterated melatonin was used as internal standard. All samples of one individual were assayed in the same batch. Mean intra- and inter-assay coefficients of variation were below 9.0%. Quantification limit for melatonin was 5.0 pmol/L.

Statistics

All statistics were performed using IBM SPSS Statistics 20 (IMB, SPSS Inc., Chicago, IL). Linear mixed model analyses were used to compare the melatonin levels of the depressed and the non-depressed group, using all observations. The same was done for morning, afternoon, and evening levels separately. Bootstrapped confidence intervals were used to account for skewness in the distribution of melatonin values. We also conducted Levene's test for homogeneity of variances to compare the variances in the depressed and non-depressed group. The level of significance was $p < .05$ for all analyses.

For each individual, the mean square successive difference (MSSD), that is, the average of the squared differences between successive observations, was calculated to investigate day-to-day variability in melatonin levels[18]. This was done for morning, afternoon and evening levels separately. We used autoregressive integrated moving average (ARIMA) modeling[19] to examine the degree of autocorrelation in the individual

time series. Autocorrelation reflects the stability of the melatonin secretion patterns. Morning and afternoon dummies were added to the models to control for daily cycles (reference category = evening) and a time variable was added to account for time trends in the series. Variables for medication use (1 for medication use, 0 otherwise), medication change (1 for change in medication use, 0 otherwise) and illness (1 for sick days, 0 otherwise) were used to model exogenous influences related to (changes in) medication use and illness. Optimal model specification of the ARIMA models was determined by inspection of the (residual) autocorrelation function and the Bayesian Information Criterion (BIC). Backward selection was used to remove non-significant control variables. Outlier values higher than three times the standard deviation of the noise residuals were accounted for by adding an outlier dummy variable (1 at the time point of the outlier, 0 otherwise) to the model. The white noise assumption (no residual autocorrelation) was tested with the Ljung-Box test. The assumption of normally distributed residuals was tested with the skewness test. A log transformation was applied to the melatonin values in case this assumption was violated. The models were adjusted, re-estimated, and re-evaluated until both assumptions were met.

Results

Group-level characteristics of depressed and non-depressed participants

Demographic, clinical and melatonin characteristics can be found in Table 1. The bootstrapped linear mixed model analyses showed that melatonin levels were significantly higher in the depressed compared to the non-depressed group (B = 87, 95% CI 56-123, p = .002). The same was true for the afternoon (B = 19, 95% CI 11-28, p = .003) and evening (B = 166, 95% CI = 95-261, p = .013) melatonin levels separately. A trend toward significance was shown for the morning levels (B = 77, 95% CI 37-125, p = .051). Levene's test showed significant differences in the variances for morning, afternoon, and evening melatonin levels between the depressed and the non-depressed group (p < 0.001 for the three measurements).

Individual patterns of melatonin time series

Mean levels

The individual melatonin time series are visualized in Figure 1. Large interindividual differences in mean levels and standard deviations were observed for all 3-day segments, with mean levels ranging from 0 to 1397 and standard deviations from 0 to 2171.

For most participants, evening values were significantly higher than morning and afternoon values, as expected. Exceptions were participants C3, C6, D6, D7, and D10. Note that participant numbers starting with a D refer to depressed participants and numbers starting with a C to their non-depressed matched controls.

Table 1. *Demographic, clinical, and melatonin characteristics*

	Non-depressed (N = 10)	Depressed (N = 10)
Female, n	7	7
Age, y	36.7 (7.9)	36.4 (10.3)
BMI, kg/m^2	22.2 (2.3)	23.9 (4.9)
Non-smoker, n	10	10
BDI at intake	2.9 (3.4)	30.7 (10.9)
Morning melatonin, pmol/L		
Mean (SD)	5.1 (8.4)	81.7 (195.4)
Median (IQR)	0.0 (7.0)	0.0 (14.8)
Afternoon melatonin, pmol/L		
Mean (SD)	1.9 (5.3)	20.3 (40.8)
Median (IQR)	0.0 (0.0)	0.0 (5.3)
Evening melatonin, pmol/L		
Mean (SD)	20.5 (18.1)	186.3 (429.1)
Median (IQR)	11.8 (29.0)	32.1 (54.3)
Missing obs, %	7.6 (7.4)	2.3 (2.3)

Note. Age, BMI, BDI at intake, and missing obs are expressed as mean (SD). BMI = Body Mass Index; BDI = Beck Depression Inventory; missing obs = missing observations; IQR = Interquartile Range.

The afternoon melatonin levels were usually close to 0, except for those of participants C6, D7, and D8. For D8, this could be explained by nocturnal intake of the antidepressant citalopram (B = 753, 95% CI 466-1039, $p < .001$). The extraordinarily high-afternoon levels of C6 and D7 could not be explained by medication use or illness.

Mean morning values were highest for D6 and D7. The high-morning values of D6 starting at day 24 could be explained by nocturnal amitriptyline intake, starting at day 23 of the research period (B = 4517, 95% CI 387-514, $p < .001$). We found no explanation for the high-morning values of D7.

Variability

The estimated MSSDs indicated substantial day-to-day variability, particularly for the evening values, though not for all individuals and not for all day segments. For D2, D6 and D7, the day-to-day variability was highest for morning values and D10 showed the highest variability for afternoon values. Depressed individuals mostly showed higher day-to-day variability than their non-depressed matched controls, except for pairs 2, 3 and 6.

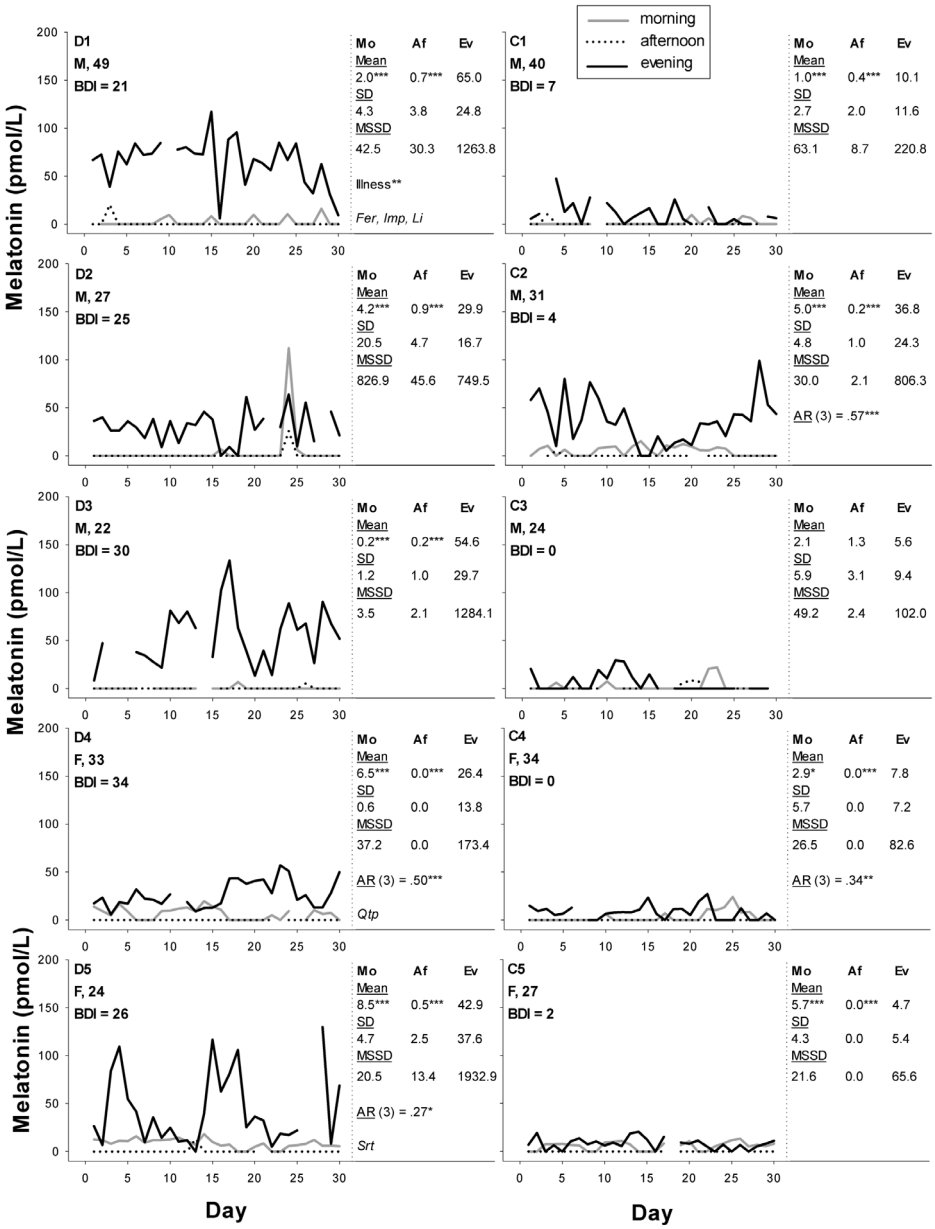
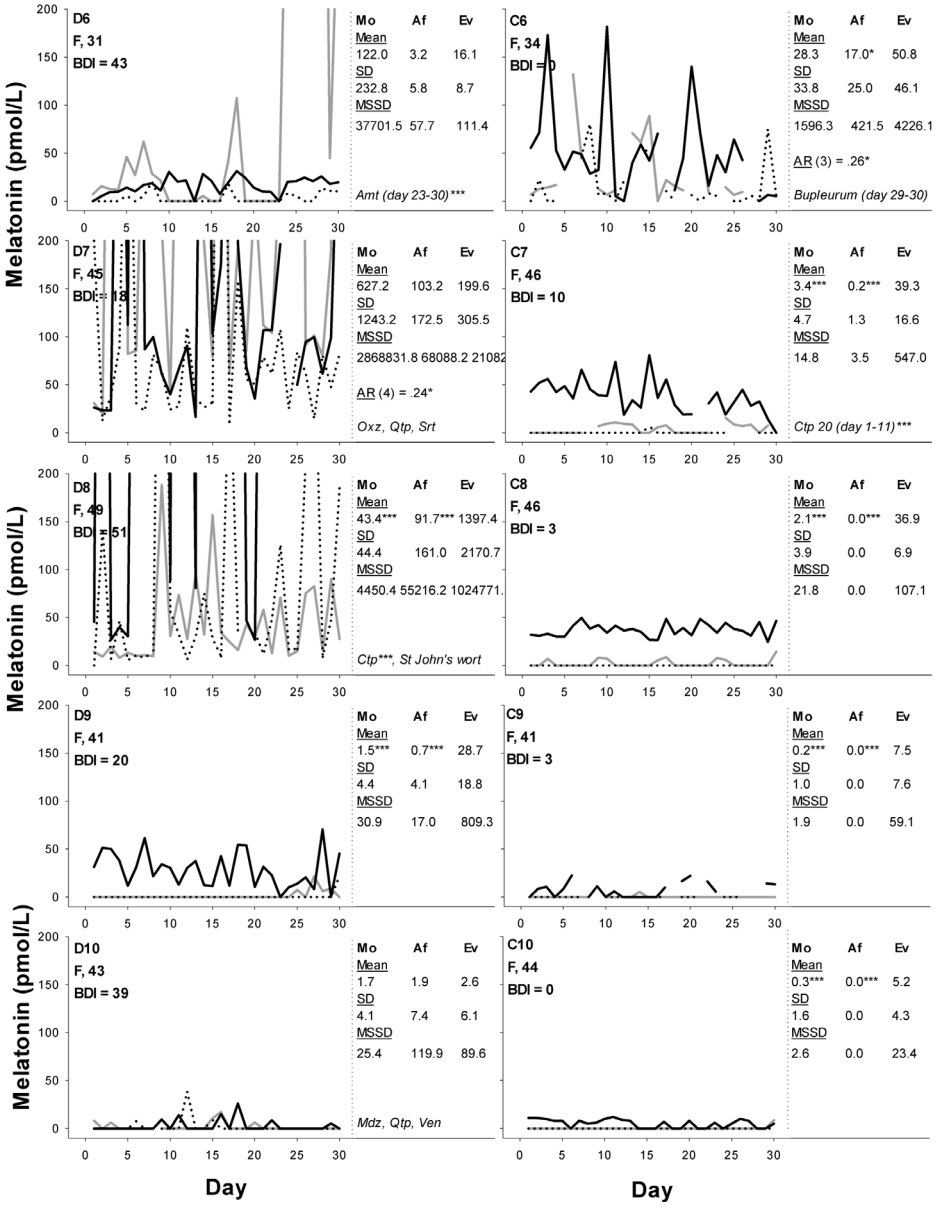


Figure 1. *30-day melatonin secretion patterns of depressed (D) and non-depressed (C) individuals*


D6
F, 31
BDI = 43
Mo Af Ev
Mean
122.0 3.2 16.1
SD
232.8 5.8 8.7
MSSD
37701.5 57.7 111.4
Amt (day 23-30)***
C6
F, 34
BDI = 0
Mo Af Ev
Mean
28.3 17.0* 50.8
SD
33.8 25.0 46.1
MSSD
1596.3 421.5 4226.1
AR (3) = .26*
Bupleurum (day 29-30)
D7
F, 45
BDI = 18
Mo Af Ev
Mean
627.2 103.2 199.6
SD
1243.2 172.5 305.5
MSSD
2868831.8 68088.2 21082
AR (4) = .24*
Oxz, Qtp, Srt
C7
F, 46
BDI = 10
Mo Af Ev
Mean
3.4*** 0.2*** 39.3
SD
4.7 1.3 16.6
MSSD
14.8 3.5 547.0
Ctp 20 (day 1-11)***
D8
F, 49
BDI = 51
Mo Af Ev
Mean
43.4*** 91.7*** 1397.4
SD
44.4 161.0 2170.7
MSSD
4450.4 55216.2 1024771.
Ctp***, St John's wort
C8
F, 46
BDI = 3
Mo Af Ev
Mean
2.1*** 0.0*** 36.9
SD
3.9 0.0 6.9
MSSD
21.8 0.0 107.1
D9
F, 41
BDI = 20
Mo Af Ev
Mean
1.5*** 0.7*** 28.7
SD
4.4 4.1 18.8
MSSD
30.9 17.0 809.3
C9
F, 41
BDI = 3
Mo Af Ev
Mean
0.2*** 0.0*** 7.5
SD
1.0 0.0 7.6
MSSD
1.9 0.0 59.1
D10
F, 43
BDI = 39
Mo Af Ev
Mean
1.7 1.9 2.6
SD
4.1 7.4 6.1
MSSD
25.4 119.9 89.6
Mdz, Qtp, Ven
C10
F, 44
BDI = 0
Mo Af Ev
Mean
0.3*** 0.0*** 5.2
SD
1.6 0.0 4.3
MSSD
2.6 0.0 23.4
Melatonin (pmol/L)
Day

Stability

Moderate to large autoregressive effects were found in three non-depressed individuals (C2: 0.57, C4: 0.34, C6: 0.26, $p < .05$) and three depressed individuals (D4: 0.50, D5: 0.27, D7: 0.24, $p < .05$), in most cases at lag 3, indicating that current melatonin levels are predicted by previous-day levels in these individuals. Otherwise we found no significant autocorrelations.

Discussion

Our investigation of individual longitudinal melatonin secretion patterns revealed systematic group-level differences between the depressed and non-depressed group. The depressed group showed higher mean levels and also more variance in melatonin levels compared to the non-depressed group, probably due to extreme heightened melatonin levels in some individuals of the depressed group. Above that, we found important inter- and intra-individual variability in melatonin secretion patterns, which would have remained unnoticed if the focus were exclusively on aggregated group results.

One of this study's strengths is the detailed information collected about individuals' melatonin secretion pattern over a period of 30 days. This information allowed us to show large interindividual differences in mean levels, variance, day-to-day variability and stability, as well as large intra-individual variability of melatonin secretion. Our results emphasize the added value of using a replicated single-subject time-series design, as mentioned before[9,12]. Inconsistencies in the literature[5] as well as small overall effect sizes in group-based studies can be interpreted in the light of the heterogeneity revealed by our study. Large intra- and interindividual differences can account for substantial variations in study results, depending on the sample and the time of assessment.

Some individuals in the depressed group showed extreme heightened melatonin levels. This in contrast to most studies, where depressed patients seem to show overall lower melatonin levels compared with healthy controls[4]. However, higher levels of melatonin in depressed patients have been reported as well[5], and earlier studies have suggested that elevated levels of melatonin might be due to different clinical characteristics between participants. Another factor that might be of influence on melatonin secretion levels is drugs use: after taking the influence of drug use into consideration, melatonin levels of depressed patients have been shown to be lower than melatonin levels of healthy controls[20].

The antidepressants citalopram and amitriptyline had a clear influence on melatonin levels in this study. Both antidepressants were developed to inhibit synaptic serotonin reuptake, and serotonin is a precursor of melatonin[21,22]. It may therefore be argued that melatonin secretion is increased by the SSRI-induced suppression of synaptic reuptake of serotonin. Interestingly, imipramine and sertraline, acting comparably to citalopram and amitriptyline, were not found to affect melatonin levels in this study. Citalopram, amitriptyline and imipramine were taken after the evening measurement, and sertraline was taken before the morning measurement. In light of the circadian rhythm of melatonin secretion, the

time of intake might mediate the effect of antidepressants on melatonin secretion. These possibly differential side effects of SSRIs might have clinical implications, particularly for depressed patients who suffer from a disturbed biological rhythm, and are additionally treated with light therapy.

Diet can have an influence on melatonin levels as well, though the influence of diet is minor compared with that of light[23]. Food with a high concentration of tryptophan provides an environment that is needed for the production of melatonin, but several vitamins and minerals have to be present to activate this production process[24]. Studying participants in their natural environment makes it hard to control their diet. However, in the current study, participants were instructed not to consume any food or beverages (except for water) within 30 min prior to the saliva sampling.

A limitation of our study was that we did not measure melatonin during the night. Technological advancement may make it possible to include nocturnal melatonin measurements in future studies. It remains to be investigated why melatonin levels can differ so substantially between individuals. In addition to medication use, possible contributors to this heterogeneity in melatonin levels include sleep, exercise and nutrition. To fully understand their relationships with melatonin, it is important not to focus on each of these factors separately, but to examine their interdynamics over time.

This is the first study that shows the potential of individual time series for the exploration of inter- and intra-individual variability in melatonin secretion. The substantial heterogeneity, both across and within groups, and the large day-to-day variability within individuals emphasize the need to account for individual heterogeneity and temporal complexity in endocrinological studies.

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