

BRIEF REPORT

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Associations between circadian, sleep, and mood disturbances in Uruguayan dance students

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Abstract

Background Current evidence supports associations between circadian, sleep, and mood disturbances. However, it is still debated to what extent different chronobiological and mood variables act independently or in synergy to impact health.

Methods This study assesses how these variables interact to affect depressive symptoms and sleep quality in 26 Uruguayan dancers (age=22.27±2.43) training in the morning ($n=9$) or in the night shift ($n=17$). Participants completed the Munich Chronotype Questionnaire, the Pittsburgh Sleep Quality Index, and the Beck Depression Inventory-II.

Results Participants reported high social jetlag (1.58±1.45h), late chronotypes (05:43±01:35), and poor sleep quality (6.77±2.67), while adequate sleep duration (7.10±1.31h) and minimal depression (7.15±3.62), without differences across shifts. Depressive symptoms increased as sleep quality worsened ($F(1,21)=20.66$, $p<0.001$, $\eta^2=0.50$). In addition, we found a marginal interaction between sleep duration and the shift to explain depressive mood ($F(1,21)=4.06$, $p=0.057$, $\eta^2=0.16$), with participants in the morning shift showing higher depressive symptoms with decreased sleep duration. Furthermore, sleep quality deteriorated as social jetlag increased ($F(1,20)=14.82$, $p=0.001$, $\eta^2=0.43$), particularly in more depressed individuals ($F(1,20)=24.09$, $p<0.001$, $\eta^2=0.55$).

Conclusions Our findings indicate that social, circadian, sleep, and mood variables are inextricably linked in this population of dancers.

Keywords Dancers, Shifts, Sleep, Depressive symptoms, Social jetlag, Chronotype

Introduction

The circadian system controls the endogenous rhythms of different body functions, being the sleep-wake cycle the most conspicuous human circadian rhythm (Aschoff, 1965). Social demands interfere with the sleep-wake cycle and can lead to a discrepancy between social and biological time. This discrepancy, called social jetlag (SJL), is used as a proxy of the amount of strain on the circadian system exerted by social timing constraints, and it is worse in late chronotypes. Youngsters usually must fit their late sleep schedules, driven by their natural tendency to eveningness, with early school/work timings. This constant adjustment puts them at risk of

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circadian misalignment and sleep disruptions (Wittmann et al. 2006). The combination of SJL and eveningness has documented detrimental effects on sleep health, but it is still debated which is the main influential factor or whether specific traits interact with them (Roenneberg et al. 2019). Engaging in physical activity can also adjust the circadian system, and exercising at appropriate times may promote chronobiological homeostasis, preventing circadian disruption (Lewis et al. 2018).

Sleep can be characterized by its duration, timing, and quality (Chaput and Shiau 2019). Sleep quality, conceived as an individual's satisfaction with one's sleep (Ohayon et al. 2017), can also be constrained by social pressures. In line with this, applying a chronotype-adjusted schedule in shift workers improves their sleep quality and decreases their SJL (Vetter et al. 2015). Therefore, it is not the chronotype *per se* but working out-of-phase with respect to the individual chronotype that explains low sleep quality. In addition, sleep quality is restrained by sleep duration, with people with short and long sleep durations being the most likely to report poor sleep quality (Bin 2016).

Sleep and circadian rhythm disturbances frequently occur in depression, the most prevalent mental disorder and the leading cause of disability to date. Sleep disturbances can precipitate or exacerbate depression and increase resistance to treatment (Zaki et al. 2018). Both short and long sleep durations have been associated with a higher risk of depression (Zhai et al. 2015). In addition, the association between depression and chronotype has been extensively reported, with studies showing that evening types are more prone to report depressive symptoms (Hidalgo et al. 2009). However, the relationship between SJL and depression is less consistent (Henderson et al. 2019).

A large amount of evidence supports a bidirectional association between sleep quality and depression (Dinis and Bragança 2018). Depressive symptoms are more prevalent in poor sleepers (Wilson et al. 2014), and the vast majority of people with depression complain about sleep quality (Tsuno et al. 2005). Furthermore, studies among university students have found that poor sleep quality was associated with depression and vice versa. Chronotype, the use of technology, and physical activity have been reported to influence this association (Dinis and Bragança 2018; Feng et al. 2014).

To contribute to the study of the multidirectional associations between social, circadian, sleep, and mood variables, it is imperative to find advantageous models that allow disentangling the effects of each component. Young Uruguayans emerge as an interesting model because they

are extremely late-oriented and exhibit strong misalignment (Coirolo et al. 2020; Estevan et al. 2020; Estevan et al. 2021; Silva et al. 2019; Tassinio et al. 2016). In fact, previous studies on Uruguayan youngsters show some of the most extreme late chronotype values reported so far, even when compared with similar sociocultural populations (Randler 2008), and more than one h of SJL, which is currently considered above the tolerable limit (Korman et al. 2020). Moreover, dancers, as competitive athletes who undergo long periods of training and high pressure, are especially vulnerable to sleep and mood disturbances. However, only a few studies examined how sleep is altered in dancers, reporting sleep efficiency and duration deficits before a premiere (Fietze et al. 2009). This study stands on previous work evaluating changes in the biological clock's function and the sleep patterns of Uruguayan dancers trained in shifts (Coirolo et al. 2020; Coirolo et al. 2022; Estevan et al. 2023). In this same model system, we aimed to investigate how social, chronobiological, and psychological factors interact to affect depressive symptoms and sleep quality.

Materials and methods

Participants

Dance students from a four-year training public program for professional contemporary and folkloric dance (Escuelas de Formación Artística, END-SODRE, Ministerio de Educación y Cultura, Uruguay) were personally invited to participate in this study through informational flyers. This program begins each year in March and ends in December. First and second-year students attend the night shift (20:00 to 24:00), and third and fourth-year students attend the morning shift (08:30 to 12:30) from Monday to Friday. This organization in shifts allows the maximization of the school infrastructure usage, and students cannot select their shifts.

Twenty-six participants attending either the morning ($n=9$) or night ($n=17$) shift (a subset of the participants previously reported (Coirolo et al. 2020; Coirolo et al. 2022; Estevan et al. 2023)) fulfilled the inclusion criteria to participate in this study. Inclusion criteria involved not taking psychiatric medication, not using alarm clocks during weekends, and having complete information in the selected questionnaires. Participants did not significantly differ in gender and age across shifts (Table 1). The data was collected in August 2019 (wintertime in the southern hemisphere) during school time. This work was conducted following the principles of the Declaration of Helsinki and was approved by the Research Ethics Committee from the School of Psychology (Universidad de la República). Written informed consent was obtained from all the participants.

Table 1 Demographic, chronobiological, and sleep characteristics between shifts

n	Total 26	Morning-shift 9	Night-shift 17	p-value	Cohen's w/d
Gender (F/M)	21/5	7/2	14/3	1.00 ns	0.06
Age (years)	22.27 ± 2.43 (min: 18, max: 28)	22.78 ± 2.17	22.00 ± 2.57	0.45 ns	0.32
MSFsc (MCTQ) ^a	05:43 ± 01:35 (min: 02:11, max: 07:47)	05:10 ± 01:36	06:00 ± 01:33	0.21 ns	0.53
SJL (MCTQ) ^b	1.58 ± 1.45 (min: -1.26, max: 3.75)	2.12 ± 1.24	1.30 ± 1.51	0.18 ns	0.57
AvSD (MCTQ) ^c	7.10 ± 1.31 (min: 4.55, max: 10.36)	7.12 ± 0.70	7.09 ± 1.56	0.96 ns	0.02

Values are reported as mean ± SD. Fisher's exact test for discrete variables and Independent-samples t-test for continuous variables. Effect sizes were calculated as Cohen's w for frequency differences and Cohen's d for differences in means between groups. ^aMSFsc: midpoint of sleep on free days corrected (presented in military format); ^bSJL: the absolute difference between the midpoints of sleep on work and free days; ^cAvSD: average sleep duration estimated from sleep onset and end on work and free days. MSFsc and SJL correlated as expected (Pearson's correlation $r=0.52$, $p=0.006$); the later the chronotype, the higher the SJL.

Instruments

Demographic data were collected through a short questionnaire applied at the beginning of the assessment. The chronobiological characterization of participants was assessed using the Munich Chronotype Questionnaire (MCTQ) (Roenneberg et al. 2003). Chronotype was assessed as the midpoint of sleep on free days corrected for sleep debt accumulated on workdays (MSFsc) (Roenneberg et al. 2004), and SJL as the absolute difference between the midpoints of sleep on work and free days (Wittmann et al. 2006). Average sleep duration (AvSD) was estimated from sleep onset and end on work and free days. Participants additionally completed the Beck Depression Inventory-II (BDI) (Beck et al. 1996) and the Pittsburgh Sleep Quality Index (PSQI) (Buysse et al. 1989). BDI and PSQI have a four-point scale ranging from 0 to 3, with higher scores representing worse severity (BDI mean scoring >14 represents significant depressive symptoms, and PSQI mean scoring >5 represents poor sleep quality). All scales were administered in their Spanish versions and fulfilled during school time.

Statistical analysis

Statistical analyses were performed using RStudio version 2022.12.0. Linear regression models (R package "lm") were used to explore the influence of shift, SJL, MSFsc, AvSD, and PSQI on BDI and the influence of shift, SJL, MSFsc, AvSD, and BDI on PSQI. The models were selected using the best subsets method. This method relies on stepwise regression to find the subset of independent variables that best explains the outcome (Kassambara 2018). We used the forward stepwise regression, which implies iteratively adding the most contributive predictors until the improvement is no longer statistically significant. The model with the higher adjusted R^2 was selected for each outcome.

Furthermore, the residuals were plotted and inspected for deviations in normality or homoscedasticity. In both models, the mean of the residuals was close to zero, and the Shapiro-Wilk test and the Non-Constant Error Variance Test did not show evidence of non-normality ($p>0.05$), and non-constant variance ($p>0.05$), respectively. Effect sizes were calculated using the partial eta-squared method (η^2). Continuous explanatory variables (MSFsc and AvSD) were mean-centered and standardized. The `emtrends` and `sim_slopes` functions were used for estimating the slopes of fitted lines, with a 0.95 confidence level. Throughout the text, values are presented as mean ± standard deviation in descriptive statistics. The statistical significance level was set at $p<0.05$.

Results

Twenty-six dancers (18-28 years old, 80.8% women) trained at END-SODRE, Uruguay, participated in this study. The demographic, chronobiological, and sleep characterization of these dancers is presented in Table 1. Participants' MSFsc, SJL, and AvSD were not significantly different between shifts. Dancers showed a mean BDI of 7.15 ± 3.62 (min: 0, max: 14; morning shift: 6.56 ± 3.24 ; night shift: 7.47 ± 3.86) and a mean PSQI of 6.77 ± 2.67 (min: 3, max: 12; morning shift: 6.78 ± 2.11 ; night-shift: 6.76 ± 2.99). These results showed that participants were minimally depressed (mean scoring <14) while their subjective sleep quality was poor (mean scoring >5). Participants' BDI and PSQI were not significantly different between shifts ($p=0.55$, $p=0.99$, respectively) (Fig. 1).

The PSQI component in which the participants reported worse difficulties was sleep latency, followed by subjective quality and disturbances (Fig. 2).

Variables associated with depressive symptomatology

A significant effect of PSQI on BDI was found ($F_{(1,21)}=20.656$, $p<0.001$, $\eta^2=0.50$), with depressive

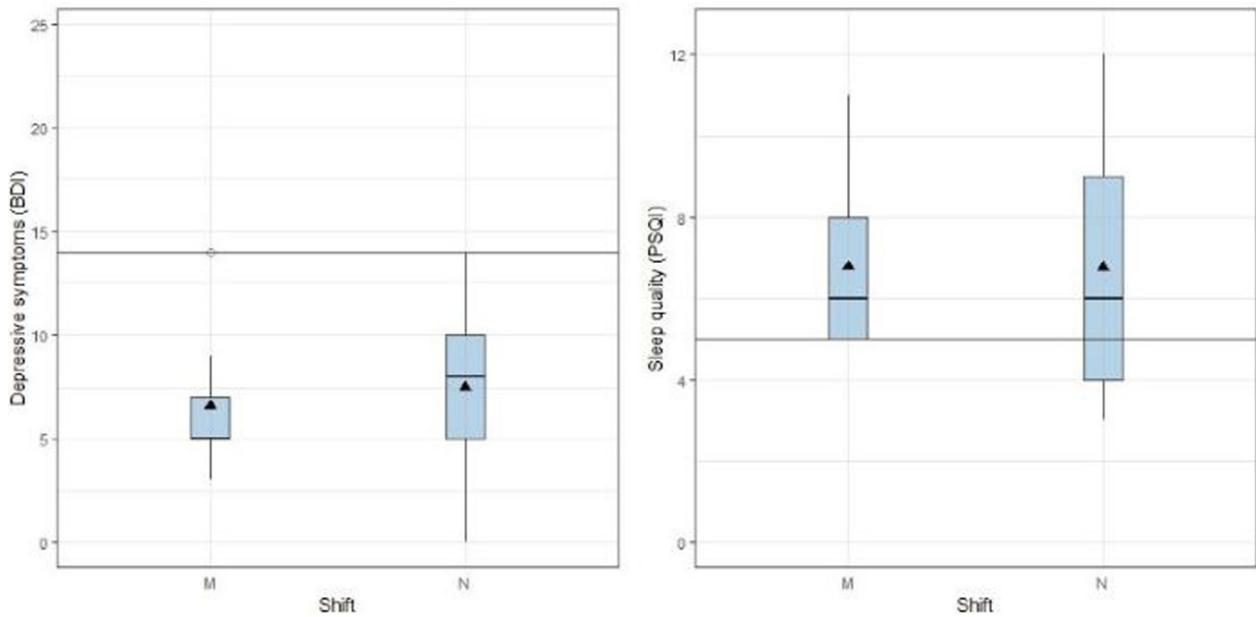


Fig. 1 Boxplots of outcome variables: depressive symptoms (BDI) and sleep quality (PSQI) between shifts

Notes: Triangles denote the mean. The horizontal line in both plots represents the reference point for considering depressive symptoms as more than minimal (BDI > 14) and sleep quality as poor (PSQI > 5)

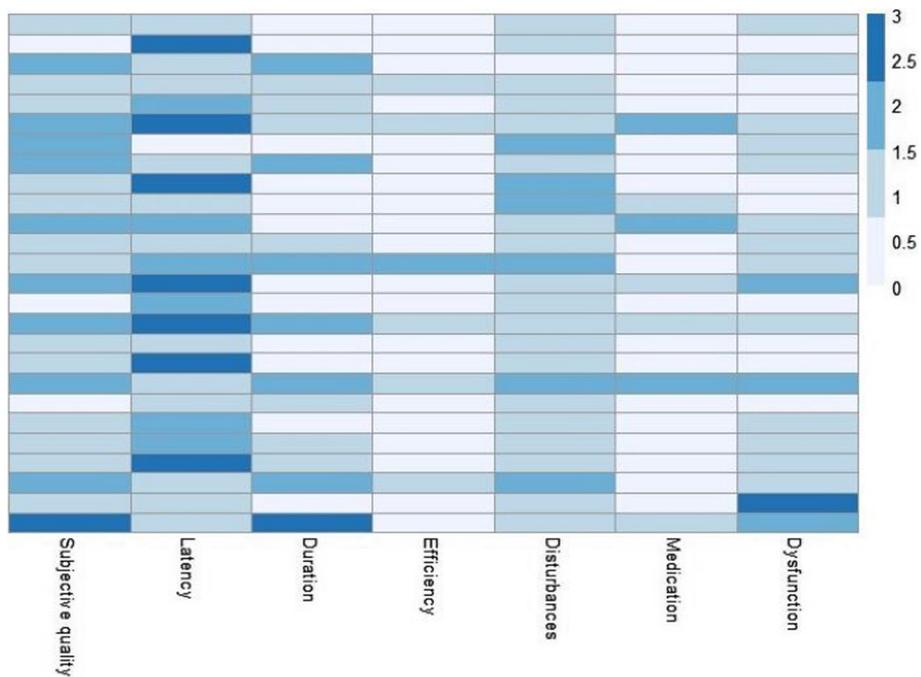


Fig. 2 Heatmap of PSQI components

Notes: The seven components are displayed on columns, and participants are displayed on rows. Stronger colors represent higher scores on the PSQI Likert scale, indicating greater difficulties

Table 2 ANOVA table for the regression model with depressive symptomatology as the dependent variable

	Df	Sum Sq	Mean Sq	F-value	P value	η^2
PSQI	1	141.402	141.402	20.656	<0.001***	0.50
MSFsc*AvSD	1	4.604	4.604	0.673	0.421	0.03
PSQI*AvSD	1	9.809	9.809	1.433	0.245	0.06
AvSD*Shift	1	27.817	27.817	4.064	0.057*	0.16
Residuals	21	143.753	6.845	-	-	
Adjusted R ²	0.48					

** denotes p -values <0.10 **** denotes p -values <0.001. η^2 = partial eta-squared. MSFsc and AvSD were mean-centered and standardized. PSQI: sleep quality; MSFsc: midpoint of sleep on free days corrected; AvSD: average sleep duration estimated from sleep onset and end on work and free days. SJL was not a relevant variable in the model based on the best subsets method

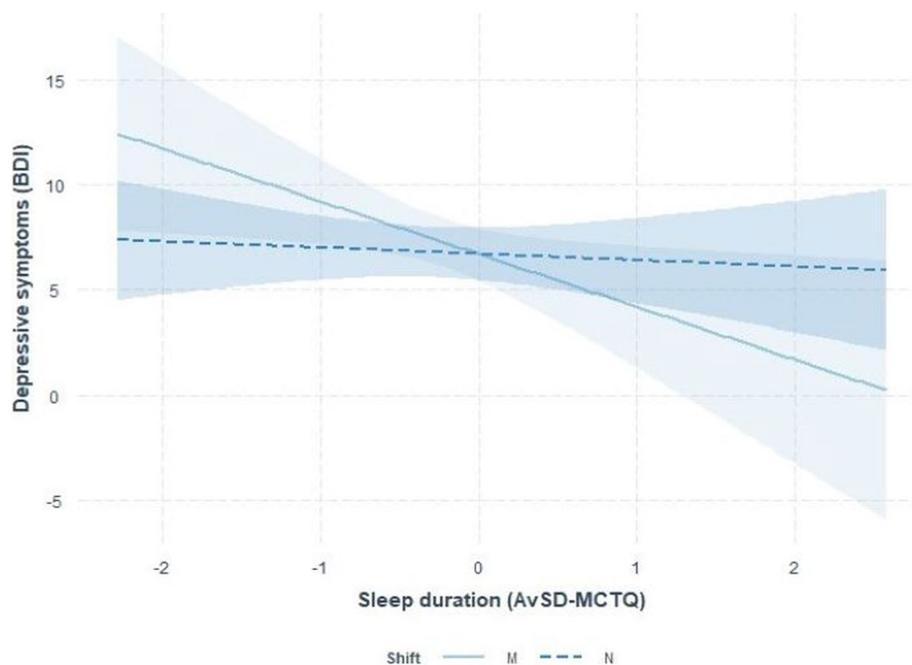


Fig. 3 Prediction from the linear model for depressive symptoms across sleep duration by shift

Notes: Sleep duration interacted with the shift to explain depressive symptoms, with participants in the morning shift showing higher depressive symptoms with decreased sleep duration. AvSD was mean-centered and standardized. The shaded area reflects 95% confidence intervals. M= morning shift, N= night shift

symptoms increasing as sleep quality worsened. In addition, a marginal interaction was found between AvSD and shift ($F_{(1,21)}=4.064, p=0.057, \eta^2=0.16$) (Table 2).

According to the slope analysis, for participants in the morning shift, the slope of AvSD was negative and different from zero ($\beta=-2.515, 95\%CI=-4.680, -0.345$), while for participants in the night shift, the slope of AvSD was negative but not different from zero ($\beta=-0.295, 95\%CI=-1.570, 0.984$) (Fig. 3). This means that only for participants in the morning shift, depressive symptoms

increased as sleep duration decreased. The remaining interactions were not significant.

Variables associated with sleep quality

A significant main effect of SJL on PSQI was found ($F_{(1,20)}=14.822, p=0.001, \eta^2=0.43$). In addition, a significant interaction was found between SJL and BDI ($F_{(1,20)}=24.099, p<0.001, \eta^2=0.55$) (Table 3). Results were similar when correcting the formula of social jetlag for sleep debt (Jankowski 2017).

Table 3 ANOVA table for the regression model with sleep quality as the dependent variable

	Df	Sum Sq	Mean Sq	F-value	P value	η^2
SJL	1	41.774	41.774	14.822	0.001**	0.43
Shift	1	3.233	3.233	1.147	0.297	0.05
SJL*BDI	1	67.922	67.922	24.099	<0.001***	0.55
Shift*MSFsc	2	9.317	4.658	1.653	0.217	0.14
Residuals	20	56.370	2.818	-	-	
Adjusted R ²	0.61					

*** denotes p-values <0.05, **** denotes p-values <0.001. η^2 = partial eta-squared. MSFsc was mean-centered and standardized. SJL: the absolute difference between the midpoints of sleep on work and free days; BDI: depressive symptoms; MSFsc: midpoint of sleep on free days corrected. AvSD was not a relevant variable to include in the model based on the best subsets method

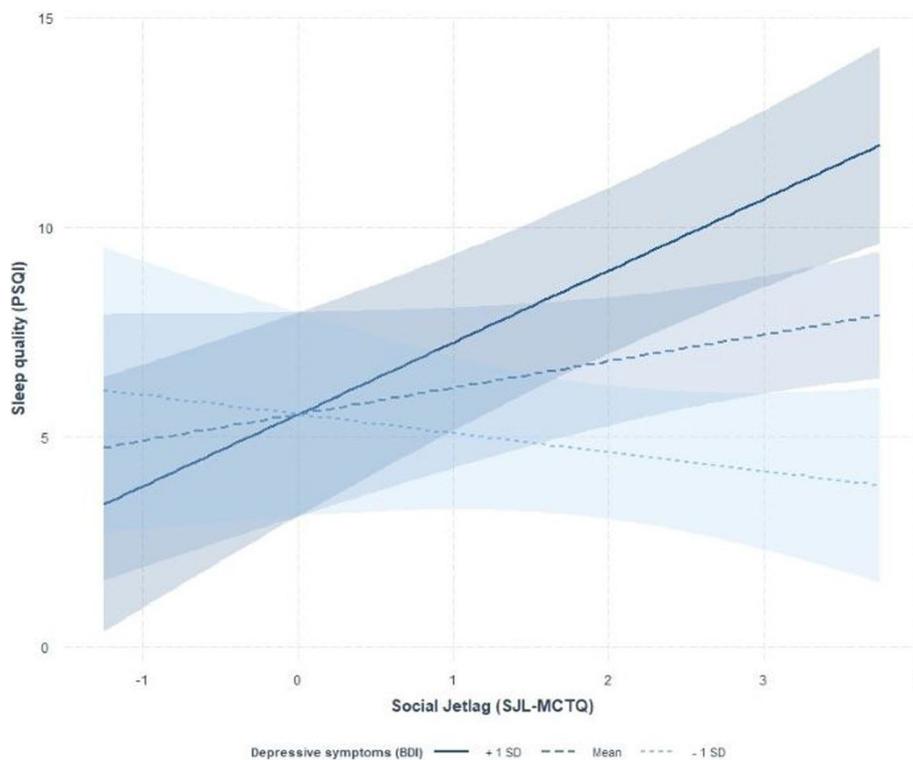


Fig. 4 Prediction from the linear model for sleep quality across social jetlag by depressive symptoms

Notes: Social jetlag interacted with depressive symptoms to explain sleep quality; the higher the depressive symptoms, the stronger the effect of social jetlag on sleep quality. Only three participants had a negative SJL. The shaded area reflects 95% confidence intervals

According to the slope analysis, for lower depressive symptoms (- 1 SD from the mean=3.54), the slope of SJL was negative but not different from zero ($\beta=-0.42$, 95%CI=-1.20, 0.35, $p=0.27$). On the contrary, for average (7.15) and higher (+1 SD from the mean=10.78) depressive symptoms, the slope of SJL was positive and different from zero ($\beta=0.67$, 95%CI=0.01, 1.33, $p=0.05$; $\beta=1.76$, 95%CI=0.84, 2.69, $p\leq 0.01$ respectively; Fig. 4). This means that the higher the depressive symptomatology, the stronger the effect of SJL on sleep quality, with worse sleep quality as SJL increases. The remaining effects and interactions were not significant.

Discussion

Although several previous reports have described crossed associations among shifts, sleep, misalignment, chronotype, and depression (e.g. Selvi et al. 2018; Choi et al. 2019), it is still unclear to what extent each variable acts independently or interacts with the others. To comprehensively analyze these associations, we took advantage of the peculiar characteristics of dancers being trained in shifts, who are extremely late-oriented, have a strong misalignment, but do not show sleep duration deficits (Coirolo et al. 2020). We showed that: 1) despite

participants' lateness, dancers exhibited low depressive symptoms, and neither depression nor sleep quality were affected by chronotype; 2) dancers had a poor sleep quality, and SJL was strongly associated with its worsening, while chronotype and sleep duration were irrelevant; 3) depressive symptoms depended on the interaction between sleep duration and shift; and 4) sleep quality and depression were strongly linked.

College students and athletes are typically at risk of having sleep problems. Several reports have documented sleep deprivation in these populations (Bolin 2019) and poor sleep quality (Dinis and Bragança 2018; Fietze et al. 2009). Our study population, college dance students, exhibited the expected poor sleep quality. However, despite the great challenge dance training entails, these dancers documented an adequate sleep duration. On the other hand, depression is prevalent in college students (Ibrahim et al. 2013) and is associated with eveningness (Hidalgo et al. 2009). In this context, the participant's low depressive symptomatology was unexpected. However, exercise has been reported as an effective treatment for depression and circadian disruption (Firth et al. 2020); thus, it may protect dancers from the deleterious effect of late chronotype and lead them to adequate sleep duration.

One of the most striking features of these dancers is that they trained either early in the morning or very late in the evening. These participants belong to a larger population in which it has been reported that those attending the morning shift have earlier chronotypes and sleep timing (Coirolo et al. 2020), advanced dim-light melatonin onset, and higher SJL (Coirolo et al. 2022) compared with night-shift dancers. Probably because of the reduced number of our subsample, many of these features were not observed, nor did we find that depression or sleep quality were shift-dependent.

Depression and sleep

The consequences of having poor sleep quality are many, including a profound impact on mental health (Dinis and Bragança 2018). Numerous studies support an association between sleep quality and depression severity, whereby depressive symptoms are more prevalent in poor sleepers (Wilson et al. 2014). Moreover, a recent meta-analysis showed that interventions for sleep hygiene could reduce depression (Gee et al. 2019). Several mechanisms underlying the connection between sleep quality and depressive mood may be speculated. Some authors have argued that chronotype could influence this association, while others point toward the use of technology or physical activity (Dinis and Bragança 2018; Feng et al. 2014). Our results confirmed these previous reports by showing a

bidirectional association between poor sleep quality and depressive symptom severity.

SJL was very high among participants, but we found that it was not a relevant variable to include as a predictor of depressive symptoms in our model. This result was not that unexpected, given that it has been argued that the association between SJL and depression is inconsistent (Henderson et al. 2019). Moreover, we found that depression symptoms were quite low among participants and sleep duration adequate, and neither of them differed between the shifts. However, an interesting marginal association emerged from the influence of sleep duration and shift on depressive symptom severity. Morning-shift participants, but not night-shift ones, showed higher depressive symptoms as sleep duration decreased. Previous studies have shown that short sleep duration increases the risk of depression (Zhai et al. 2015). Short sleep durations are also known to affect mood regulation processes and increase negative emotions (Watling et al. 2017). Although sleep deficit was not an issue for dancers on either shift on average, we observed an association between sleep duration and depressive symptomatology in morning-shift dancers, in which short sleep was likely associated with misalignment. However, this finding should be taken with caution as it did not reach conventional levels of statistical significance.

Misalignment associated with sleep quality

Sleep quality was evaluated with one of the most cited indexes to assess this construct, the PSQI (Buysse et al. 1989). This instrument allows deriving different components. In our sample, the component of PSQI that contributed the most to the poor sleep quality reported by participants was sleep latency, followed by subjective quality and disturbances. Usually, sleep duration is a major dimension for measuring sleep quality (Mendonça et al. 2019). Nonetheless, the restorative effects of sleep also depend on other aspects besides its duration (Hirshkowitz et al. 2015). In line with this, we found that PSQI component sleep duration was not a core factor to explain the poor sleep quality reported by our participants. Furthermore, these dancers reported poor sleep quality but an adequate sleep duration measured by the MCTQ.

Consistent with past findings, we confirmed that sleep quality was influenced by SJL (Kang et al. 2021), indicating that sleep occurring in a different circadian window on work and free days may lead to poorer sleep quality. Previous studies have highlighted that in college students, sleep habits are constantly shifting between weekdays and weekends to meet the demands of college life, disrupting sleep or lessening their quality (Buboltz et al. 2001). Moreover, we found that SJL interacted with depressive

symptomatology to explain sleep quality in these dancers. In particular, we found that the most severe symptoms of depression are associated with the greatest effect of SJL on sleep quality. Importantly, this effect was found even when the higher scores of depressive symptoms among participants corresponded to minimally depressed individuals (Beck et al. 1996).

Circadian misalignment is a chronic stress factor that renders the organism an allostatic overload (Karatsoreos and McEwen 2011). Thus, it is not surprising that it is linked to various health problems (Caliandro et al. 2021). Interestingly, previous studies in populations with minor or sub-clinical depressive symptoms did not find an association between SJL and depression severity (Schmitt et al. 2013). However, we showed that in dancers training in shifts, the additive effect of depressive symptoms and SJL adversely affect sleep quality. This result provides an excellent example of how circadian disruption and mood can have deleterious effects on sleep.

We did not find that sleep duration was a relevant variable to include as a regressor of sleep quality. These dancers who reported a good sleep duration on average presented a strong misalignment and poor sleep quality. These conditions that seem contradictory can also be an opportunity to test factors that usually interact and are difficult to disentangle. We demonstrated that the poor sleep quality observed in these adequate sleepers could be explained by sleep latency. Other factors such as the use of technology before sleeping, several wake episodes at night, or intense physical activity at the dim light melatonin onset time window could also collaborate to increase the high circadian disruption in this dancer population, leading to a deteriorated sleep quality.

Limitations

This study has several limitations, most of them derived from being an ecological study on a particular population in which influential variables were not controlled. First, the sample size was relatively small, which led to fragile associations that most likely cannot be extrapolated to other populations, and more than 80% of participants were women. Regardless, when controlling for gender in the models, the results remained the same. Second, the observational nature of the study precludes inference about causality. Third, we only used self-reported questionnaires; future studies combining objective and subjective measures should be performed. Fourth, we did not differentiate between PSQI scores on work and free days, which may have affected our results. Fifth, we did not take into account naps when measuring sleep duration. Sixth, we did not collect data regarding the daily activities of students; thus, we lacked information about other synchronizers (such as dietary habits) that could

influence their circadian system and emotional state. Finally, we aimed to study the END-SODRE population, which led to not capturing participants with severe depressive symptoms.

Conclusions

Using dance training in shifts as an advantageous real-world model, we assessed the interplay of social, chronobiological, and mood variables influencing depressive symptoms and sleep quality. The unique characteristics of these dancers, being extremely late-oriented, having strong circadian disruption and poor sleep quality but not reporting sleep duration deficits or high depressive symptoms, created the perfect setting to explore how these factors interact. We found that a) circadian misalignment predicted the poor sleep quality dancers displayed; b) although participants had poor sleep quality but not depression, these variables were inextricably linked; c) despite participants were not sleep deprived or depressed, the shorter the sleep duration, the higher the depressive mood in morning-shift dancers; d) social jetlag did not predict dancers' depressive symptomatology, but the higher the symptoms the stronger the effect of circadian misalignment on sleep quality. Our findings contribute novel evidence on the impact of the interaction of diverse factors on mood and sleep quality in the daily life of a normal population of dancers. It would be interesting for future studies to replicate these findings among other athletes susceptible to sleep and emotional disruptions as those who frequently travel internationally for competitions. Future work shall focus on understanding the mechanisms behind these effects in developing depression and poor sleep quality.

Abbreviations

SJL	Social jetlag
MCTQ	Munich chronotype questionnaire
MSFsc	Midpoint of Sleep on free days (corrected for sleep debt accumulated on workdays)
AvSD	Average sleep duration
BDI	Beck depression inventory
PSQI	Pittsburgh sleep quality index

Acknowledgments

We thank all the dancers of the END-SODRE who volunteered as participants in this study and Martín Inthamoussou (Director of the School of Performing Arts, SODRE), Andrea Salazar, and Emiliano D'Agostino (Coordinators of the END-SODRE) for their support and logistic help. We are very grateful to Micaela De Mori, Agustín Laguardia, Betania Martínez, and Luna Perla for their help in data collection and pre-processing. We finally thank Álvaro Cabana for his suggestions on the statistics and Victoria Garfield for the suggestions and English proofreading.

Authors' contributions

VP: conceptualization; data curation; formal analysis; methodology; visualization; writing (original draft, review & editing). NC: conceptualization; data curation; investigation; methodology; writing (review & editing). BT:

conceptualization; funding acquisition; investigation; methodology; supervision; writing (review & editing). AS: conceptualization; funding acquisition; investigation; methodology; supervision; writing (review & editing). All authors approved the final manuscript.

Funding

This research was funded by Comisión Sectorial de Investigación Científica (CSIC, UdelaR, Uruguay) and Comisión Académica de Posgrados (CAP, UdelaR, Uruguay).

Availability of data and materials

The datasets generated and analyzed during the current study are available in the OSF repository (<https://osf.io/pkw28/>).

Declarations

Ethics approval and consent to participate

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Research Ethics Committee, School of Psychology, Universidad de la República. Informed consent was obtained from all subjects involved in the study.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Received: 21 August 2023 Accepted: 27 October 2023

Published online: 10 December 2023

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