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Regular Article

Relationship between depressive mood and chronotype in healthy subjects

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Aim: The endogenous circadian clock generates daily variations of physiological and behavior functions such as the endogenous interindividual component (morningness/eveningness preferences). Also, mood disorders are associated with a breakdown in the organization of ultradian rhythm. Therefore, the purpose of the present study was to assessed the association between chronotype and the level of depressive symptoms in a healthy sample population. Furthermore, the components of the depression scale that best discriminate the chronotypes were determined.

Methods: This cross-sectional study involved 200 volunteers, aged 18–99 years, 118 women and 82 men. The instruments were the Montgomery–Äsberg Depression Rating Scale (MADRS), the Morningness/Eveningness Questionnaire, the Self-Reporting Questionnaire-20, and the future self-perception questionnaire.

Results: Logistic regression showed that subjects with the eveningness chronotype had a higher chance of reporting more severe depressive symptoms compared to morning- and intermediate-chronotypes, with an odds ratio (OR) of 2.83 and 5.01, respectively. Other independent cofactors associated with a higher level of depressive symptoms were female gender (OR, 3.36), minor psychiatric disorders (OR, 3.70) and low future self-perception (OR, 3.11). Younger age, however, was associated with a lower level of depressive symptoms (OR, 0.97). The questions in the MADRS that presented higher discriminate coefficients among chronotypes were those related to sadness, inner tension, sleep reduction and pessimism.

Conclusion: Identification of an association between evening typology and depressive symptoms in healthy samples may be useful in further investigation of circadian typology and the course of depressive disease.

Key words: chronobiology, chronotype, circadian rhythm, depressive mood, morningness.

THE ENDOGENOUS CIRCADIAN clock generates daily variations of physiological and behavior functions. Biological rhythms are cyclic changes that correspond to the temporal organization of the environment.[1] Many biological variables, such as body temperature, heart rate, blood pressure and hormone levels, show a definite periodicity with a cycle length of 24 h. Psychological and behavioral variables, such as mood, alertness, appetite and task performance, also demonstrate circadian effects.[2] These variables have been shown to be associated with individual differences in circadian rhythm. This endogenous

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interindividual component of the circadian clock is the circadian typology (morningness/eveningness preferences).

Morning types are those people who consistently prefer diurnal activity, while evening types are those who prefer nocturnal activities.[3] The morningness/eveningness dimension is regulated by a complex interaction among social, geographic and genetic factors. Studies have demonstrated that increasing age and being female are associated with morningness.[4] In addition, individuals who are born during autumn and short photoperiods of 8–10 h showed stronger morningness tendencies, probably as a reflection of the influence of light intensity and variation in photoperiods during early development.[5] During constant routines, significant differences have been observed between morning and evening types in the circadian phases of body temperature and alertness. Also, it has been demonstrated that chronotype depends on a circadian gene polymorphism. In a UK study it was reported that a polymorphism of *PER3* genes may influence the morningness/eveningness dimension.[6] The four-repeat allele was associated with eveningness and delayed sleep-phase syndrome (DSPS; which may be considered an extreme form of eveningness) and the five-repeat allele was associated with morningness; the same association with diurnal preference was also found in a Brazilian population but the five-repeat allele was associated with DSPS, probably reflecting the latitude of influence.[7] The impact of this phase shift on health has been debated. Eveningness seems to be related to morbidity and especially to sleep and mood disorder.[8]

Studies have suggested that mood disorders are associated with a breakdown in the organization of ultradian rhythm.[9] There is a debate as to whether depression is related to circadian rhythms. Three lines of evidence suggest that depressive disorder has a circadian component: (i) improved outcomes in individuals exposed to light treatment; (ii) association between the timing of light and phase shift; and (iii) report of a difference in NPAS2 genes, a gene family involved in circadian rhythm generation, between seasonal affective disorder and healthy controls.[9] A phase delay has also been described in affective disorders. Previous studies found an association between evening type and depressive symptoms in restricted samples such as students and patients with mood disorder.[10,11]

The main hypothesis under investigation was that evening-type subjects present higher rates of symptoms of depression not only in depressed patients, as demonstrated in previous studies, but also in healthy people. Thus, we assessed the association between chronotype and the range of depressive symptoms, controlling for the effect of potential confounding variables. Components of the depression scale that best discriminate chronotypes were also analyzed.

METHODS

Subjects

The study protocol was approved by the Ethics Committee at the institution in which the work was carried out. All participants signed an informed consent form. From October to November, a systematic sample of urban census tracts was conducted. In each tract a starting point was randomly selected and interviewers searched the neighborhood visiting one out of every three houses. These subjects were selected from a community that is situated in an urban circumscribed geographic area that constitutes the catchment area of the Basic Health Unit linked to the Hospital de Clínicas de Porto Alegre, which serves a population of approximately 6000 living nearby. For the present study, all members of the nuclear family selected who met the criteria of inclusion were assessed. The rate of participation was >98%. The sample was composed of 200 volunteers, ranging in age from 18 to 99 years (mean ± SD, 34.35 ± 16.39 years).

Exclusion criteria were shift work, self-report of past or current history of psychiatric disorder and medical conditions, and use of drugs known to influence sleep, as well as a medical history of organic brain damage, mental retardation, not speaking Portuguese and difficulty in understanding verbal commands. Drug use and diagnosed disease were assessed by the following questions: 'Do you have any diagnosed diseases? If yes, what kind of disease?' and 'Do you use any drug(s)? If yes, what kind of drug(s)?' Moreover, at the end of the testing the subject read a list of diseases that included psychiatric disorders, habitual snoring, apnea, and thyroid disorders, among others. Then they answered a yes or no questionnaire about the diseases and their use of substances (benzodiazepines, antidepressants, illicit drugs [cocaine, cannabis], tea, and caffeine). An affirmative answer was used as an exclusion criterion.

Instruments

All of the psychological tests used in the present study were validated and adapted for the Brazilian population.

The main outcome was the depressive symptoms as assessed on the Montgomery–Äsberg Depression Rating Scale (MADRS).[12] This scale contains 10 questions ranging from zero to 6 points. The final score ranges from zero to 60 points. An intraclass correlation of 0.96 was observed for the agreement between different evaluators. The highest quartile, which was 8, was used as the cut-off point to classify patients in terms of moderate–intense or absence/mild depressive symptoms. Individuals with a score >8 were classified as having moderate–intense depressive symptoms, and those with a score ≤8 were classified as having absence or mild depressive symptoms.

In the present study the main factor of interest was the chronotype, which was assessed on a Brazilian Portuguese adaptation of the Morningness/Eveningness Questionnaire.[3] The Morningness/Eveningness Questionnaire is a construct developed to estimate phase tendencies in circadian rhythm from self-description. Scores range from 16 to 86, with higher scores indicating greater morningness tendencies. Scores can also be divided into five categories of chronotypes: definite evening type, 16–30; moderate evening type, 31–41; intermediate type, 42–58; moderate morning type, 59–69; and definite morning type, 70–86.[3] For the analysis of the differences among chronotypes, moderate evening types and definite evening types were grouped together under evening type; the morning-type category encompassed definite morning type and moderate morning type.[3]

Subjects completed a questionnaire about demographic characteristics. Also the subjects completed a questionnaire in which they answered items about use of drugs, diagnosed diseases affecting sleep, and sleeping habits (estimated bedtime, estimated wake-up time, estimated number of hours of sleep [amount of sleep]).

The hopelessness scale,[13] based on the Beck Hopelessness Scale,[14] was applied. It consists of 10 simple questions addressing the participant's perceptions of the future. Individual scores varied from −2 to +2, such that the scale of the total scores ranges from −20 to +20. Higher scores denote higher future self-perception. The cut-off point for classifying high future self-perception or low future self-perception was the lowest quartile (i.e. 8), so that individuals with a score above the lowest quartile were classified as high future self-perception, reflecting an optimistic view, and those with a score equal to or below the lowest quartile reflected a negative future perception (hopelessness).

The World Health Organization self-reporting questionnaire (SRQ-20) has been used for screening of minor psychiatric disorders: somatic symptoms, depressive mood, depressive thoughts and decreased energy. All the questions are of the yes/no type, and the score is given by the number of questions that are answered as 'yes.' Minor psychiatric disorders were defined by an SRQ-20 score of ≥6 for men, and ≥8 for women. Using these cut-offs, the sensitivity of the method to detect minor psychiatric disorders is 89% for men and 86% for women, and the specificity is 81% for men and 77% for women.[15]

Procedure

The investigation was designed as a population-based cross-sectional study. It was performed in the city of Porto Alegre, which is located in the extreme south of Brazil (30°05′ south, 51°10′ west).

The data were collected by four research assistants (blinded to the objective of the study and previously trained in order to avoid biases in measurement), within a period of 2 months, in order to avoid major variances in the duration of the day and night periods. Participants were not aware of the study's objective. To guarantee blinding, the questionnaires contained several questions that were not related to depressive symptoms or morningness/eveningness dimension. Questionnaires were presented in a random order to prevent order effects and were answered individually, following the instructions for each question.

Statistical analysis

The sample size was estimated based on the data from an internal pilot study with 40 subjects, who met the same inclusion criteria described in the present study. These subjects were included in the overall analysis of the data. Taking into account that we found a prevalence of moderate–intense depressive symptoms that was 15% in subjects who were not evening type in the pilot study, an analysis indicated that 196 subjects were required to obtain a power of 80% and α set at 0.05.

A one-way ANOVA with Tukey post-hoc test was used to analyze parametric variables related to

chronotype. The difference between absent or mild and moderate–severe depressive symptoms for continuing data were examined on *t*-test for independent samples and categorical data were examined using Pearson's χ^2 or the Mantel–Haenszel method. Univariate and multivariate analyses were carried out using SPSS for Windows.[16] In order to control potential confounding variables, and to determine the independent factors associated with moderate–intense depressive symptoms, logistic regression was used. Factors that had a significant association with depressive symptoms or chronotype were then included in multivariate logistic regression following the stepwise forward procedure.[17] $P < 0.20$ was required for a factor to be included in the analysis. The independent variables included in the model as continuous variables were age, education, wake up hour, time of falling asleep and sleep length. Chronotype, gender, future self-perception and minor psychiatric disorders were included in the model as categorical variables. The Hosmer–Lemeshow goodness-of-fit test was used to evaluate the degree of correspondence between a model's estimated probability of moderate–intense depressive symptoms and the actual moderate–intense depressive symptoms. Statistical significance was assessed through the likelihood ratio test.

Discriminant analysis was also applied to evaluate which of the questions in the MADRS presented higher coefficients to discriminate between evening and intermediate type, and also between evening and morning type. The significance level was set at $P < 0.05$ (two-tailed).

RESULTS

Table 1 lists the comparison of demographic and sleep characteristics among chronotypes. In this sample 71 subjects (35.5%) were classified as evening, 71 (35.5%) as morning, and 58 (29%) as intermediate type. The relationship between chronotypes and sleep characteristics, age and gender are presented in Table 1. Usual waking-up and falling-asleep times were significantly different among chronotype, and older age was linked to a morning typology.

A significant association was seen on univariate analysis, between the rate of depressive symptoms and the following variables: gender, age, falling asleep, future self-perception, minor psychiatric disorders and evening chronotype (Table 2).

On logistic regression circadian typology for evenings was found to be an independent factor associated with a higher risk ratio for reporting moderate–intense depressive symptoms compared to morning and intermediate chronotypes (Table 3). Furthermore, it also demonstrated other independent cofactors associated with a higher risk ratio of reporting moderate–intense depressive symptoms in healthy subjects, such as female gender and age. Age was included in the model as a continuous variable, thus, in the interpretation of the odds ratio it was observed that the risk ratio for reporting moderate–severe depressive symptoms was reduced 3.3% for each year younger. Presence of minor psychiatric disorders and low future self-perception were also associated with a higher chance of reporting moderate–intense depressive symptoms. The variables of formal education, wake up hour, time of falling asleep and sleep length were not retained in the final model. The Lemeshow goodness-of-fit test of the present model was $P = 0.72$. This P indicates that the model is a good fit, because it correctly classifies 83.3% of cases when comparing the expected rates of moderate–intense depressive symptoms with the observed.

Table 1. Subject characteristics vs chronotype

Variable	Evening ($n = 71$)	Intermediate ($n = 58$)	Morning ($n = 71$)	χ^2/F	*P*
Gender (female/male)†	41/30	31/27	46/25	1.77	0.41
Formal education (years)‡	14.82 ± 3.94[a]	12.71 ± 3.77[b]	12.76 ± 4.91[b]	5.45	0.00
Age (years)‡	29.80 ± 11.87[a]	33.76 ± 16.89	39.39 ± 18.53[b]	6.47	0.00
Wake-up time (hh : mm)‡	6.17 ± 1.804[a]	5.37 ± 1.324[b]	5.23 ± 1.418[c]	6.24	0.00
Going to sleep (hh : mm)‡	23.58 ± 1.489[a]	23.26 ± 1.142	23.10 ± 1.486[b]	5.99	0.00
Sleep length (hh : mm)‡	6.19 ± 2.029	6.11 ± 1.699	6.14 ± 1.700	0.09	0.91

†χ^2 test; ‡one-way ANOVA.
[a,b,c]Different superscripts indicate significant difference among chronotypes using Tukey test.

Table 2. Baseline subject characteristics vs level of depressive symptoms

		Depressive symptoms				
Variable	Categories	Moderate–intense ($n = 48$)	Absence or mild ($n = 152$)	OR	95%CI	*P*
Gender‡	Female/Male	37/11	81/71	2.95	(1.40–6.20)	0.00
Age (years)†		39.08 ± 17.01	32.86 ± 15.95	–	–	0.02
Formal education (years)†		12.87 ± 5.17	13.66 ± 4.07	–	–	0.27
Wake-up time (h)†		5.86 ± 1.85	5.74 ± 1.49	–	–	0.67
Falling asleep time (h)†		24.27 ± 1.57	23.14 ± 1.36	–	–	0.04
Sleep length (h)†		8.47 ± 2.79	7.73 ± 2.48	–	–	0.08
Future self-perception‡						
	Low/High	20/28	21/131	4.46	(1.13–9.30)	0.00
Minor psychiatric disorders‡						
	Yes/No	15/33	13/138	4.83	(2.1–11.11)	0.01
Chronotype‡	Evening	25	46	1.00	–	0.01
	Morning	17	54	1.73	(0.83–3.59)	
	Intermediate	6	52	4.71	(1.64–14.14)	

†Unpaired *t*-test; ‡Pearson's χ^2 test or Mantel–Haenszel test.
CI, confidence interval; OR, odds ratio.

The MADRS questions relating to inability to feel and concentration difficulties were the items with the highest discriminating coefficients for the distinction between evening and intermediate, and evening and morning chronotypes (Table 4). Using the complete MADRS, the percentage of correctly classified cases between evening and intermediate was 64.34% and 58.45% when the comparison was between evening and morning-types.

DISCUSSION

In the current study the evening typology was associated with a higher risk of reporting moderate–intense depressive symptoms, despite the use of the 'report of psychiatric disorders' as an exclusion criterion. The present findings corroborate, to some extent, the hypothesis raised by Drennan *et al*. and Chelminski *et al*. that the eveningness dimension cannot simply be a characteristic of the depressed patient but rather may reflect a pre-morbid trait.[10,11] The present finding may indicate that the chronotype is a biological characteristic that itself constitutes a trait or behavioral symptom that is related to affective disorders. Although early circadian rhythm theories of depression focused on primary disturbances in the intrinsic properties of circadian clocks and their entrainment pathways, since the 1990s behavioral and physiologi-

Table 3. Potential factors associated with moderate–intense depressive symptoms in healthy subjects ($n = 200$)†

Variable in the equation	β	SE	*P*	OR	95%Cl
Gender					
Female/male	1.249	0.431	0.00	3.36	(1.46–7.74)
Age	–0.026	0.012	0.03	0.97	(0.95–0.99)
Chronotype					
Evening/morning	1.153	0.459	0.00	2.83	(1.15–6.97)
Evening/intermediate	1.685	0.557	0.00	5.01	(1.68–14.93)
Low future self-perception					
Low/High	1.135	0.455	0.01	3.11	(1.27–7.59)
Minor psychiatric disorders					
Yes/No	1.30	0.51	0.01	3.70	(1,36–10,04)

†Stepwise forward logistic regression.
CI, confidence interval; OR, odds ratio.

Table 4. Discriminant analysis: MADRS score vs chronotype ($n = 200$)

MADRS items	Discriminant index between chronotypes groups	
	Evening × intermediate ($n = 129$)	Evening × morning ($n = 142$)
1. Apparent sadness	0.13	−0.04
2. Reported sadness†	0.59	0.41
3. Inner tension†	0.31	0.41
4. Reduced sleep†	0.34	0.35
5. Reduced appetite	−0.05	−0.46
6. Concentration difficulties†	0.71	0.52
7. Lassitude	0.50	0.13
8. Inability to feel†	0.74	0.55
9. Pessimistic thoughts†	0.47	0.37
10. Suicidal thoughts	0.39	0.21
Percentage of 'grouped' cases correctly classified	64.34%	58.45%

†Discriminate coefficient >0.3 on comparison between chronotypes.
MADRS, Montgomery-Äsberg Depression Rating Scale

cal changes have been recognized as being regulated by circadian clocks and as a strong feedback control for the structures that compose the clock.[1] This feedback can be direct; for example, behavioral activation clock disturbance, the daily cycle of which is driven by circadian pacemakers.[2] Therefore, affective symptoms (depression and mania), such as changes in the level of arousal and in the frequency of exposure to the natural environment, definitely feed back to the pacemaker (i.e. the circadian clock). Therefore, in the presence of a primary disturbance it seems likely that interactions between clocks and symptoms would be bidirectional.[18] The affective symptoms appear to interweave with the function of the circadian clock and rhythms in both seasonal and non-seasonal forms of depression.[19]

Eveningness was an independent factor associated with depressive symptoms, in spite of the effect of potential confounding variables such as low future self-perception and minor psychiatric disorders. The potential colinearity (i.e. information redundancy) among these variables was investigated on likelihood ratio tests so as to determine which variables would be dropped from the model.[20] This is an important strategy to control the effect of several variables on clinical phenomena and increase the internal validity of the investigation. Future self-perception was considered as a confounding variable because evening-type individuals have been reported to be more pessimistic than morning types.[21] This is attributed to a possible relationship between negative outlook on life and reluctance to start the day at an early hour with anticipation of unfavorable outcome.[21] Also, so far, it has been widely demonstrated that a pessimistic future self-perception and minor psychiatric disorders are associated with the level of depressive symptoms, independent of subject chronotype.

The results of the discriminant analysis were shifted in the same direction as those of the logistic multivariate analysis. This agreement demonstrates the consistency of the present data. Upon deeper analysis of the association between depressive symptoms and chronotype, it was observed that the MADRS items such as inability to feel and concentration difficulties, were the most effective at discriminating between the circadian evening typology of morning and intermediate chronotypes. Low future self-perception has been associated with chronotype in previous studies;[21] in the present study pessimistic thought had only a moderate–lower effect on the ability to differentiate between chronotypes. Although we do not know the reasons for this finding, we suggest that this symptom is more strongly linked to depressive symptoms than to a specific chronotype.

The fact that evening-type individuals woke up and went to bed later reinforces the circadian hypothesis of the chronotype, that is, that the evening type is characterized by a phase delay, and the morning type by a phase advance. Nonetheless, in the present sample the phase difference did not interfere with the number of hours slept, because the chronotype did not differ according to self-reported sleep duration. This finding is supported by a prior large epidemiological morningness/eveningness study.[22] Because, on univariate analysis, some of these variables related to sleep characteristics were associated with depressive symptoms, they were included in the multivariate analysis to control their potential confounding effect in the association between main outcome and the factor of particular interest (i.e. chronotype). They were dropped from the multivariate model, however, because it is possible that the result of univariate analysis is explained by the confounding effects of other covariates. This finding is important because it underscores the idea that these characteristics alone

define the chronotype typology, as presented in Table 1, but it is possible that they are not specifically associated with depressive symptoms.

Younger people have a lower probability of reporting moderate–intense depressive symptoms, that is, each year younger reduce the probability of reporting higher levels of depressive symptoms by 3.3%. Moreover, the present study supports the reported trend for increasing morningness tendencies with age. A direct effect of age on pacemaker has been observed in several rodent species.[23] In humans it has been shown that circadian amplitude is lowered and the timing of rhythms varies with age. Also, it has been demonstrated that score distribution for the Morning–Evening Questionnaire changes towards morningness with age.[4,10,21,24–28] In the present study aging was associated with depressive symptoms in spite of elderly people having more morningness tendencies. To the best of our knowledge this is the first investigation that expands on the data in the literature, providing additional evidence that the relationship between evening typology and depressive symptoms occurs independently of age.

Although the level of depressive symptoms presented by these subjects is considered to be mild, according to the cut-off used to initiate pharmacological treatment in clinical depression,[31] we think that it is imperative that this be taken into account in future longitudinal studies to verify the hypothesis of a causal relationship between evening chronotype and psychiatric disorder. Finally, the identification of an association between evening typology and depressive symptoms in healthy samples may be useful in further investigation of circadian typology and the course and treatment of depressive disease. Furthermore, it could be used to plan early interventions in patients with a higher susceptibility to depression, to change the course of psychiatric disorder, while reducing the cost and social burden of their care.

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