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TEMPO EM MOVIMENTO:
RELEVÂNCIA DO ESTUDO DA ORGANIZAÇÃO CIRCADIANA E SUA RELAÇÃO COM ESTADOS DE SAÚDE E DOENÇA

LUÍSA KLAUS PILZ

PORTO ALEGRE
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Orientadora: Profa. Dra. Maria Paz Loayza Hidalgo
Orientador estrangeiro: Prof. Dr. Till Roenneberg
Coorientadora: Profa. Dra. Rosa Levandovski

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“I love science, and it pains me to think that so many are terrified of the subject or feel that choosing science means you cannot also choose compassion, or the arts, or be awed by nature. Science is not meant to cure us of mystery, but to reinvent and reinvigorate it”.

Robert Sapolsky

“Everything in the universe has a rhythm, everything dances”.

Maya Angelou

RESUMO

Introdução: Organismos de praticamente todos os filos possuem um sistema temporizador que orquestra seus ritmos e os sincroniza aos ritmos de 24 horas do ambiente. Estes ritmos foram mantidos ao longo da evolução por pressões de seleção que favoreciam a sincronização ao dia externo; se não for por ela, o sistema perde sua principal vantagem: otimizar as funções do organismo de acordo com as alterações previsíveis do ambiente. A luz é o principal sinal externo a sincronizar os ritmos biológicos, provavelmente por refletir as mudanças cíclicas que ocorrem no ambiente. Com o advento da eletricidade, o ser humano deixou de organizar suas rotinas de acordo com as transições dia-noite. Numa escala evolutiva, estes novos estilos de vida são tão recentes que nossa biologia ainda não está adaptada, trazendo como consequência perturbações de ritmos e do sono (cronorruptura). Considerando que praticamente toda a fisiologia está sob controle do relógio, o estudo dessas alterações e como estão relacionadas a diferentes estados de saúde e doença provavelmente nos permitirá propor estratégias de prevenção e tratamento com foco na sincronização circadiana. **Objetivo:** Avaliar a utilização de métodos que investiguem variáveis relacionadas a ritmos biológicos e sua aplicabilidade e conveniência em estudos clínicos e epidemiológicos. **Metodologia:** *Estudo 1:* Investigamos a confiabilidade de uma escala desenvolvida com o objetivo de avaliar ritmos de variáveis frequentemente alteradas em transtornos de humor (Instrumento de Ritmos de Humor). *Estudo 2:* Avaliamos a capacidade do Instrumento de Ritmos de Humor em diferenciar indivíduos com e sem risco para transtornos psiquiátricos em uma amostra brasileira e em uma amostra espanhola. *Estudo 3***:** Investigamos diferenças em horários e duração de sono em comunidades quilombolas rurais com diferentes históricos de acesso à eletricidade utilizando actimetria e o Questionário de Cronotipos de Munique (Munich ChronoType Questionnaire, MCTQ), testando a aplicabilidade e correspondência entre estes instrumentos no contexto de estudos epidemiológicos de campo. *Estudo 4:* Avaliamos, nas comunidades quilombolas, quais variáveis derivadas do MCTQ estão associadas a sintomas depressivos. **Resultados:** *Estudo 1:* Observamos que o Instrumento de Ritmos de Humor não é sujeito a viés de memória significativo e que a soma de itens em que a presença de um pico foi reportado está associada a escores de sintomas de depressão e bem-estar. *Estudo 2:* A presença de picos em determinados sinais e comportamentos que estão alterados em transtornos de humor apresentou diferentes prevalências em indivíduos com risco para transtornos psiquiátricos (tristeza no Brasil, motivação na Espanha, pessimismo e motivação para exercício em ambos países). Além disso, na amostra brasileira, que apresentava altos níveis de desalinhamento circadiano, mais indivíduos em risco reportaram que seu pico de

sonolência acontecia pela manhã. Na amostra espanhola, por outro lado, a diferença entre o pico de apetite e motivação para se exercitar foi significativamente menor em indivíduos em risco, tendo uma maior proporção deles reportado que seu pico de apetite ocorre antes do pico de motivação para o exercício. Tais achados sugerem que eles podem estar em uma condição de cronorruptura. *Estudo 3:* A actimetria e o MCTQ demonstraram correspondência entre si e, no contexto da pesquisa de campo, se mostraram como instrumentos ideais para medida contínua e retrospectiva de ritmos de atividade-repouso e sono-vigília. Em comunidades com acesso à eletricidade, os indivíduos dormem, em média, mais tarde e, em algumas das comunidades, a duração de sono também é mais curta. *Estudo 4:* O jetlag social, controlando-se para idade e sexo, está significativamente associado a sintomas depressivos nas comunidades quilombolas. **Conclusões:** Nesta tese, a aplicabilidade e a relevância da avaliação de ritmos em estudos epidemiológicos foi demonstrada. Tanto a actimetria, quanto o MCTQ se mostraram bons instrumentos na avaliação de ritmos de atividade-repouso e sono-vigília em estudos de campo. O jetlag social, aferido pelo MCTQ, parece estar associado a sintomas depressivos e é uma medida interessante em estudos que investiguem as consequências dos estilos de vida atuais para a saúde. Estudos longitudinais que demonstrem como o desalinhamento circadiano em diferentes níveis pode estar envolvido na etiologia de estados patológicos são essenciais para que possamos propor estratégias de prevenção e desenvolver tratamentos que atentem para estes aspectos. A avaliação de ritmos de humor apresenta potencial para auxiliar no diagnóstico de transtornos psiquiátricos. Futuros estudos em pacientes deprimidos podem comprovar tal aplicabilidade. Estudos adicionais que avaliem as diferenças de fase entre os itens do Instrumento de Ritmos de Humor podem contribuir no entendimento de estados de cronorruptura.

PALAVRAS-CHAVE: Cronobiologia, ritmos biológicos, eletricidade, sono, transtornos psiquiátricos

ABSTRACT

Introduction: Organisms from practically all phyla have a temporal system that orchestrates its rhythms and synchronize them to the 24-hours rhythms of the environment. These rhythms were preserved through evolution by selection pressures that favoured entrainment; if it is not for entrainment, the system loses its main advantage: optimising the organism functions according to predictable external changes. Light is the main external cue to synchronize biological rhythms, probably because it mirrors diverse cyclic changes of the environment. With the possibilities brought by electricity, humans do not organise their routines around day-night transitions anymore. In an evolutionary scale, these new lifestyles are so recent that our biology has not adapted yet, leading to rhythm and sleep disruption (chronodisruption). Taking into account that practically all physiology is under the clock control, studying these changes and how they are related to different states of health and disease will probably help us in proposing prevention and treatment strategies targeting the circadian organization. **Objective:** To evaluate methods of assessment of circadian rhythms and to explore how feasible and useful they might be when applied to epidemiological studies. **Methodology:** *Study 1:* We investigated the reliability of a scale developed to investigate rhythms of factors often altered in mood disorders (Mood Rhythm Instrument). *Study 2:* We tested the ability of the Mood Rhythm Instrument to discriminate individuals at risk for psychiatric disorders in both a Brazilian and a Spanish sample. *Study 3:* We investigated timing and sleep duration differences in rural quilombolas communities with different histories of access to electricity using actimetry and the Munich ChronoType Questionnaire (MCTQ), testing the applicability and correspondence between both methods in the context of epidemiological field studies. *Study 4:* We evaluated, in quilombolas communities, which variables derived from the MCTQ are associated to depressive symptoms. **Results:** *Study 1:* The Mood Rhythm Instrument is not subject to significant memory bias and the count of items where individuals report to have a peak was significantly correlated to scores of depression symptoms and wellbeing. *Study 2:* The prevalence of reported peaks in some symptoms and behaviours that are usually altered in mood disorders was significantly different in individuals at risk for psychiatric disorders (sadness in Brazil, motivation in Spain, pessimism and motivation to exercise in both countries). Furthermore, in the Brazilian sample, which showed high levels of social jetlag, a higher number of individuals at risk reported their peaks of sleepiness to be in the morning. On the other hand, in the Spanish sample, the difference between the peak of appetite and motivation to exercise was significantly lower in individuals at risk, and a higher proportion of subjects reported their peak of appetite to happen earlier than the peak of motivation to

exercise. These findings suggest these individuals might be suffering from chronodisruption. *Study 3:* Actimetry and MCTQ showed good correspondence and, in the context of field work presented themselves as ideal instruments to continuously and retrospectively measure rest-activity and sleep-wake rhythms. In communities that have access to electricity, individuals sleep on average later and, in some communities, sleep duration is shorter. *Study 4:* Social jetlag is significantly associated to depressive symptoms in quilombolas communities when controlling for age and sex. **Conclusion:** In our studies, we have confirmed the feasibility and usefulness of rhythms assessment in epidemiological studies. Both actimetry and the MCTQ were good instruments to assess rest-activity/sleep-wake rhythms in fieldwork. Jetlag as assessed by the MCTQ, seems to be associated to depressive symptoms and is an interesting measure for studies that investigate the consequences of modern lifestyles to health. Longitudinal studies showing how circadian misalignment at different levels might contribute to the aetiology of pathological states is key for us to propose prevention strategies and develop treatments that focus on chronobiological aspects. The study of the rhythmicity of mood and behaviours related to mood disorders has the potential to help in the diagnosis of psychiatric disorders. Future studies in depressed patients should prove the applicability of the Mood Rhythm Instrument in such context. Additional studies that investigate the phase differences between the Mood Rhythm Instrument items might contribute in the understanding of chronodisruption states.

KEYWORDS: Chronobiology, biological rhythms, electricity, sleep, psychiatric disorders.

LISTA DE ABREVIATURAS
(REVISÃO DE LITERATURA)

BMAL1: do inglês, *Brain and Muscle Arnt-like protein 1*

CLOCK: do inglês, *Circadian Locomotor Output Cycles Kaput*

Cry: do inglês, *Cryptochromes*

CoG: centro de gravidade

DLMO: início da secreção de melatonina / *dim light melatonin onset*

E-Box: do inglês, *Enhancer-box*

MCTQ: Questionário de Cronotipos de Munique / *Munich ChronoType Questionnaire*

MEQ: Questionário de Matutinidade-Vespertinidade / Morningness-Eveningness Questionaire

MSF_{sc}: ponto médio de sono em dias livres corrigido para a dívida acumulada em dias de trabalho

NSQ: núcleo supraquiasmático

Per: do inglês, *Period*

ROR: do inglês, *Retinoic Acid Receptor-Related Orphan Receptor*

SUMÁRIO

CAPÍTULO 1

Referencial teórico e delineamento dos objetivos da tese.

1 INTRODUÇÃO

O despertador toca. Segunda-feira, 6h40. Já é hora de acordar. Talvez 10 minutos de "modo soneca" e o atraso não seja tão grande. Talvez perca o ônibus das 7h20. Ainda assim, se embarcar no das 7h30, é possível que chegue no trabalho a tempo de bater o ponto antes das 8h. Se não fosse o tempo... Se não fosse o ponto... Se o ponto não ditasse o tempo...

Entraram os primeiros raios de luz pelas frestas da parede de casa. Os primeiros cantos do galo já haviam anunciado que o dia estava pra nascer. Já se percebe que hoje é dia de tempo bom. Ah, o tempo... Aqui parece que passa diferente! Aqui quem dita o tempo é o sol.

Luísa K. Pilz

Durante a maior parte da sua história, o ser humano associava a passagem do tempo a fenômenos naturais: astros, cheias de rios, eventos meteorológicos. A religião também teve sua participação na organização temporal, em especial com a chegada da Idade Média: toque dos sinos, calendário litúrgico... Na transição para a Idade Moderna, porém, com uma nova visão de mundo chega uma nova visão de tempo e espaço, um calendário que, com as tecnologias das grandes navegações, passa a ser mercantilista. Com a Revolução Industrial, surge uma nova paisagem urbana: a produção sistematizada e massificada faz com que o relógio da fábrica organize o ritmo do trabalhador. "Tempo é dinheiro". As ferrovias promovem o uso do sistema de fusos horários e o horário solar pode chegar a ter uma diferença de até duas horas do tempo marcado pelo relógio. Quando a luz elétrica começa a ser comercializada, nem o horário do relógio importa, o dia pode ser tão longo quanto o indivíduo quiser ou o trabalho requeira. Mas, afinal, o tempo do ser humano é o tempo marcado pelo relógio?

1.1 Das vantagens e desvantagens de um sistema circadiano

"Nothing in biology makes sense except in light of evolution". – T. Dobzhansky, 1973.

Organismos de praticamente todos os filos possuem um sistema temporizador responsável por conferir ritmos a funções fisiológicas em todos os níveis: da expressão gênica a comportamentos complexos, como o sono. A esse sistema chamamos circadiano por sua principal função, sincronizar os ritmos endógenos que gera aos ritmos de 24h da Terra. É esta

função que provavelmente foi evolutivamente mantida por pressões de seleção, uma vez que orquestrar as funções do organismo de acordo com o ambiente permite que elas se adaptem e sejam otimizadas de acordo com as alterações cíclicas previsíveis do meio externo (ASCHOFF, 1951, 1960; PITTENDRIGH, 1960; ROENNEBERG; MERROW, 2016). Apesar de não ter respostas primárias, o sistema circadiano influencia praticamente toda a fisiologia.

A nível molecular, um conjunto de genes identificado como *"genes relógio"* é responsável por conferir ritmicidade à expressão gênica e consequentemente às funções fisiológicas. O fato de cada célula possuir uma maquinaria molecular responsável pela geração de ritmos é uma descoberta recente: estudos pioneiros identificando as bases genéticas de fenótipos com alterações de ritmos datam dos anos 70 e 80 em diferentes espécies: *Drosophila melanogaster* (KONOPKA; BENZER, 1971), *Neurospora Crassa* (FELDMAN; HOYLE, 1973) e hamsters (RALPH; MENAKER, 1988). O mecanismo molecular do relógio identificado em todos os eucariotos estudados até o momento é baseado em alças de *feedback* de transcrição e tradução e foi inicialmente proposto por Paul Hardin, Jeff Hall e Michael Rosbash (HARDIN; HALL; ROSBASH, 1990). Entretanto, os componentes recrutados são espécie-específicos. Os elementos deste relógio molecular (Figura 1) regulam e conduzem a expressão rítmica de uma série de genes, o que, em última instância, confere ritmicidade a funções fisiológicas e comportamentais (LIU; CHU, 2013; MCCLUNG, 2013). Ilustrando a relevância da ritmicidade ao organismo, recente estudo em babuínos (*Papio anubis*) demonstrou que 65,5% de todos os genes expressos e até 81,7% dos genes que codificam proteínas apresentam expressão rítmica (MURE et al., 2018). Como previamente observado em moscas (CERIANI et al., 2002) e camundongos (ZHANG et al., 2014), quantos e quais genes são expressos de forma rítmica varia de tecido para tecido (MURE et al., 2018).

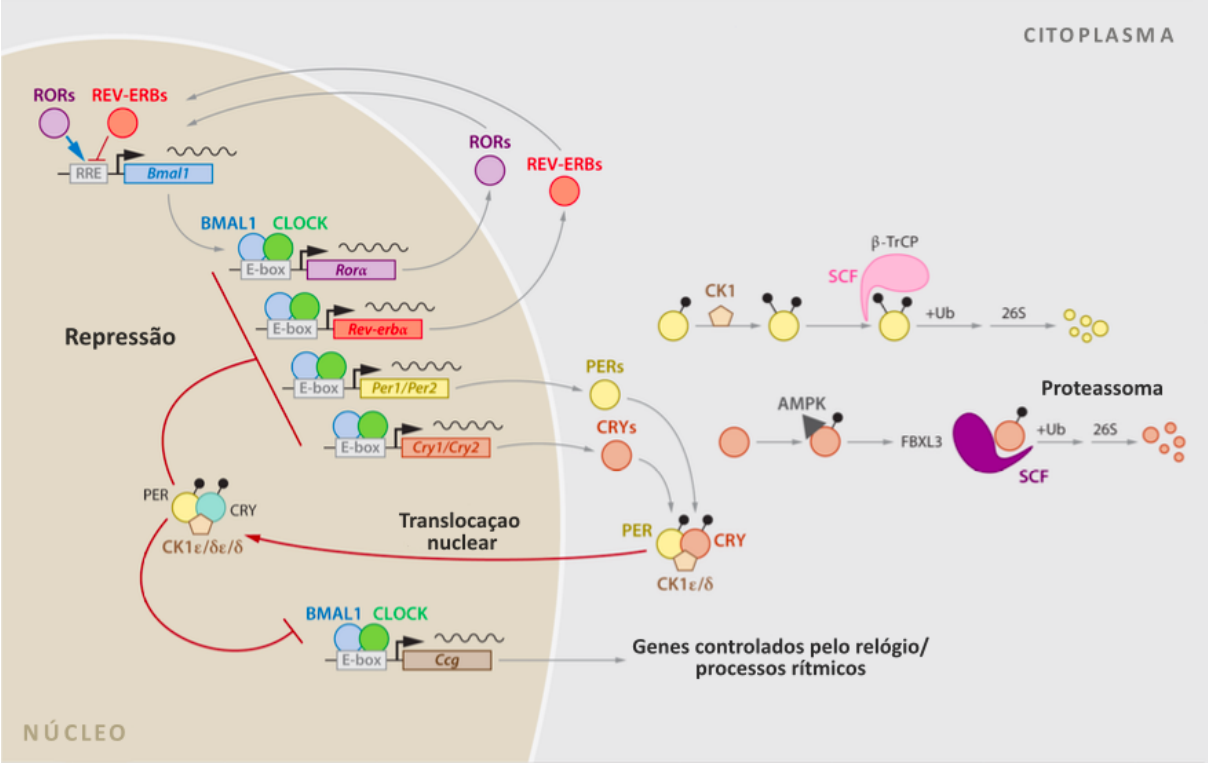

Figura 1. Maquinaria molecular do relógio circadiano. Alça de *feedback* transcricional auto-regulatória envolvendo os ativadores CLOCK e BMAL1 e seus genes alvo Per1, Per2, Cry1 e Cry2, cujos produtos gênicos formam um complexo repressor de *feedback* negativo. Outras alça de *feedback* reguladas por CLOCK:BMAL1 representada é a alça formada com Rev-erbα e Rorα. Outros mecanismos pós-traducionais também estão envolvidos na regulação deste relógio molecular e ele controla a expressão de uma série de genes alvo. Adaptado de Mohawk, Green, Takahashi (2012)

A nível sistêmico, em mamíferos, o relógio biológico é composto por osciladores periféricos, cujos ritmos são sincronizados por um oscilador central (Figura 2). Este marcapasso localiza-se no núcleo supraquiasmático (NSQ) em mamíferos (MOORE; LENN, 1972; STEPHAN; ZUCKER, 1972). A luz sincroniza o sistema circadiano ao ambiente principalmente através de projeções diretas das células fotorreceptoras da retina, células ganglionares intrinsicamente fotossensíveis (BERSON; DUNN; TAKAO, 2002; HATTAR et al., 2002). A fase dos relógios moleculares dos neurônios do NSQ são, desta forma, sincronizados ao ambiente e cabe a este orquestrar os osciladores periféricos encontrados em todo o organismo. Os relógios periféricos são sincronizados via sinalização autônoma e outras pistas sistêmicas, incluindo temperatura corporal e sinalização humoral (SILVER et al., 1996; BROWN et al., 2002; GUO et al., 2005). Apesar de o NSQ ser o principal sincronizador de todo o sistema, a ingestão de alimentos é capaz de desacoplar os relógios periféricos deste controle (DAMIOLA et al., 2000).

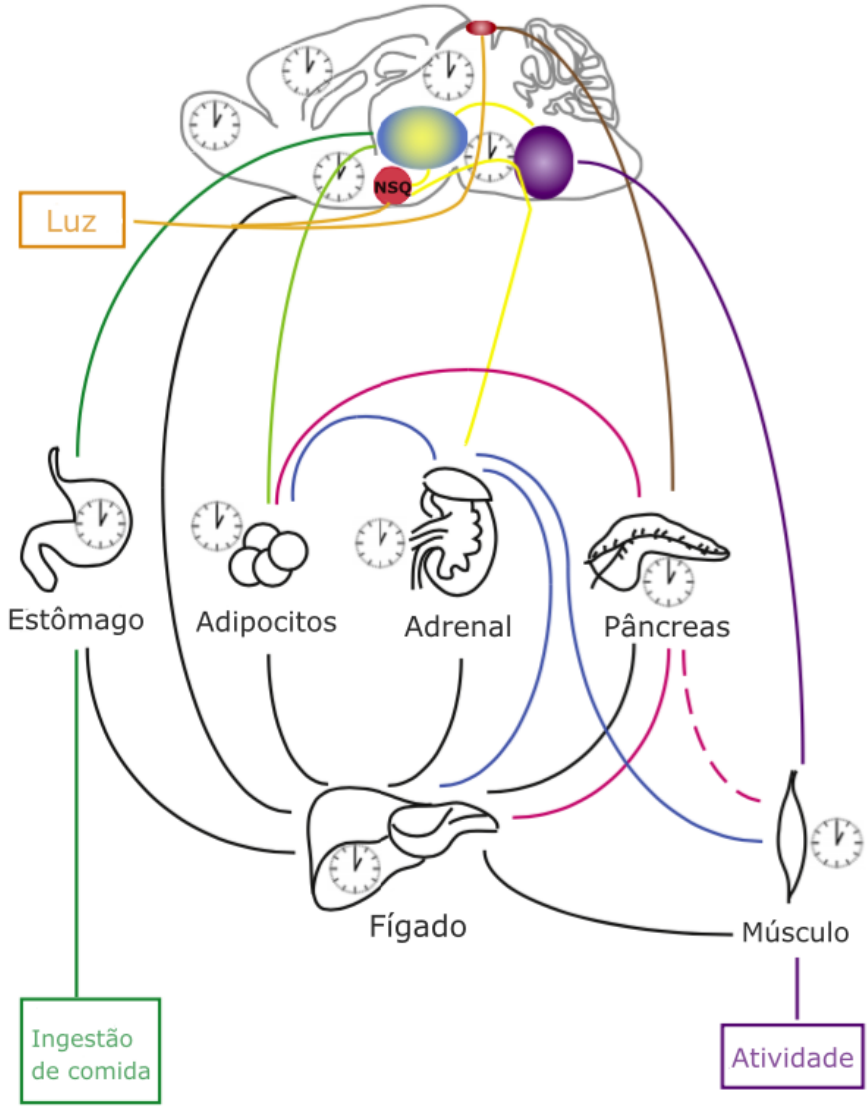

Figura 2. Sistema circadiano. Osciladores circadianos são encontrados em todas as células de vários órgãos. O marcapasso, ou relógio central (núcleo supraquiasmático, NSQ), sincroniza outros relógios no sistema nervoso central relacionados, por exemplo, à integração metabólica e de recompensa (oval amarelo-azul) e coordenação motora (oval roxa) por vias diretas e indiretas (linhas amarelas). O NSQ é sensível à luz (linhas laranjas) e organiza seus ritmos a partir dela. No esquema, a sinalização humoral importante para a organização temporal é mostrada: melatonina (linha marrom), grelina (verde escuro), leptina (verde claro), insulina/glucagon (rosa, linha tracejada: apenas insulina), adrenalina (azul). A sinalização metabólica também está representada: carboidratos, ácidos graxos, aminoácidos (preto). Roxo: conexões neuronais entre cérebro, medula espinhal e músculos. Adaptado de Albrecht, 2012.

Esta organização do sistema circadiano é capaz de gerar relações de tempo (fase) complexas entre os ritmos do organismo. Estas relações são provavelmente críticas para o funcionamento ótimo e sobrevivência. Apesar disso, diferentes organizações podem ser vistas

em outras espécies: osciladores periféricos também são sensíveis à luz na *Drosophila melanogaster* e no *Danio rerio* (peixe zebra), por exemplo (PLAUTZ et al., 1997; WHITMORE; FOULKES; SASSONE-CORSI, 2000). Ainda assim, independente de suas propriedades e conexões, a existência de múltiplos osciladores é uma característica comum a todos os organismos multicelulares descritos até então. Esta organização, que determina que uma série de componentes rítmicos do organismo se sincronizem a um sinal externo, passando ou não por uma regulação de um oscilador central, confere flexibilidade ao sistema. Também pode-se supor que diferenças filogenéticas neste sistema sejam resultado da seleção natural.

No caso dos mamíferos, um sistema organizado hierarquicamente (com um relógio central que orquestra os demais osciladores a partir do sinal luminoso) é capaz de se ajustar sem dificuldades a alterações pequenas e graduais na fase do sinal sincronizador (luz), como mudanças sazonais no comprimento do dia, por exemplo. Entretanto, quando a mudança é abrupta, este sistema fica temporariamente e gravemente desorganizado. É o que ocorre, por exemplo, no caso do *jetlag* (WATERHOUSE et al., 2007) ou mesmo do avanço/atraso de fase de uma hora no horário de verão (KANTERMANN et al., 2007). Em experimentos *in vitro*, observou-se que a fase dos osciladores centrais e periféricos necessitam de tempos diferentes para se ajustar (YAMAZAKI et al., 2000), resultando em um período de desorganização temporal do sistema, cuja duração pode variar de acordo com a magnitude (quantas horas) e a direção (atraso/avanço de fase) da alteração. Além dos sintomas do *jetlag*, este fenômeno também pode explicar as consequências do trabalho noturno, associado a risco aumentado para diabetes mellitus (GAN et al., 2015) e eventos vasculares (VYAS et al., 2012).

Para além da expressiva taxa de trabalhadores noturnos ou em turnos, outro fenômeno que atinge ainda mais indivíduos atualmente, em especial nas áreas urbanas, é o *jetlag social*, desalinhamento circadiano consequência da discrepância entre o relógio endógeno e os tempos impostos pela sociedade (WITTMANN et al., 2006). Com as possibilidades trazidas pela eletricidade, tanto tempo quanto intensidade da exposição à luz têm sido diferentes: a vida em ambientes fechados, com pouca luz durante o dia e exposição à luz durante a noite, enfraquece o *zeitgeber* (do alemão, "doador de tempo"). Nesta situação, a fase de sincronização da maioria das pessoas acaba atrasando, ou seja, os indivíduos se tornam mais vespertinos. Em sociedades industrializadas, apesar de a organização das rotinas não seguir mais a transição natural de dias e noites e a fase de sincronização ser atrasada, os horários de trabalho ainda não se modificaram. Nos dias de folga, as pessoas podem dormir no horário ditado por seu relógio biológico, porém em dias de trabalho são forçadas a acordar mais cedo

ao som do despertador. Além de, em geral, serem privadas de sono, é como se vivessem em fusos horários diferentes em diferentes dias da semana, como em uma situação crônica de jetlag - *jetlag social* (WITTMANN et al., 2006; ROENNEBERG et al., 2007; ROENNEBERG; MERROW, 2016). Evidências apontam que este fenômeno tão comum, estimado pelo cálculo da diferença do ponto médio de sono entre dias de trabalho e dias livres, está associado à obesidade, sintomas depressivos, risco cardio-metabólico, e propensão a se tornar fumante (LEVANDOVSKI et al., 2011; ROENNEBERG et al., 2012; WONG et al., 2015; BEAUVALET et al., 2017).

1.2 Função e regulação do sono

"For do but consider what an excellent thing sleep is: it is so inestimable a jewel, that, if a tyrant would give his crown for an hour's slumber, it cannot be bought (...) for sleep is that golden chain that binds health and our body together". – T. Dekker, 1609.

Um dos processos sob controle circadiano mais extensivamente estudados é o ciclo sono-vigília. O sono permanece, de certa forma, um enigma científico. O ser humano passa cerca de um terço de sua vida dormindo, mas ainda não há um consenso sobre as principais funções deste estado. Do ponto de vista comportamental, o sono pode ser caracterizado por 1) quiescência ou diminuição da atividade; 2) resposta diminuída a estímulos durante o estado quiescente; 3) regulação homeostática do estado quiescente (ALLADA; SIEGEL, 2008). Apesar de uma série de pressões de seleção negativas estarem associadas ao sono (enquanto dorme, o indivíduo não pode comer, dormir, reproduzir e está vulnerável a predadores), ele é filogeneticamente antigo: acredita-se que estados *"tipo-sono"* possam ser encontrados em qualquer organismo com uma rede neural (RAIZEN et al., 2008; KRUEGER et al., 2016; NATH et al., 2017). Portanto, o sono deve apresentar importantes vantagens adaptativas. Evidências apontam para um papel do sono na manutenção da resposta imune (IMERI; OPP, 2009; BESEDOVSKY; LANGE; BORN, 2012), da performance cognitiva (VAN DONGEN et al., 2003), da plasticidade sináptica (TONONI; CIRELLI, 2014) e do *clearance* metabólico no encéfalo (XIE et al., 2013).

Uma das razões para assumir que o sono tem importantes funções para o organismo é o fato de o horário em que ocorre e sua duração serem estritamente regulados. Uma série de modelos para explicar a regulação do sono foram propostos, tendo o modelo de dois processos servido como importante base conceitual para a pesquisa em sono (BEERSMA, 1998; BORBÉLY et al., 2016). O modelo propõe que a interação de um processo homeostático e um processo controlado pelo sistema circadiano determinam aspectos cruciais

da regulação do sono (Figura 3). O processo homeostático representa a dívida de sono que se acumula durante a vigília e diminui durante o sono, mas as transições do aumento para diminuição de dívida de sono e vice-versa (horários de dormir e acordar) são determinados por limiares influenciados fortemente pelo processo circadiano (BORBÉLY, 1982).

No contexto atual, o sono não apenas é regulado, como influencia a organização circadiana, uma vez que, muitas vezes, é apenas durante o sono que o organismo está exposto ao escuro. Como descrito anteriormente, este triângulo sistema circadiano-sono-comportamento tem papel importante no balanço saúde-doença (ROENNEBERG; MERROW, 2016).

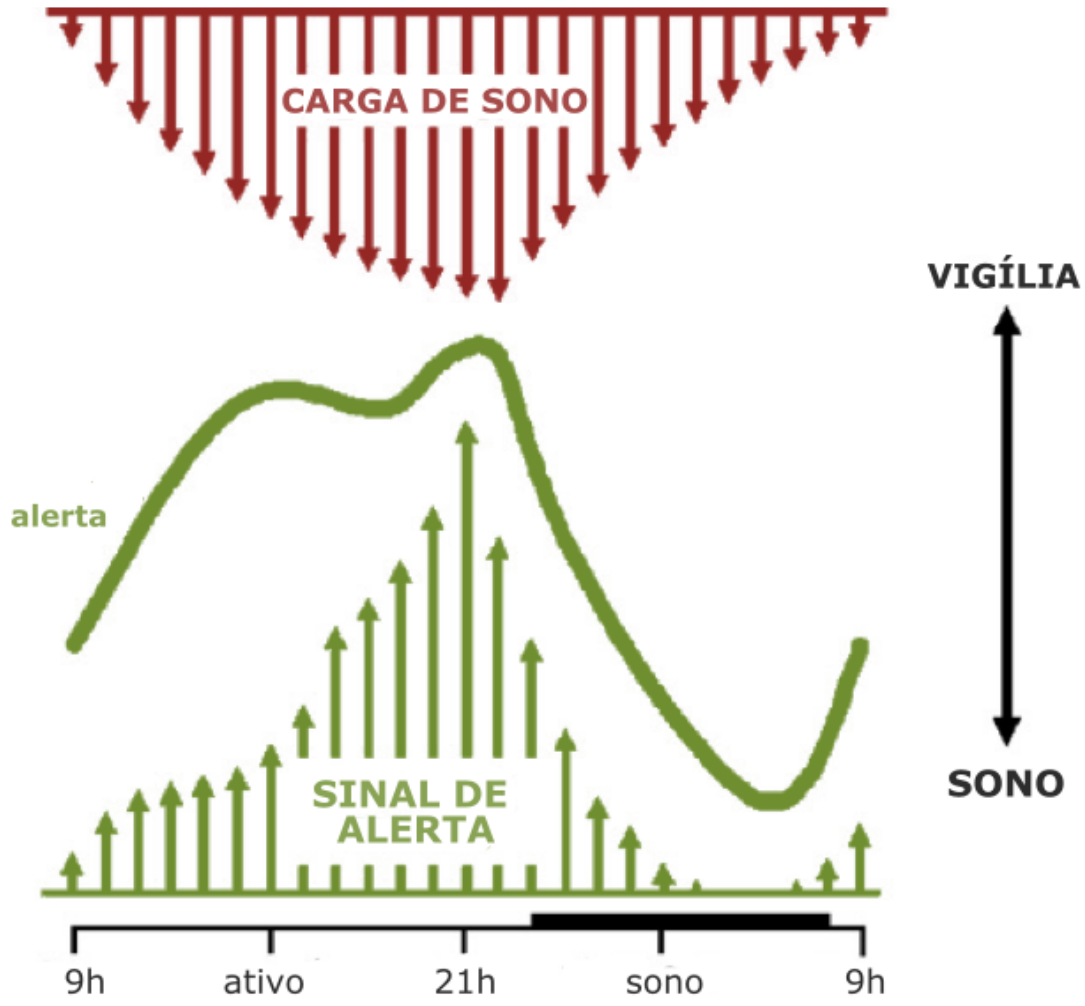

Figura 3. Modelo de dois processos. Representação esquemática da interação entre a pressão de sono (processo homeostático) e sistema circadiano (processo circadiano). Adaptado de Schmidt e cols (2007).

1.3 Alterações de ritmos e sono em transtornos psiquiátricos

"Time cools, time clarifies, no mood can be maintained quite unaltered through the course of hours". – T. Mann, 1924.

Considerando a ubiquidade do sistema circadiano (MURE et al., 2018) e seu papel regulatório, é de se esperar que um relógio funcional tenha importante participação na manutenção da saúde. Em estados patológicos, frequentemente são observadas alterações de ritmos biológicos, incluindo o ciclo sono-vigília Entender até que ponto e como estas alterações são parte da sintomatologia e/ou contribuem em sua etiologia é um dos principais objetivos da pesquisa em Cronobiologia atualmente.

Apesar de a direção ainda não ter sido elucidada, uma série de evidências aponta para associações entre alterações de ritmos e transtornos neuropsiquiátricos (MCCLUNG, 2013; BECHTEL, 2015; ZAKI et al., 2018): polimorfismos em genes relógios estão associados a manifestações clínicas (CHARRIER et al., 2017), modelos animais com ruptura de ritmos apresentam comportamento tipo-depressivo (ROYBAL et al., 2007; DE BUNDEL et al., 2013; LOGAN et al., 2015) e há alta prevalência de alterações de sono e ritmos em pacientes com transtornos psiquiátricos (ROSENWASSER; WIRZ-JUSTICE, 1997; SOEHNER; KAPLAN; HARVEY, 2014). Neste contexto, o estudo de ritmos de atividade/repouso tem o potencial de auxiliar no diagnóstico e tratamento de transtornos psiquiátricos. Tonon e cols (2017) demonstraram que a atividade noturna, aferida através da actigrafia, é capaz de diferenciar pacientes depressivos melancólicos dos não-melancólicos.

Apesar do grande interesse na intersecção entre sistema circadiano e regulação do humor, ainda se faz necessário desenvolver conhecimento mais aprofundado sobre a ritmicidade de sintomas de humor e sua relação com estados patológicos. Sentir-se pior na manhã é listado no DSM-5 como uma das características de depressão de caráter melancólico (AMERICAN PSYCHIATRIC ASSOCIATION, 2013). Pioras na segunda metade do dia também são reportadas e sugeriu-se que fossem indicativo de depressões subclínicas, distimia ou tratos de personalidade associados a afetividade negativa (RUSTING; LARSEN, 1998). Associações entre variação de humor (medida duas vezes ao dia, pela manhã e à noite, por um período de 23 dias) e severidade dos sintomas não foram encontradas em pacientes internados (HAUG; FÄHNDRICH, 1990). Ainda assim, a presença de variações de humor foram sugeridas como potencial preditor de uma depressão caracterizada por base biológica ou endógena; mais indivíduos que reportaram tais variações responderam normalmente ao tratamento (farmacológico + psicoterápico), sugerindo, pelo tempo de latência da remissão,

uma resposta ao tratamento farmacológico (CARPENTER; KUPFER; FRANK, 1986). Morris e cols (2007) investigaram diferentes padrões de variações diurnas de humor e verificaram que, independente do momento da piora (manhã, tarde ou noite), estas variações estavam significativamente associadas a outros critérios para o subtipo melancólico.

Indivíduos saudáveis, em protocolo de rotina constante, apresentaram ritmicidade circadiana em afeto positivo congruente com a ritmicidade de temperatura corporal central. Tal ritmicidade não foi observada em afeto negativo. Este achado faria sentido, ao passo que o afeto positivo é uma manifestação do sistema de recompensa, que operaria em parte sob o princípio de homeostase preditiva, sendo o sistema circadiano obviamente parte integral desta função. Por outro lado, o afeto negativo funcionaria reativamente, devido à relativa imprevisibilidade de ameaças e intensas demandas de energia (MURRAY; ALLEN; TRINDER, 2002). O ciclo sono-vigília parece também ter efeito nas alterações de humor ao longo do tempo (DANILENKO; CAJOCHEN; WIRZ-JUSTICE, 2003) e, a partir de resultados em estudo experimental, concluiu-se que a variação no humor pode ser explicada pela influência de uma interação complexa e não-aditiva de fase circadiana e duração da vigília (BOIVIN et al., 1997).

Wefelmeyer e Kuhs (1996) investigaram a ritmicidade do humor ao longo de 3 dias, coletando dados em quatro horários. Observaram que dias com piora de humor pela manhã ou sem variação de humor ocorriam com a mesma frequência em indivíduos saudáveis e pacientes; piora de humor na segunda metade do dia eram menos frequentes, especialmente em pacientes. Entretanto, seu achado mais interessante é que quase todos os indivíduos saudáveis atribuíam as variações ao longo do dia a suas atividades e circunstâncias externas, enquanto mais da metade dos pacientes reportavam que estas eram espontâneas. Desta forma, variações circadianas de humor talvez sejam mais percebidas por indivíduos deprimidos.

1.4 Laboratórios naturais

"Também sou da paisagem... Vago, solúvel no ar, fico sonhando... E me transmuto... iriso-me... estremeço... Nos leves dedos que me vão pintando!" – M. Quintana, 1938

Muito do conhecimento que se tem sobre ritmos e biológicos e sono vem de estudos no ambiente controlado do laboratório. Entretanto, estas características foram moldadas por fatores ambientais e forças ecológicas que resultaram em diferenças entre espécies. Além disso, o sono é uma característica altamente plástica, que responde rapidamente a alterações locais, inclusive o ambiente artificial do laboratório (RATTENBORG et al., 2017). O chamado 'efeito da primeira noite' – sono perturbado num ambiente desconhecido – já foi

demonstrado, sendo assimetria inter-hemisférica na profundidade do sono uma característica observada. As evidências apoiam a hipótese de que o sono perturbado em um ambiente não-familiar seria uma estratégia de sobrevivência (TAMAKI et al., 2016). Estudos também mostram que o sono de certas espécies pode ser muito diferente no cativeiro do que é no habitat natural: a preguiça (*Bradypus variegatus*) dorme em média 15,85 horas em cativeiro (DE MOURA FILHO; HUGGINS; LINES, 1983) e 9,63 horas no habitat natural (RATTENBORG et al., 2008).

Para entendermos a função do sono e do sistema circadiano e sua relação com os processos de saúde-doença, é importante que entendamos mecanismos regulatórios investigando-os no laboratório, mas também é importante que estudemos o sono e os ritmos biológicos no contexto ecológico em que eles se desenvolveram. Métodos recentes, descritos na seção a seguir, permitem o estudo de ritmos biológicos no dia a dia dos seres humanos em diferentes contextos, em "laboratórios naturais", por exemplo: em comunidades que vivem em regiões afastadas, na internação hospitalar, em casos clínicos específicos ou indivíduos que recentemente sobreviveram a algum trauma ou catástrofe. Entender quais parâmetros rítmicos estão alterados em estados patológicos distintos potencializaria a aplicabilidade do conhecimento em Cronobiologia à prática clínica.

1.5 Aferindo o tempo: actigrafia e marcadores de fase

"It sounds plausible enough tonight, but wait until tomorrow. Wait for the common sense of the morning." – H.G. Wells, 1895

Considerando o papel do sistema circadiano na regulação de praticamente toda fisiologia, não apenas o horário de medida das variáveis biológicas torna-se evidentemente importante, como a medida de sua variação ao longo do tempo. Um exemplo clássico da potencial aplicabilidade dos conhecimentos provenientes do estudo da Cronobiologia na clínica/ Farmacologia são as diferenças nas taxas de sobrevivência em pacientes de câncer de acordo com o momento em que são tratados com quimioterápicos (HALBERG et al., 2003; REFINETTI; LISSEN; HALBERG, 2007; PEEPLES, 2018). Ainda assim, precisa-se mais informação para que este conhecimento possa ser utilizado e, de acordo com os registros, uma proporção mínima dos ensaios clínicos atuais inclui considerações sobre cronoterapia (SELFRIDGE et al., 2016).

A relação entre a funcionalidade do sistema circadiano e a saúde, entretanto, envolve uma rede complexa de influências e confundidores. Por exemplo, problemas de saúde podem surgir tanto do desalinhamento circadiano como da privação de sono, que ocorrem

simultaneamente em trabalhadores de turno. Os osciladores do sistema circadiano, tanto central como periféricos, podem estar em diferentes estados: otimamente sincronizados, atrasados/adiantados em relação a outros osciladores, em livre-curso (não encarrilhado-*entrained*), ou arrítmicos. As diversas combinações de alterações em diferentes osciladores precisam ser compreendidas em termos de consequências para saúde: quais são bem toleradas e quais imprimem altos riscos? Desta maneira, será possível implementar os conhecimentos de Cronobiologia à área da saúde.

Alguns ritmos e marcadores de fase são comumente utilizados em Cronobiologia para avaliar o funcionamento do sistema circadiano, sua sincronização ao ciclo claro-escuro, diferenças individuais e potenciais alterações de ritmos. Alguns ritmos muito utilizados são atividade-repouso, sono-vigília, temperatura, concentração plasmática de hormônios. Em modelos animais e estudos *in vitro*, também é comum investigar a expressão rítmica dos genes relógio.

Diferentes métodos para avaliação destes ritmos muitas vezes se complementam e a aferição de diferentes ritmos pode fornecer interessantes informações quando, por exemplo, diferenças de fase podem ser calculadas e comparadas em diferentes estados de saúde doença. Os estudos apresentados nesta tese se baseiam em dados coletados através de actigrafia e escalas.

Actimetria / actigrafia

Actimetria é a medida da atividade locomotora que, em humanos é principalmente realizada com actímetros utilizados no pulso. Estes equipamentos normalmente contêm acelerômetros de um a três eixos que podem ainda medir outros parâmetros com exposição à luz, temperatura e pulso (ROENNEBERG et al., 2015). Quanto mais longo o intervalo entre as medidas ("*epoch"*), menos memória e bateria são utilizadas. Porém, intervalos mais longos podem interferir na inferência de parâmetros relacionados ao sono a partir dos dados de atividade. Os intervalos mais utilizados e validados são 30 segundos e 1 minuto. Pelo menos 7 dias de coleta são recomendados para que a medida reflita adequadamente o padrão de atividade-repouso do indivíduo, uma vez que dias de semana e fins de semana são incluídos na coleta. No caso de se desejar comparar dias de semana e fins de semana, 14 dias de coleta são recomendados, de forma que dois fins de semana sejam coletados (ANCOLI-ISRAEL et al., 2015).

Apesar de a polissonografia ser o padrão ouro para a avaliação do sono, a actigrafia é capaz de fornecer informações impossíveis de serem capturadas em uma única noite de

laboratório. Por exemplo, a regularidade dos horários de dormir e acordar no "habitat" natural de cada indivíduo só poderia ser aferida em casa. Uma série de algoritmos capazes de aferir quando o indivíduo está dormindo e características do seu sono já foram validados contra a polissonografia (COLE et al., 1992; SADEH; SHARKEY; CARSKADON, 1994; ROENNEBERG et al., 2015; WINNEBECK et al., 2018).

Entre os desafios encontrados na utilização de actigrafia estão:

- Adesão dos participantes, uma vez que o actímetro precisa ser usado todo o tempo em dias de coleta. A Figura 4 traz exemplos de utilização inadequada do equipamento.
- Dados faltantes: a detecção e tratamento de "*missing data*" precisa ser realizada previamente à análise. Embora alguns actígrafos possuam mecanismos de detecção ("off-wrist"), muitas vezes este processo precisa ser realizado manualmente e, de certa forma, subjetivamente, pelo pesquisador.
- Perda do equipamento, uma vez que o actímetro, como um relógio, pode ser perdido ou esquecido quando retirado do pulso.

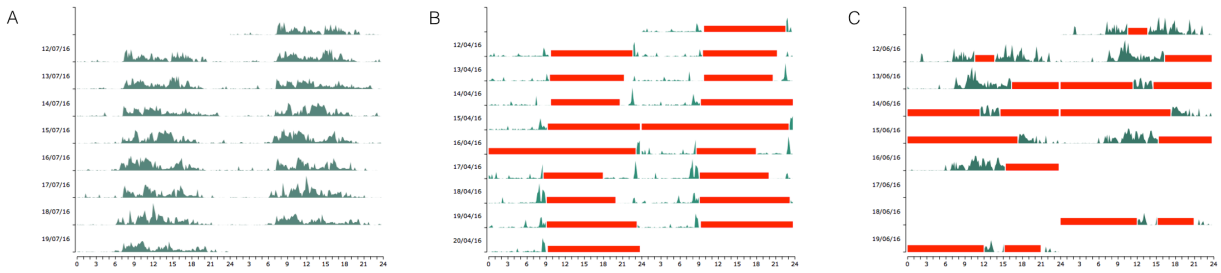

Figura 4. Exemplos de utilização inadequada do actímetro. As linhas vermelhas representam dados faltantes. O eixo y de cada linha representa contagens de atividade. Nestes gráficos duplos, dois ciclos de coleta estão plotados de maneira escalonada em cada linha (linha 1: ciclos 1 e 2, linha 2: 2 e 3, etc). As linhas vermelhas representam dados faltantes, quando os indivíduos não estavam utilizando o equipamento. O gráfico A é um exemplo de boa adesão. Os gráficos B e C mostram longos períodos sem utilização. No caso de B, o indivíduo utilizava o actímetro apenas durante a noite.

Escalas

Algumas escalas se propõem a avaliar características relacionadas ao sistema circadiano de forma a entender as diferenças individuais do relógio biológico e possíveis associações entre perturbações do sistema circadiano e estados patológicos (LEVANDOVSKI; SASSO; HIDALGO, 2013). Um dos mais importantes aspectos destas escalas é que elas avaliem a dimensão tempo.

Munich ChronoType Questionnaire (MCTQ): em 2003, Roenneberg e colaboradores propuserem o uso do Questionário de Cronotipo de Munique (Munich ChronoType

Questionnaire, MCTQ) para avaliar o cronotipo como uma variável contínua que representa a fase de sincronização do relógio biológico ao dia de 24h. Ele utiliza como marcador os horários de sono em dias livres, uma vez que nestes dias o sono, em geral, não está ou está menos subjugado a "adequações" estabelecidas por horários de trabalho. O MCTQ pergunta, separadamente para dias de trabalhos e dias livres, horários de deitar, dormir, acordar e levantar. Assim, a partir do questionário é possível derivar uma série de variáveis: incluindo duração média de sono, diferença de duração de sono entre dias de trabalho e livre (dívida de sono), ponto médio do sono (marcador do horário de sono, calculado como hora de dormir + duração do sono/2) e a diferença entre ponto médio de sono em dias livres e de trabalho. Esta última variável fornece uma estimativa do jetlag social, fenômeno descrito na sessão 1. A variável descrita como cronotipo é o ponto médio de sono em dias livres, corrigido para a dívida de sono acumulada ao longo da semana de trabalho (MSF_{sc}). Mais de 200.000 dados já foram coletados utilizando a plataforma online do MCTQ (ROENNEBERG et al., 2015). O MSF_{sc} apresenta uma distribuição normal e adolescentes são os tipos mais tardios (ROENNEBERG; WIRZ-JUSTICE; MERROW, 2003; ROENNEBERG et al., 2004; ROENNEBERG, 2015). O MCTQ também se propõe a avaliar o desalinhamento circadiano, através do jetlag social. Erren e Reiter (2013) propõem que a pesquisa epidemiológica em cronorruptura no futuro seja focada em três pontos: primeiro, podem utilizar o MCTQ que facilita a comparação entre populações e considerar as diferenças de idade. Segundo, perguntar sobre atividades em relação aos horários de trabalho, como faz o MCTQ. Terceiro, medir a intensidade de cronorruptura e o quanto os horários dos relógios externos e internos se sobrepõem.

Instrumento de ritmos de Humor (Mood Rhythm Instrument, MRI): O Intrumento de Ritmos de Humor é um questionário de 15 itens auto-preenchido, que foi desenvolvido com o objetivo de avaliar alterações no ritmo de variáveis fisiológicas e comportamentais associadas a transtornos de humor. A escala é composta por duas questões que caracterizam cada item: a primeira pergunta se os comportamentos ou sintomas tiveram um pico diário nos últimos 15 dias (resposta dicotômica: sim/não) e, na segunda, os participantes indicam em uma escala análogo-visual o horário deste pico, em caso afirmativo (resposta temporal unimodal). A versão espanhola (CARISSIMI et al., submetido; FRANCISCO et al., 2017) e portuguesa (DE SOUZA et al., 2016) estão sendo validadas.

1.6 Investigando o tempo: métodos de análise e medidas em Cronobiologia

"But what is the past? Could it be, the firmness of the past is just an illusion? Could the past be a kaleidoscope, a pattern of images that shift with each disturbance of a sudden breeze, a laugh, a thought? And if the shift is everywhere, how would we know? In a world of shifting past,..." – A. Lightman, 1993

Assim como no caso dos métodos de aferição de ritmos biológicos, é importante que métodos de análise de dados na pesquisa circadiana sejam padronizados para que o conhecimento desenvolvido tenha significado. É importante que pesquisadores da área e de diferentes campos tenham a oportunidade de conhecer os métodos já desenvolvidos e a que se propõem, antes de desenhar seus estudo e coletar os dados.

1.6.1 Séries temporais

Uma série temporal é um conjunto de observações feitas ao longo do tempo. Neste caso, a ordem dos dados é de extrema importância e as observações vizinhas são dependentes. As análises de séries temporais levam em consideração que os dados podem ter uma estrutura interna que deve ser considerada (HALBERG, 1960; DÍEZ-NOGUERA, 2013). Um ritmo é um componente de uma série temporal biológica; é comprovadamente um fenômeno recorrente e periódico (ASCHOFF, 1960; PITTENDRIGH, 1960; HERMIDA, 1987).

1.6.2 Amostragem e tratamento dos dados

Existem essencialmente três tipos de amostragem de séries temporais: longitudinais, transversais e híbridas. A *amostragem longitudinal* é a realizada continuamente no mesmo indivíduo por *n* ciclos; é muito útil no entendimento da estrutura temporal também a nível individual. Quanto mais longa a coleta, mais fácil distinguir componentes da série, em especial no caso de períodos mais próximos. A *amostragem transversal* é a realizada em diferentes horários, mas apenas uma vez por indivíduo. Os dados são então agrupados por horário e mede-se o comportamento rítmico da população. Neste caso, quanto mais sujeitos incluídos, melhor a representação da população geral. A limitação desse método são as diferenças entre indivíduos. Certos desfechos, porém, podem ser medidos apenas uma vez (por exemplo, mortalidade) ou não podem ser medidos com muita frequência, pois uma medida interferiria na próxima (por exemplo, coletas de sangue em intervalos curtos em ratos) e neste caso amostragens transversais precisam ser utilizadas. Os *desenhos híbridos* são aqueles em que um número de indivíduos é acompanhado por um número de ciclos (HAUS; TOUITOU, 1992; REFINETTI; LISSEN; HALBERG, 2007).

Frequência

As dificuldades financeiras, éticas e logísticas para a coleta de séries temporais muitas vezes limitam o número de coletas que podem ser realizadas ao longo de ciclos. Entretanto, restrições na frequência da amostragem podem influenciar inferências sobre os ritmos das variáveis coletadas (REDFERN; WATERHOUSE; MINORS, 1991). A amostragem deve ser realizada de acordo com a regularidade do ritmo a ser estudado e a importância relativa do ritmo em questão em relação a possíveis frequências maiores ou menores que possam estar sobrepostas. O número de amostras necessárias para caracterizar parâmetros de um ritmo difere de uma variável para outra. Em variáveis com respostas marcadas e rápidas a estímulos ambientais, ou com muitas altas frequências sobrepostas, mais medidas podem ser necessárias. Muitas vezes, quando coletas não podem ser realizadas adequadamente, a avaliação de certas frequências precisa ser omitida. Neste caso, é preciso escolher o período de maior interesse na variável a ser estudada e amostrar em intervalos suficientemente curtos que demonstrem os ritmos de alta frequência, ou amostrar por um intervalo de tempo longo suficiente para que as informações sobre as frequências mais baixas sejam acuradas (HAUS; TOUITOU, 1992).

No estudo de ritmos circadianos, ritmos ultradianos podem causar *aliasing* (identificação não acurada de um sinal: exemplo na Figura 5) se intervalos de frequência de amostragem muito longos são utilizados. Quanto mais rápidas e erráticas se espera que as flutuações sejam, maior deve ser a frequência de coleta se o objetivo é uma aferição acurada.

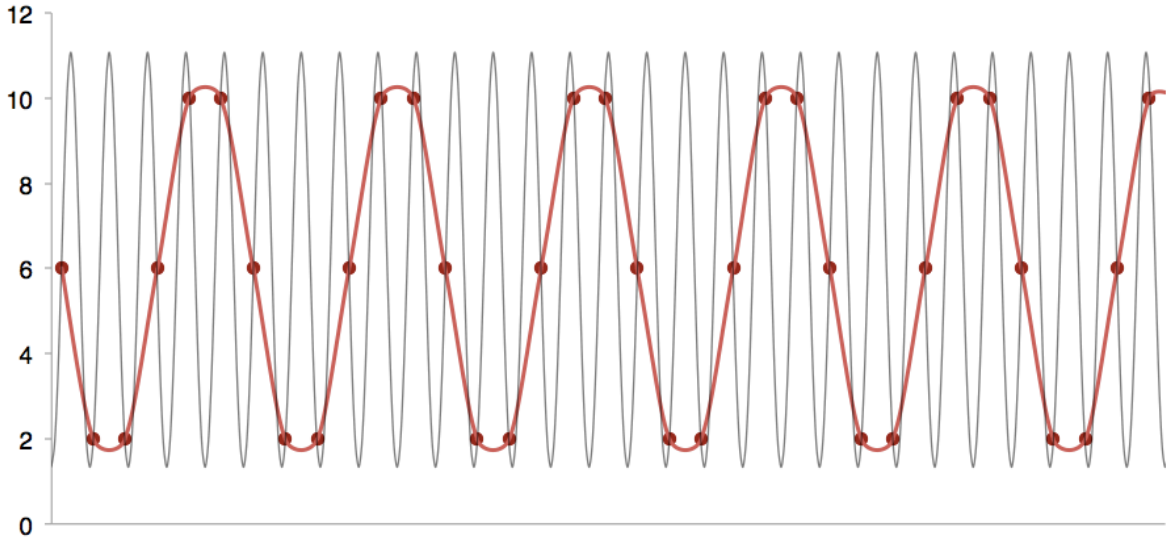

Figura 5. Aliasing. *Amostragem com frequência insuficiente pode causar a reconstrução de um sinal diferente do original.*

A Figura 6A mostra concentrações de cortisol plasmático com dados coletados a cada meia hora. Os mesmos dados são plotados como se tivessem sido coletados em frequências diferentes (Figura 6B-F). Muitos picos transientes deixam de aparecer nesse caso e podemos ver alguma diferença quando a coleta é apenas deslocada (Figura 6C-D, intervalos de 2 horas, início de coleta uma hora depois em D em relação a C). Com amostras menos frequentes, apenas a natureza geral do ritmos pode ser observada. Dosagens urinárias podem fornecer informações integradas (Figura 6G-H), ao contrário de variáveis como o pulso ou a temperatura, cuja medida pela manhã não reflete um valor acumulado do que ocorreu à noite. Porém, perdem-se informações potencialmente importantes sobre o momento das alterações noturnas; não é possível perceber que a concentração de cortisol aumenta na segunda metade da noite, por exemplo (REDFERN; WATERHOUSE; MINORS, 1991).

Poucas informações estão disponíveis sobre recomendações de coletas para a avaliação de ritmos de funções comumente medidas. Em variações ultradianas, como ocorre, por exemplo, com muitos hormônios pituitários e esteroides, intervalos de coleta de 10-15 minutos seriam adequados. Raramente tal coleta é factível. Alguns procedimentos de detecção de ritmos podem auxiliar em séries que normalmente estão sujeitas a mascaramento e ruído e fornecer uma estimativa da frequência de coleta ou ainda do tamanho de amostra do grupo de sujeitos que seriam necessários para descrever um ritmo pelo procedimento adequado. Também é importante lembrar que repetidas coletas com técnicas invasivas (como coleta de sangue) podem mascarar o ritmo sendo estudado e que é necessário tomar precauções para reduzir a interferência do investigador que pode enviesar os resultados, dando preferência, por exemplo, a medidas não invasivas (HAUS; TOUITOU, 1992).

Para ajustar uma curva a um ritmo, é necessário ter no mínimo três pontos cobrindo o período de interesse (dois pontos seriam apenas uma reta). O teorema de Nyquist define que o período da análise não pode ser inferior a três vezes a frequência de amostragem. Por exemplo, se dados são coletados a cada 4 horas, o menor período que pode ser testado é de 12h; com dados coletados a cada 8 horas, o menor período que pode ser testado é 24h. Para a análise mais aprofundada de ritmos, mais do que 3 coletas são necessárias: um ajuste de uma curva cosseno com apenas 3 pontos vai simplesmente sobrepô-los, sem possibilidade de testar quão adequado é o modelo (qualidade do ajuste, normalidade dos resíduos). Para descrever uma forma de onda ou outra característica do ritmo, 3 pontos não são suficientes e uma quantidade maior de coletas é essencial. Métodos padrões de investigação de ritmos normalmente exigem que os dados sejam coletados em intervalos equidistantes, a cada 10 minutos, duas horas, ou um dia por exemplo. A série também não deve ter intervalos (*missing*

data) que interferem na análise (KOUKKARI; SOTHERN, 2007). No caso de *missing*, ou em que dados claramente espúrios tenham que ser excluídos, o valor pode ser substituído por interpolação linear ou de spline. Esta medida é aceitável no caso de poucos *outliers* ou dados faltantes. Se a série possui muitos dados espúrios, a melhor decisão pode ser repetir a coleta. A detecção de *missing data* deve ser cuidadosa e verificar se há um padrão associado aos dados faltantes ou *outliers* ou a interpolação pode criar periodicidades (REFINETTI; LISSEN; HALBERG, 2007).

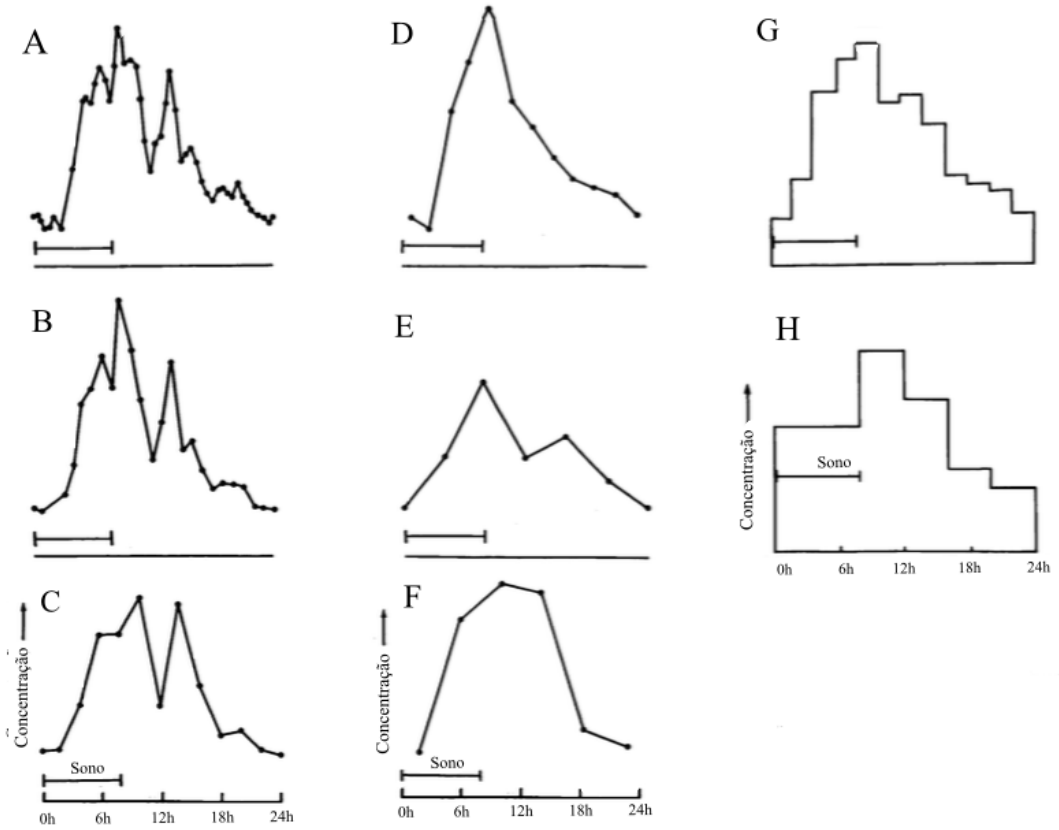

Figura 6. Ritmo circadiano de cortisol plasmático *(baseado em Krieger, 1979, disponível e adaptado de Redfern, Waterhouse, Minors, 1991). (A) dados plotados em intervalos de 30 minutos. Mesmos dados mas plotados: em intervalos de (B) 1 hora, (C) 2 horas em horas pares, (D) duas horas em horas ímpares, (E) 4 horas iniciando às 0h, (F) 4 horas iniciando às 2h. Amostras integradas (urinárias) em intervalos (G) de 2 horas e (H) de 4 horas durante o dia com apenas uma amostra integrada do período noturno.*

Extensão (tempo de coleta)

Outro importante fator a ser considerado na amostragem, além da frequência, é por quanto tempo a coleta deve ser feita. É recomendado que se colete pelo menos duas vezes o comprimento do período a ser estudado (por exemplo, 48 h para um período de 24 h) para demonstrar reprodutibilidade no padrão dos dados (KOUKKARI; SOTHERN, 2007). Esta

série temporal pode ser usada para descrever as características dos dados da série geral (média, mediana...) ou por momentos separados (horário do dia, dia da semana, por exemplo). Os dados podem ser comparados utilizando testes estatísticos adequados para avaliar se há diferença significativa entre horários.

Testes que detectam padrões rítmicos em séries temporais muitas vezes tem outras exigências quanto a frequência e tamanho da série e a recomendação é que coletas mais longas sejam realizadas. Para obter estimativas de parâmetros rítmicos acurados, uma quantidade considerável de ciclos precisa ser estudada. O número de ciclos e quantos sujeitos são necessários só podem ser previstos uma vez que o ritmo tenha sido caracterizado (quanto a amplitude, ruído...). No caso de ritmos com periodicidades desconhecidas, a coleta de ainda mais dados deve ser necessária (HAUS; TOUITOU, 1992).

Suavização de dados (filtros)

Algumas vezes, a identificação de padrões em séries temporais longas exigem a suavização dos dados para remoção de ruídos que atrapalham a detecção do sinal. Médias ou medianas móveis com intervalos adequados podem ser úteis neste sentido (Figura 7). A mediana móvel é bastante efetiva em eliminar dados espúrios e, na maioria dos casos, o método mais recomendado (DÍEZ-NOGUERA, 2013).

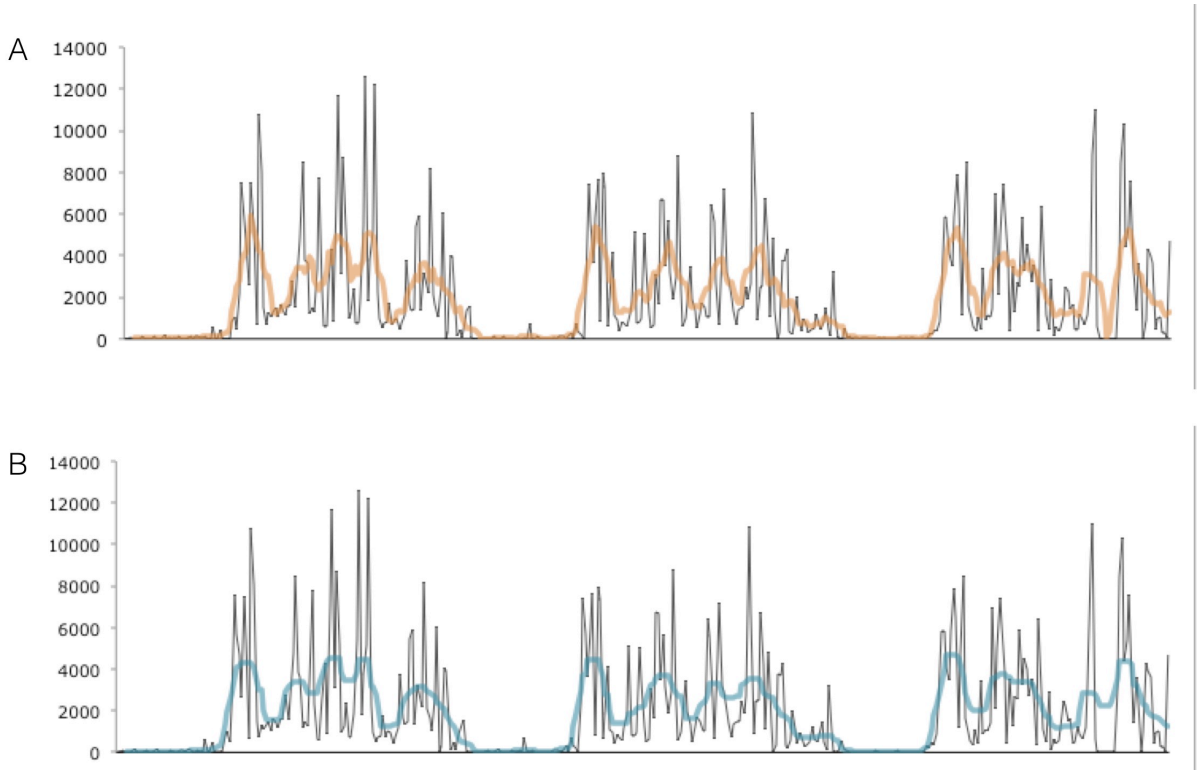

Figura 7. Suavização dos dados. O gráfico A mostra a média móvel em laranja e o gráfico B, a mediana móvel em azul.

1.6.3 Inspeção visual

Certos fenômenos podem ser reconhecidos como rítmicos imediatamente, suas repetições previsíveis podem ser detectadas a olho nu. É por esta razão que normalmente o primeiro passo após a coleta de dados é sua inspeção visual.

As séries temporais normalmente são plotadas da esquerda para direita e em gráficos lineares (Figura 8A), nos quais já é possível detectar o caráter rítmico da variável. Porém alterações de fase e período em longas séries são difíceis de serem detectados em tais gráficos. Uma demonstração gráfica muito utilizada para verificar a variação de dados quando a coleta é realizada por um longo período, como no caso da actigrafia, são os actogramas (Figura 8B). O eixo y de cada linha representa, no caso da figura 8B, contagens de atividade. No caso de gráficos duplos, vemos dois ciclos de coleta em cada linha. Os blocos de cada linha são escalonados, de forma que na primeira linha podem ser vistos os ciclos 1 e 2, na segunda os ciclos 2 e 3 e assim por diante (ROENNEBERG et al., 2015). Na figura 8, por exemplo, pode-se perceber claros indícios de manifestação de jetlag social no ritmo de atividade/repouso (horários diferentes em dias de semana e fim de semana) apenas quando os dados são plotados no actograma (8B).

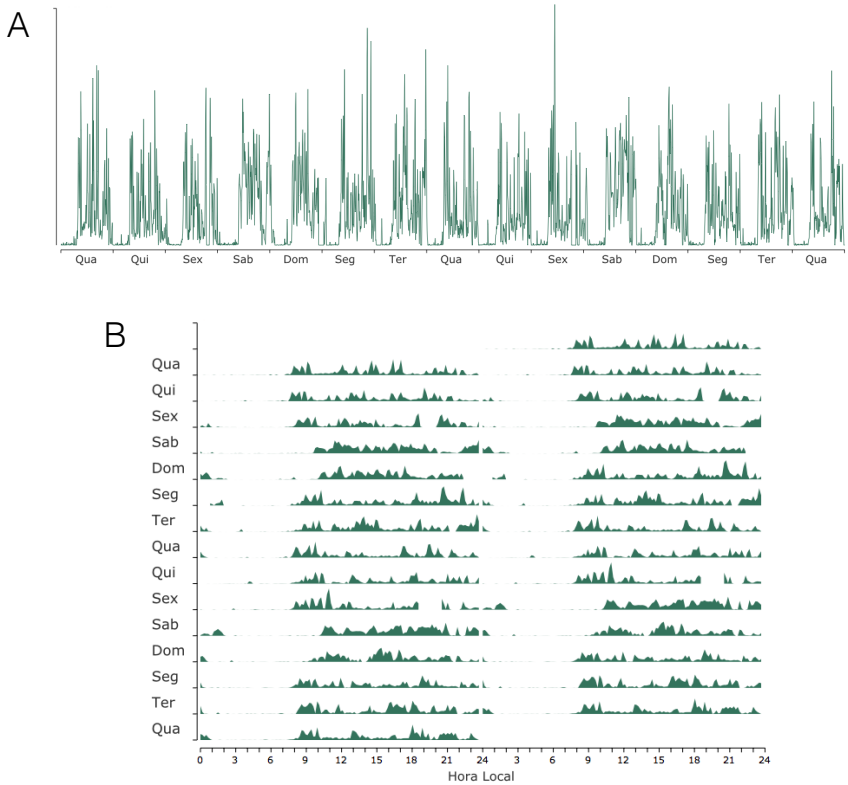

Figura 8. Gráfico linear (A) e actograma (B) *de 15 dias de dados de atividade/repouso coletados através de actigrafia.*

1.6.4 Métodos em análise de séries longas

Alguns testes permitem analisar, avaliar propriedades e interpretar séries longas (por exemplo, uma série de atividade, coletada com actigrafia a cada minuto por um mês).

Análise de cosinor

O método de cosinor foi desenvolvido por Franz Halberg na década de 1960 (HALBERG; TONG; JOHNSON, 1967). Quando desenvolvido, outras técnicas disponíveis geralmente necessitavam de dados equidistantes e coletados por mais de um ciclo. Métodos de mínimos quadrados em geral não apresentam esse problema e, portanto, são úteis quando é desejável obter uma função que se ajuste a um conjunto de medidas (CORNELISSEN, 2014).

O método é baseado na ideia de que ritmos circadianos podem ser pensados como ritmos suavizados mas sujeitos a ruído. Desta forma, um modelo de curvas cossenos com períodos conhecidos (24 horas apenas, ou mais harmônicos – componentes com outros períodos) pode ser ajustado aos dados pelo método de mínimos quadrados e representaria uma estimativa deste ritmo sem ruído. No caso de apenas uma curva cosinusoidal, a equação do modelo é:

$$y(t) = M + A\cos(\theta_i + \phi) + e_i$$

Y(t): dados coletados em t*i* (i=1, ... , N)
M: mesor (Midline Estimating Statistic Of Rhythm, valor médio do ritmo ajustado)
A: amplitude (medida da extensão da mudança prevista para acima ou abaixo do valor médio)
***ϕ*:** acrofase (fase em que a função ajustada atinge o valor máximo)
θi: ângulo trigonométrico correspondente ao horário da coleta computado como $2\pi t/\tau$ em que τ é o período (duração de um ciclo)
e_i: erro a cada horário. Assume-se que sejam independentes, normalmente distribuídos, com média 0.

No caso de o intervalo de confiança da amplitude não sobrepor o mesor, há evidência estatística para um padrão rítmico com o período testado (REFINETTI; LISSEN; HALBERG, 2007). Os parâmetros derivados da análise de cosinor (mesor, acrofase, amplitude) podem ser comparados entre indivíduos e grupos utilizando testes estatísticos adequados.

Os pressupostos para a realização do teste de cosinor são: 1) dados coletados tenham um padrão sinusoidal; 2) normalidade e independência dos erros; 3) erros não devem depender do nível esperado do sinal. Além disso, o período utilizado precisa ser adequado (DE PRINS; WALDURA, 1993).

Análise espectral ou de Fourier

A representação gráfica das amplitudes de diferentes harmônicos é denominada espectro da série e representa os componentes de diferentes frequências envolvidos na definição do padrão. A análise de Fourier decompõe a forma de onda nos seus diferentes harmônicos (oscilações com diferentes períodos). Ela baseia-se na ideia de que qualquer forma de onda pode ser compreendida quando dividida em sinais simples, ou uma série de ondas senoidais e cossenoidais. No caso do componente principal ser identificado no intervalo circadiano, pode-se inferir que o processo exibe ritmicidade circadiana (REFINETTI; LISSEN; HALBERG, 2007; NATURE EDITORIAL, 2018).

É importante que o comprimento das seções analisadas sejam iguais ou múltiplos inteiros do período usado na análise principal para garantir que a amplitude dos harmônicos não afete a magnitude dos demais. A magnitude de cada espectro pode também ser representada como poder: a fração que o quadrado da amplitude do espectro representa em relação a soma dos quadrados das amplitudes de todos os espectros. Assim, é possível verificar a importância de cada um deles (DÍEZ-NOGUERA, 2013).

Periodogramas

Outro método na identificação de ritmicidade são os periodogramas. O método foi de Enright proposto nos anos 60 e pode ser pensado como uma ANOVA adaptada para testar ritmicidade. O procedimento divide a série em segmentos de diferentes períodos e calcula um índice de variabilidade para cada um. A significância de cada período é testado com testes F para identificar o período significativo (ENRIGHT, 1965). Sokolove e Bushell (1978) propuseram utilizar no lugar de F, a distribuição χ^2. Para que a significância estatística possa ser confiável, recomenda-se que a série tenha no mínimo 10 dias quando este periodograma é usado. Por fim, o periodograma de Lomb-Scargle tem a vantagem de permitir a análise de dados coletados em intervalos irregulares ou com *missing*. O método é derivado da análise espectral de Fourier clássica e é bastante sensível; foi desenvolvido para detectar mesmo ritmicidades fracas (LOMB, 1976; RUF, 1999). A menos que a série tenha mais de um componente, o pico mais alto é seu período estimado.

Variáveis “não paramétricas”

Van Someren e colaboradores (1999) defenderam a utilização de “variáveis não-paramétricas” (WITTING et al., 1990) no estudo de ritmos biológicos, por estas serem indicadores sensíveis de perturbações aos ritmos. No estudo em questão, os autores demonstraram que tais dados refletiram melhoras em pacientes com Doença de Alzheimer

quando tratados com terapia de luz que outros métodos clássicos (amplitude do cosinor, poder normalizado – periodograma de Lomb-Scargle) não foram capazes de aferir. Enquanto a definição em Estatística determina que testes não paramétricos são aqueles que independem de suposições acerca da distribuição a que os dados pertencem, o termo não-paramétrico aqui refere-se ao fato de tais análises não assumirem qualquer pressuposto sobre a forma de onda do ritmo.

Autocorrelação em 24h: A função de autocorrelação é uma correlação da série com uma cópia atrasada dela mesma como uma função do atraso (Figura 9). No caso da autocorrelação em 24 horas, o coeficiente de correlação é calculado em relação a correlação da série com ela mesma atrasada em um dia.

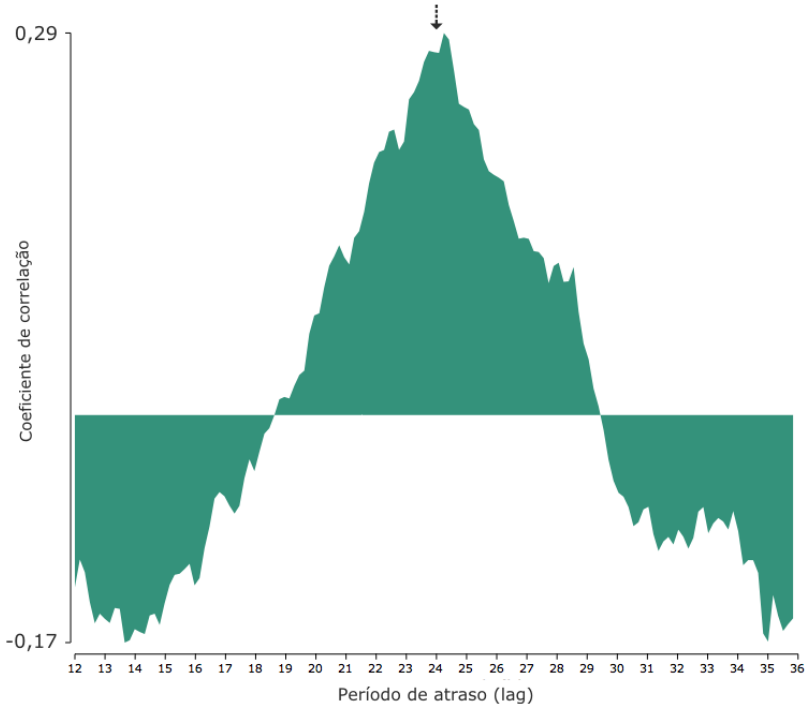

Figura 9. Função de autocorrelação. Coeficiente de correlação conforme o atraso da série correlacionada a ela mesma. É possível ver que o valor máximo é aproximadamente com o intervalo de um dia (seta), enquanto que as séries estão menos correlacionadas com um intervalo de 12 horas (em anti-fase).

Estabilidade (*interdaily stability* – IS): A IS quantifica a regularidade entre dias, ou seja, quanto o ritmo está sincronizado ao dia de 24 horas. Equivale ao valor de 24 horas do periodograma de qui-quadrado, normalizado para o número de dados. Ela é calculada como a razão entre a variância do padrão médio de 24h e a variância total. Os valores de IS vão de 0 a 1, com maiores valores indicando maior estabilidade.

$$IS = \frac{n \sum_{h=1}^{p} (\overline{x_h} - \bar{x})^2}{p \sum_{i=l}^{n} (x_i - \bar{x})^2}$$

$\boldsymbol{n}$**:** número total de dados
$\boldsymbol{p}$**:** número de dados por dia
$\boldsymbol{\overline{x}_h}$**:** médias por hora
$\boldsymbol{\overline{x}}$**:** média de todos os dados
$\boldsymbol{x_i}$**:** dados individuais

Variabilidade (intradaily variability – IV): A IV estima a fragmentação do ritmo, ou seja, a frequência e extensão de transições entre repouso e atividade. É calculada como a razão dos quadrados das diferenças entre dados sucessivos e a variância geral. A IV chega perto de 0 para uma onda sinusoidal, a aproximadamente 2 para ruídos Gaussianos e até mais na presença de um componente ultradiano de 2 horas. Ou seja, valores maiores indicam maior fragmentação.

$$IV = \frac{n \sum_{i=2}^{p} (x_i - x_{i-l})^2}{(n-1) \sum_{i=l}^{n} (x_i - \bar{x})^2}$$

n: número total de dados
p: número de dados por dia
$\overline{x_h}$: médias por hora
$\bar{x}$: média de todos os dados
x_i: dados individuais

Amplitude relativa (AR): Pode ser calculada a partir da atividade média nas 10 horas mais (M10) e 5 horas menos ativas (L5).

$$AR = \frac{M10 - L5}{M10 + L5}$$

Medidas que podem ser derivadas da actigrafia estão resumidas na tabela 1.

TABELA 1 - Variáveis derivadas de análises de actigrafia

Variável	Definição
Medidas angulares (fase)	
Acrofase	Fase em que a função sinusoidal ajustada atinge o valor máximo.
Centro de gravidade	Horário médio dos registros do ritmo.
Medidas relacionadas ao período/frequência	
Período	Tempo após o qual uma fase definida da oscilação ocorre novamente

Amplitude	Diferença entre o valor máximo ou mínimo e o valor médio em uma oscilação sinusoidal; num sentido menos rígido, o termo também é usado para oscilações com uma forma geral, mas neste caso, o termo correto deve acrescentar "positiva" ou negativa".
Amplitude relativa	Razão entre: média nas 10 horas mais ativas subtraída a média nas 5 horas mais ativas / média nas 10 horas mais ativas adicionada a média nas 5 horas mais ativas.
Extensão da oscilação (range of oscillation)	Diferença entre o valor máximo e mínimo.
Poder dos harmônicos	Magnitude de um harmônico/frequência fundamental: fração que o quadrado da amplitude do espectro representa em relação a soma dos quadrados das amplitudes de todos os espectros.
Autocorrelação em 24 h	Coeficiente de correlação em relação a correlação da série com ela mesma atrasada em um dia. Reflete quão sincronizada aos dias de 24h uma série está.
Variáveis relacionadas a estabilidade do ritmo	
Estabilidade (interdaily stability – IS)	Regularidade entre dias, ou seja, quanto o ritmo está sincronizado ao dia de 24 horas.
Variabilidade (intradaily variability – IV)	Fragmentação do ritmo, ou seja, a frequência e extensão de transições entre repouso e atividade.
Variáveis relacionadas ao sono*	
Horário de deitar	Diferente do horário de dormir, representa o horário em que o indivíduo vai para a cama.
Horário de dormir	Horário em que o indivíduo adormece
Latência do sono	Tempo necessário para adormecer. Diferença entre o horário de deitar e adormecer ou diferença entre o momento em que o indivíduo começa a tentar adormecer e em que adormece.
Horário de acordar	Horário em que o indivíduo acorda.
Horário de levantar	Diferente do horário de acordar, representa o horário em que o indivíduo sai da cama.
Inércia do sono	Representa o período em que o indivíduo está em um estado de baixa performance e cognição debilitada imediatamente após acordar. Apesar de frequentemente ultrapassar esse momento, pode ser aferida como a diferença entre o horário de levantar e acordar.
Duração de sono	Tempo dormindo: diferença entre o horário de acordar e de dormir.
Ponto médio do sono	Diferença entre horário de dormir e duração de sono/2.
Eficiência do sono	Reflete a dificuldade em adormecer e permanecer dormindo. Proporção (%) do tempo gasto dormindo quando se está na cama. Apesar de nem sempre considerado, recomenda-se atualmente que se desconsidere o tempo que se está na cama antes de tentar dormir ou quando se permanece na cama sem tentar dormir.

*Estas variáveis podem também ser aferidas, apesar de apresentarem certas limitações, através de registros actigráficos de atividade com algoritmos validados contra polissonografia. A acurácia varia conforme a medida, neste caso. Um dos inconvenientes de algumas das medidas atuais é que elas normalmente consideram o sono noturno como um episódio único. É importante que sejam consideradas diferenças entre dias de trabalho e dias livres no cálculo de valores médios.

1.4.5 Estatística circular

Muitas vezes, em Cronobiologia, trabalhamos com medidas de momento (fase) ou horário: hora de dormir, ponto médio de sono, início de secreção de melatonina (dim light melatonina onset, DLMO), horário do pico de apetite, acrofase, etc. Outro exemplo de variável de fase, que como a acrofase, afere o centro temporal da série, é o centro de gravidade (CoG). O CoG nada mais é que o horário médio dos registros do ritmo, como, por exemplo, de atividade (KENAGY, 1980).

Esse tipo de variável (de fase) pode ser aferido para cada indivíduo, mas nem sempre pode ser comparado utilizando estatística linear. Uma maneira de perceber como a estatística linear pode ser inadequada neste caso é pensar que dormir às 23h significa dormir uma hora mais cedo que 0h: a distância entre os dados não é de 23 horas. Neste caso, poderíamos comparar horários "corrigindo" o horário (para -1h no exemplo acima). Mas quando os dados são muito dispersos, tal correção nem sempre pode ser realizada e as análises de estatística circular para medidas angulares ou cíclicas se tornam a melhor opção.

A média e mediana circulares seriam o equivalente a média e mediana lineares. A média circular é calculada como a arco tangente da média dos senos pelos cossenos:

$$\bar{\phi} = \arctan\frac{\bar{y}}{\bar{x}} \qquad \bar{y} = \frac{\Sigma sin\phi_i}{n} \qquad \bar{x} = \frac{\Sigma cos\phi_i}{n}$$

O comprimento do vetor (R) pode ser utilizado como medida de dispersão dos dados e, a partir dele, podem ser calculadas outras medidas, como a variância circular (v):

$$R = \sqrt{\bar{x}^2 + \bar{y}^2} \qquad v = 1 - R$$

A tabela 2 mostra alguns dos testes de estatística circular análogos aos testes em estatística linear.

TABELA 2 - Parâmetros e testes em estatística linear e circular

	Linear	**Circular**
Distribuição	Distribuição normal: Shapiro-Wilk, Kolmogorov-Smirnov	Distribuição de Von Mises: Stephens modified Watson's test, Cox test
Comparação: dois grupos		
Não paramétrico, não pareado	Mann-Whiney test	Wheeler-Watson test
Não paramétrico, pareado	Wilcoxon signed-rank test	Moore's paired test
Paramétrico, não pareado	Student's t test	Watson-Williams test
Paramétrico, pareado	Paired t test	Hotellings paired test
Comparação: n grupos		
Não paramétrico	Kruskal-Wallis	Mardia-Watson-Wheeler uniform-scores test
Paramétrico	ANOVA	Watson-Williams high concentration F test
Correlação	Pearson, Spearman	Linear-circular: Johnson-Wehrly-Mardia correlation coefficient, Mardia's rank correlation coefficient Circular-circular: Jammalamadaka-Sarma correlation coefficient
Regressão	Regressão Linear	Predizer variável circular com fatores lineares Predizer variável circular com fatores circulares Modelos de regressão múltipla (em estudo)

2 OBJETIVOS

Geral

Avaliar a utilização de métodos que investiguem variáveis relacionadas a ritmos biológicos e sua aplicabilidade e conveniência em estudos na área da saúde.

Específicos

1. Avaliar a confiabilidade do Instrumento de Ritmos de Humor.
2. Avaliar diferenças na identificação de picos em comportamentos e variáveis fisiológicas relacionadas a sintomas de humor entre indivíduos sem risco e em risco para transtornos psiquiátricos.
3. Avaliar possíveis alterações nos ritmos sono-vigília causados pelas mudanças que ocorreram com as possibilidades trazidas pela luz elétrica.
4. Avaliar a correspondência entre uma medida objetiva (actigrafia) e uma subjetiva (questionário - MCTQ) de horários de sono em comunidades rurais Quilombolas.

3 HIPÓTESE

Os estudos da tese apresentavam as seguintes hipóteses:

Estudo 1. (Capítulo 2) O Instrumento de Ritmos de Humor não apresenta viés de memória.

Estudo 2. (Capítulo 3) A ritmicidade/horário de pico em sintomas de humor é diferente em indivíduos com risco para transtornos psiquiátricos.

Estudo 3. (Capítulo 4) As possibilidades trazidas pela eletricidade estão associados a um atraso nos horários de sono e um encurtamento de sua duração.

Estudo 4. (Apêndice 1) O desalinhamento circadiano (jetlag social) está associado a sintomas depressivos.

CAPÍTULO 2:

Confiabilidade do Instrumento de Ritmo de Humor

Artigo 1: em revisão no periódico *Frontiers in Psychiatry*.

**As figuras, legendas e tabelas foram incorporadas ao texto para facilitar a avaliação da banca.

Prospective Daily Assessment of Daily Patterns of Mood-related Symptoms

Luísa K. Pilz [1,2,+], Alicia Carissimi [1,2,+], Ana Paula Francisco[1,2], Melissa A. B. Oliveira[1,2], Anastasiya Slyepchenko[3,6], Kristina Epifano[4], Luciene Garay[1,2], Raul C. Fabris[1], Marina Scop[1], David L. Streiner[5], Maria Paz Hidalgo[1,2], Benicio N. Frey[3,5,6]

[1] Laboratório de Cronobiologia e Sono do Hospital de Clínicas de Porto Alegre (HCPA), Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, Rio Grande do Sul, Brasil;

[2] Programa de Pós-Graduação em Psiquiatria e Ciências do Comportamento – Faculdade de Medicina, UFRGS, Porto Alegre, Rio Grande do Sul, Brasil;

[3] MiNDS Neuroscience Graduate Program, McMaster University, Hamilton, ON, Canada;

[4] Department of Psychology, Neuroscience and Behaviour, McMaster University, Hamilton, ON, Canada;

[5] Department of Psychiatry and Behavioural Neurosciences, McMaster University, Hamilton, ON, Canada;

[6] Mood Disorders Program and Women's Health Concerns Clinic, St. Joseph's Healthcare, Hamilton, ON, Canada;

+these authors equally contributed to this study.

ABSTRACT

Background: The Mood Rhythm Instrument (MRI) is a new self-report questionnaire that aims to assess, the presence and timing of daily patterns of mood-related symptoms. Here, we examined the reliability of the MRI against a prospective daily investigation over the course of 15 days. As a secondary aim, we examined whether the number of items with a perceived daily pattern correlated with severity of depressive symptoms and psychological well-being.

Methods: 32 participants recruited from the general population were asked to prospectively fill out a daily version of the MRI (MRI-d) for 15 days. On the 16th day, they filled out the MRI, the Beck Depression Inventory (BDI) and the World Health Organization 5-item well-being index (WHO-5).

Results: The MRI showed high agreement with the MRI-d, which suggests that the MRI is a valid tool to assess daily patterns of mood symptoms. The number of mood symptoms perceived as having daily peaks correlated positively with BDI scores and negatively with WHO-5 scores.

Conclusion: The MRI might be a valid tool to investigate the presence of daily patterns and the timing of mood-related factors. The MRI does not seem to be influenced by recall or recency biases. Future studies should test the usefulness of this new clinical instrument in individuals with mood disorders, as well as its ability to detect changes in the daily timing of mood symptoms before and after treatment.

Keywords: Chronobiology, Circadian rhythms, Clinical Assessment, Mood, Mental health, Self-report questionnaire

INTRODUCTION

Several lines of evidence highlight the mechanistic and phenomenological links between mood symptoms and circadian rhythms (1–3). For instance, the presence of certain polymorphisms in clock genes has been associated with vulnerability and clinical manifestations of mood disorders (4). Animal studies have shown that reduced expression of *Bmal1* in the suprachiasmatic nuclei led to depressive- and anxiety-like behaviors (5). In addition, it has been well established that sleep and circadian disruptions are very prevalent in individuals with major mood disorders such as major depression and bipolar disorder (6–8). Chronotherapies targeting the synchronization of biological rhythms, such as bright light therapy, interpersonal and social rhythm therapy, sleep deprivation and sleep hygiene, are useful tools in the management of mood disorders (9–12).

Despite the well-established link between alterations in circadian rhythms and the development and clinical presentation of mood disorders, the majority of previous studies have focused on the circadian fluctuation of sleep/appetite patterns, hormonal levels, and sexual/social behaviors. Little is known about circadian fluctuations of psychological symptoms such as sadness, irritability, and mood swings. A better understanding of the circadian rhythmicity of mood symptoms will help to identify individuals whose severity of mood symptoms follow an altered circadian rhythm, which can ultimately guide more accurate treatment decisions. To address this gap, we have recently developed the Mood Rhythm Instrument (MRI). The MRI is a self-report questionnaire that assesses the presence of unimodal daily patterns (i.e, rhythmicity with a peak every 24h) and the peak timing of physiological and behavioral variables across affective, cognitive and somatic domains that are often altered in mood disorders in the last 15 days.

The MRI has been validated in Brazilian Portuguese (13) and in Spanish (14), and the English version is currently being validated in Canada. In a large study using the MRI to

examine community samples (N = 708), we have recently shown that the presence of daily patterns of specific MRI items was significantly associated with higher risk for psychiatric disorders (15). Results also suggested that the timing of some items or the phase angle differences between them might also be related to risk for psychiatry disorders (15). However, one of the main limitations of self-reported questionnaires is the reliance on recall when reporting the presence and severity of past/recent symptoms (e.g., "*past week*", "*last 15 days*"). Thus, in this study, we aimed to test the reliability of the MRI against a prospective daily investigation over the course of 15 days. We hypothesized that the MRI would have a fair-to-good agreement with a daily version of the MRI (MRI-d). As a secondary aim, we tested whether the number of MRI items where a daily pattern was perceived correlated with severity of depressive symptoms and psychological well-being.

MATERIAL AND METHODS

Participants and Procedures

Study participants were recruited from the general population through convenience-snowball sampling between November-December 2017 (16). They were asked to fill out a daily version of the MRI (MRI-d) for 15 days. To ensure participants compliance, we met them on day 8 to collect the first week of the MRI-d data and handed the material for the last 7 days. On the 15th day, the remaining MRI-d material was collected, and they were asked to complete the Mood Rhythm Instrument, Beck Depression Inventory (BDI, Beck et al., 1961), the World Health Organization 5-item well-being index (WHO-5, Bech, 2004; de Souza and Hidalgo, 2012) on the next day. Paper-based questionnaires were used. All participants gave their informed consent. The study was approved by the Ethics Committee of Hospital de Clínicas de Porto Alegre (#15-0539 GPPG/HCPA) and was conducted in accordance with the Declaration of Helsinki.

Instruments

Mood Rhythm Instrument (MRI)

The MRI is a self-report questionnaire that assesses the presence of daily patterns and the peak timing of its 15 items in the previous 15 days. Each MRI item represents a mood-related physiological/behavioral variable. For each item, two questions are asked: 1) whether each item has had a daily peak in the past 15 days (yes/no; *dichotomous variable*) and 2) only for the questions that were answered "yes", when that peak was (*time variable*).

Mood Rhythm Instrument diary (MRI-d)

The daily version of the MRI-d consists of the same 15 items from the MRI, except that participants are asked to answer the questions on a daily basis instead of "in the last 15 days".

Beck Depression Inventory (BDI)

The BDI is a 21-item self-report questionnaire that assesses severity of current depressive symptoms (17). In this study, the validated Brazilian-Portuguese version was used (20). The BDI is one of the most widely used self-report instruments in the study of severity of depressive symptoms, with high internal consistency in both psychiatric and non-psychiatric samples (21). Higher BDI scores indicate higher severity of depressive symptoms.

World Health Organization 5-item well-being index (WHO-5)

The WHO-5 is a 5-item self-report questionnaire that assesses psychological well-being over the last 2 weeks (19). The WHO-5 has high internal consistency and has been used in several studies as a screening for depression (22). The higher the score, the better the perceived psychological well-being.

Statistical analysis

Initially, we calculated the mode of each dichotomous MRI-d variable for each participant across the 15 days of the prospective daily charting (*dichotomous variable*). The agreement rates between the MRI and MRI-d items were determined by calculating the proportion of participants in whom the response on the MRI agreed with the mode of the same variables in the MRI-d. Agreement rates <0.4 were considered poor, 0.4-0.59 were fair, 0.6-0.74 were good, and ≥0.75 were considered excellent (23). In order to assess potential memory bias due to recency effects, the agreement rates were calculated between the MRI and the cumulative mode of the last 3, 5, 7, 11 and 13 days of the MRI-d. If memory bias were present, the agreement rates would be higher on the days closer to the second study visit (when participants filled out the MRI).

Similarly, we calculated participants' median times for each MRI-d variable using data from the days where timing was reported in the prospective diary (*time variable*). The time differences between the peaks reported in the MRI and MRI-d were calculated across the 15 days, and across the cumulative median of the last 3, 5, 7, 11 and 13 days in order to assess possible memory bias. If memory bias was present, the difference between the peaks reported on the MRI and the median peaks of the MRI-d would be significantly lower on the days closer to when the MRI was filled. All time variables were tested for normality using the Shapiro-Wilk test. We tested the correlation between the median MRI-d and MRI variables using Pearson's or Spearman's correlation as appropriate.

Finally, we summed the number of dichotomous items scored "yes" for each of the participants and correlated the number of items where a daily pattern was perceived with BDI and WHO-5 scores using Spearman and Pearson correlations, respectively. IBM SPSS Statistics 24 (IBM, NY, US) and GraphPad 6 (GraphPad Software, La Jolla, US) were used

for data analysis. GraphPad 6 and El Temps (Antoni Díez-Noguera, Barcelona, ES) were used to plot linear and angular data, respectively.

RESULTS

Participants

Thirty-two individuals completed the MRI, MRI-d, WHO-5 and BDI questionnaires. The mean age of the study participants was 35.3 ± 15.9 (age range: 21 - 71); 63% of the participants were women; and the mean number of years of education was 15.6 ± 2.6. The majority of the participants (88%) were currently working or studying.

MRI-d: Presence of a Daily Peak - Differences Between Weekdays and Weekends

Figure 1 shows the number of days where participants reported experiencing a peak for each MRI item. Overall, the vast majority of participants reported peaks in cognitive symptoms such as alertness, problem-solving and concentration, as well as in somatic symptoms, such as sleepiness and appetite. A high frequency of perceived daily patterns was also seen in general motivation. On the other hand, peaks in affective symptoms such as sadness, pessimism or irritability were not as often reported. There were no differences between weekdays and weekends in any of the items ($p> 0.05$; *Chi-square test*).

Figure 2 shows the distribution of the MRI and MRI-d time variables on weekdays and weekends. Most individuals reported a morning peak for cognitive items (alertness, memory, concentration, problem-solving) and an afternoon/evening peak for affective items (irritability, anxiety, sadness, pessimism). Sexual arousal consistently peaked late at night, whereas sleepiness had a more variable distribution with peaks not only early in the morning and late at night but also after lunchtime. No significant differences were detected between weekdays and weekends ($p> 0.05$; *paired t-test/Wilcoxon matched pairs signed-rank test*).

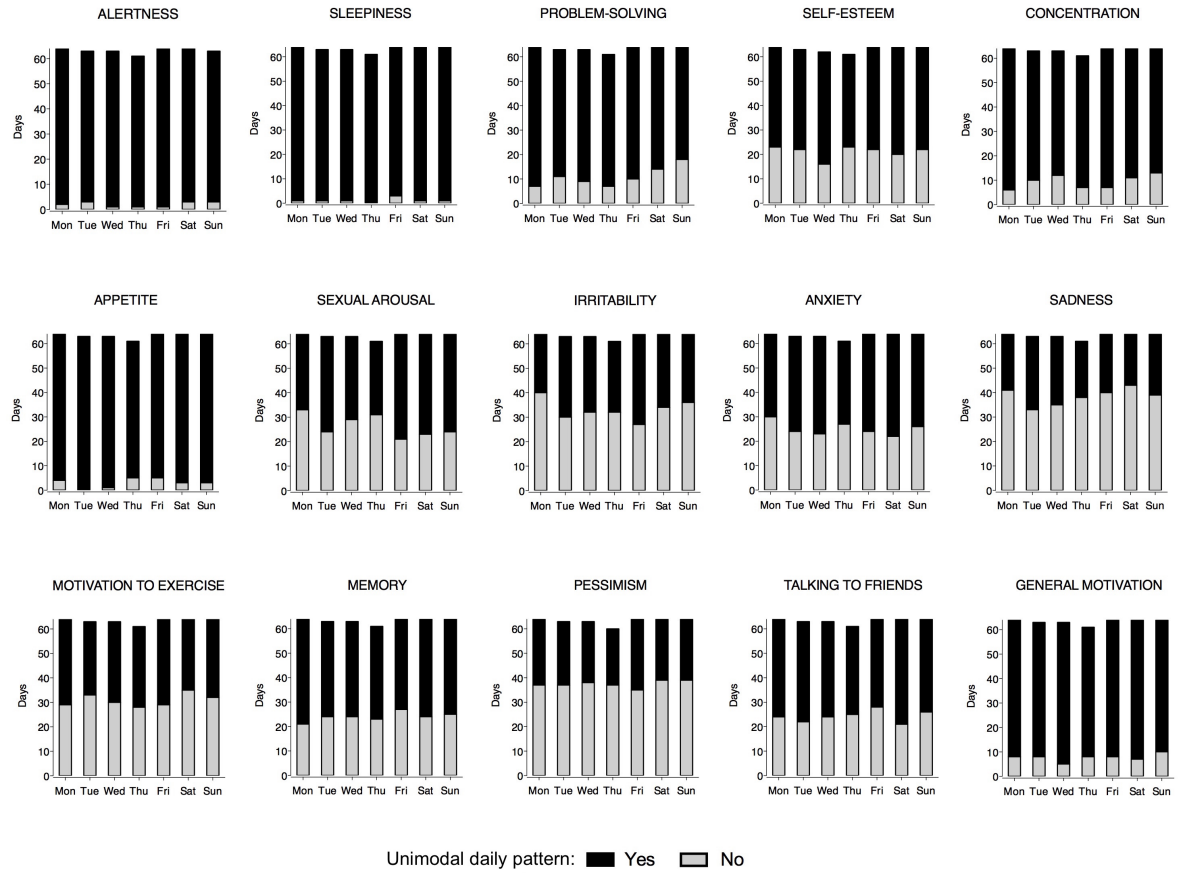

FIGURE 1 | Frequency of days reported as having a peak by weekday for each Mood Rhythm Instrument diary (MRI-d) item. The black proportion of the bars represents days where a peak was reported, whereas the gray proportion represents days where a peak was not reported. For each MRI-d item, *y-axis:* sum of days from all participants; *x-axis:* weekday. No difference was detected between weekdays vs. weekends (Chi-square).

Agreement rates between the MRI and MRI-d: Dichotomous variables

Agreement rates for each item are shown in Table 1. All items showed fair to excellent agreement rates, with fair agreement observed for irritability (0.59), good agreement for talking to friends (0.63) and sadness/anxiety (0.69), and excellent agreement observed for sleepiness (0.97), sexual arousal (0.91) and appetite/alertness (0.88). Notably, higher agreement rates were observed in the MRI items where most participants perceived having a daily pattern, whereas lower agreement rates were observed for items that fewer participants reported as having a 24h peak. Figure 3 shows the agreement rates between the MRI and the cumulative mode of the last 3, 5, 7, 11, 13 and 15 days from the MRI-d. We did not observe an overall effect of memory bias (or recency), with the exception of irritability (Figure 3).

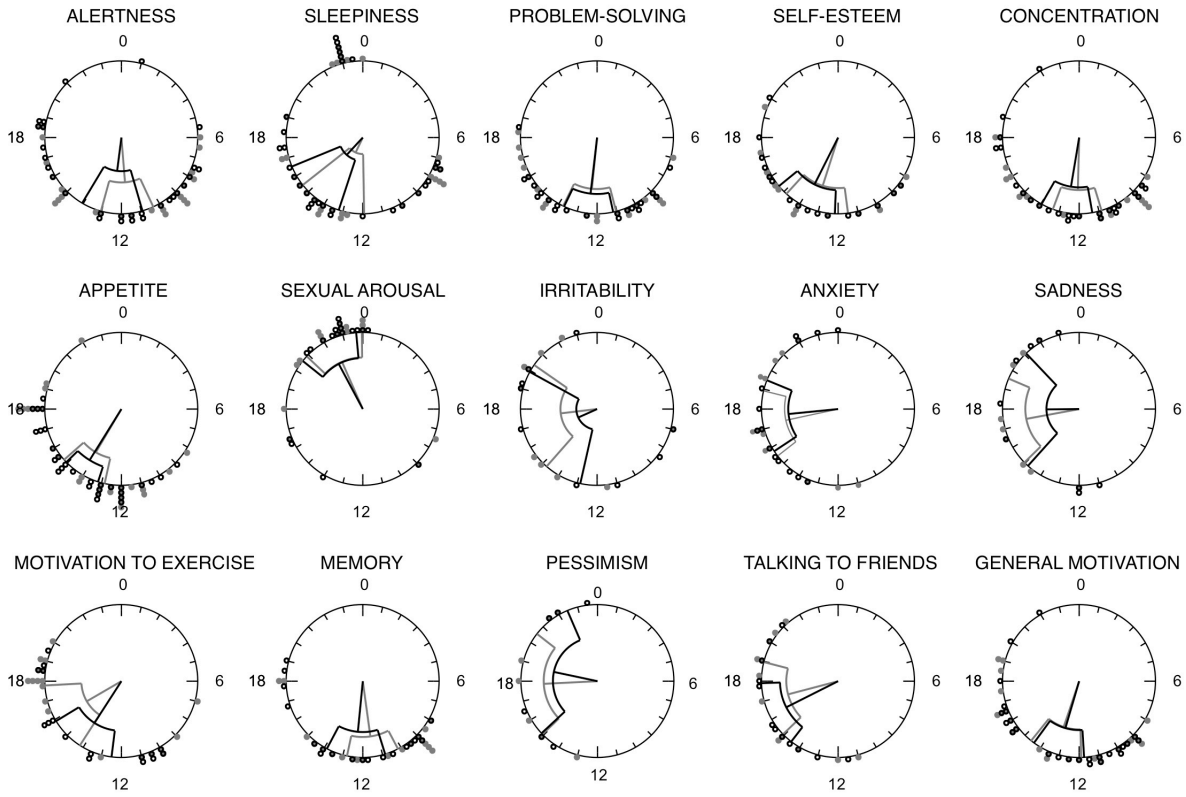

FIGURE 2 | Rayleigh plots for each Mood Rhythm Instrument diary (MRI-d) item time variable. Circles represent the 24h day. Circles along the outermost circumference represent individuals timing: full gray circles represent the median timing of reported peaks on weekdays, whereas open circles represent the median timing of reported peaks on weekends. Fiducial limits are represented.

Agreement between the MRI and MRI-d: Time variables

The time differences between the peaks reported for each item of the MRI and MRI-d are shown in Table 1 and in Bland-Altman plots (Supplementary Figure 1). For the majority of items, the average difference was less than an hour, suggesting high agreement between the prospectively- and retrospectively-recorded questionnaires. Interestingly, items related to affective symptoms – irritability, anxiety and pessimism – were the only ones with an average difference greater than an hour. Table 1 also shows the correlation coefficients between the median time variables of the MRI and MRI-d. With the exception of the affective symptoms of pessimism and anxiety, all other items had correlations ≥0.5, which further indicates high concordance between the MRI and MRI-d. Figure 4 depicts the time differences between the

MRI and the cumulative median of the last 3, 5, 7, 11, 13 and 15 days of the MRI-d. Similar to the analyses of dichotomous variables, we did not observe an overall effect of memory bias, except for irritability (Figure 4).

TABLE 1 | Agreement rates between the MRI and MRI-d.

	Agreement rate[#]	MRI_{diff} (h)[##]	MRId median vs. MRI[###]	
	Peak: y/n	Time of peak	r	n
Alertness	0.88	- 0.65 (-1.63 – 0.33)	0.82^{****}	28
Sleepiness	0.97	- 0.75 (-1.89 – 0.39)	0.80^{****}	31
Problem-solving	0.74	- 0.34 (-1.16 – 0.48)	0.82^{****}	22
Self-esteem	0.72	- 0.02 (-2.59 – 2.56)	0.54^{*}	15
Concentration	0.78	- 0.34 (-1.28 – 0.60)	0.75^{****}	25
Appetite	0.88	- 0.48 (-1.18 – 0.22)	0.77^{****}	29
Sexual Arousal	0.91	- 0.33 (-3.46 – 2.80)	0.56^{*}	18
Irritability	0.59	- 1.95 (-4.38 – 0.48)	0.80^{**}	10
Anxiety	0.69	- 1.18 (-3.94 – 1.58)	0.46^{+}	14
Sadness	0.69	- 0.58 (-3.90 – 2.73)	0.53	9
Motivation to exercise	0.72	0.42 (-0.77 – 1.61)	0.72^{***}	19
Memory	0.72	- 0.56 (-1.32 – 0.19)	0.80^{****}	20
Pessimism	0.69	1.29 (-4.01 – 5.67)	0.39	7
Talking to friends	0.63	0.54 (-0.88 – 1.96)	0.68^{*}	12
General Motivation	0.81	- 0.64 (-1.86 – 0.58)	0.56^{**}	25

[#]MRI vs. MRI-d; [##]MRI_{diff}: average MRI-d days median – MRI (95% CI); [###]Correlation coefficient of MRI-d median vs. MRI. *Pearson:* self-esteem, irritability, anxiety, pessimism, talking to friends. *Spearman:* alertness, sleepiness, problem solving, concentration, appetite, sexual arousal, sadness, physical exercise, memory, general motivation. $^{*}p< 0.05$, $^{**}p< 0.01$, $^{***}p< 0.001$, $^{****}p< 0.0001$, $^{+}p< 0.10$

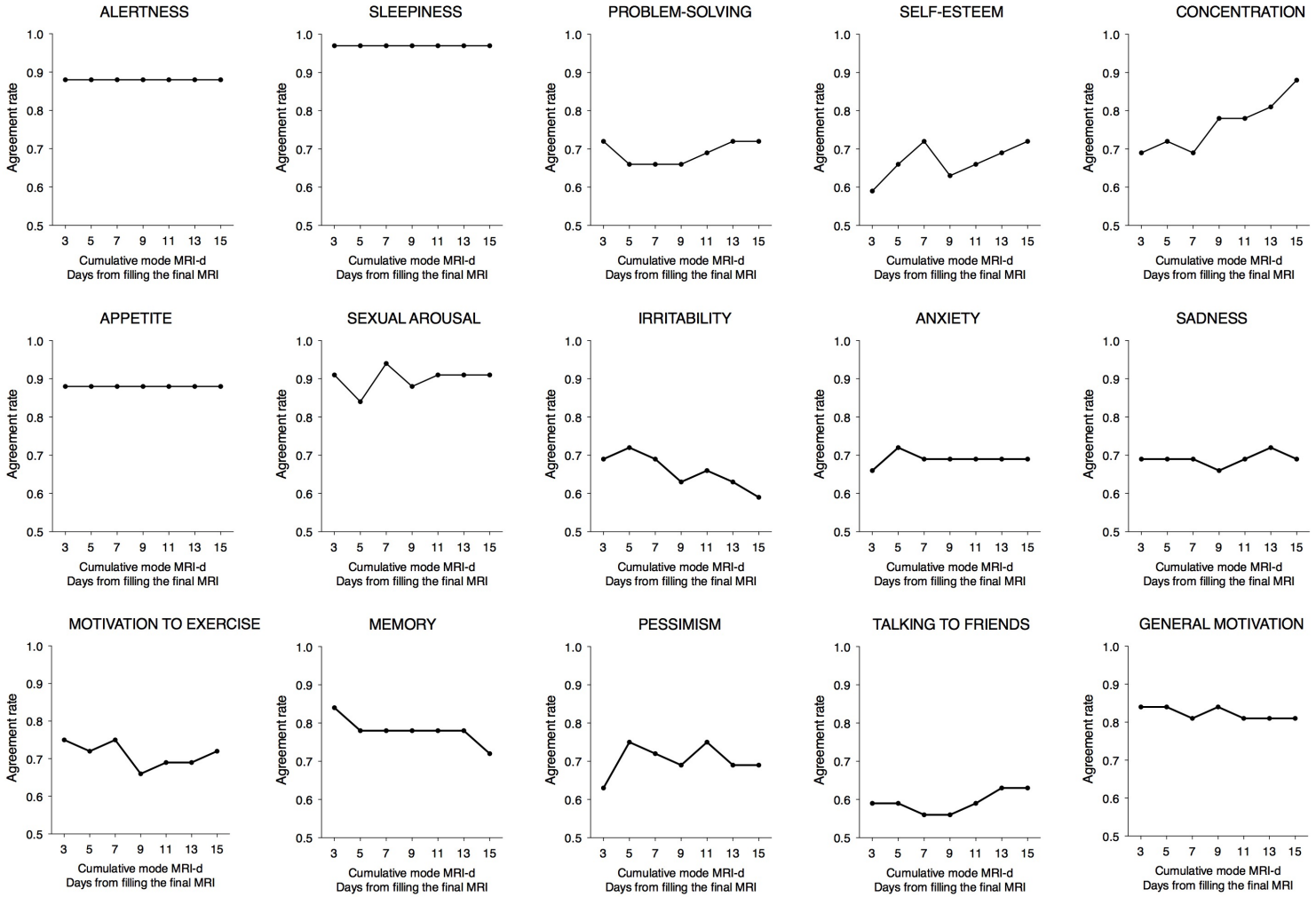

FIGURE 3 | Agreement rates between the Mood Rhythm Instrument (MRI) and the cumulative mode of the last 3, 5, 7, 11, 13, and 15 days from the Mood Rhythm Instrument diary (MRI-d). Dots show group means and whiskers represent standard deviations. Dichotomous data on each item derived from MRI and MRI-d.

Correlation between the MRI, BDI and WHO-5

The number of MRI items with a perceived daily pattern correlated positively with BDI scores (r_S= 0.44; $p < 0.05$) and negatively with WHO-5 scores (r_P= -0.55; $p < 0.01$; Figure 5). These results suggest that the higher the number of variables perceived as having a daily pattern, the greater the severity of current depressive symptoms and the lower the perceived psychological well-being.

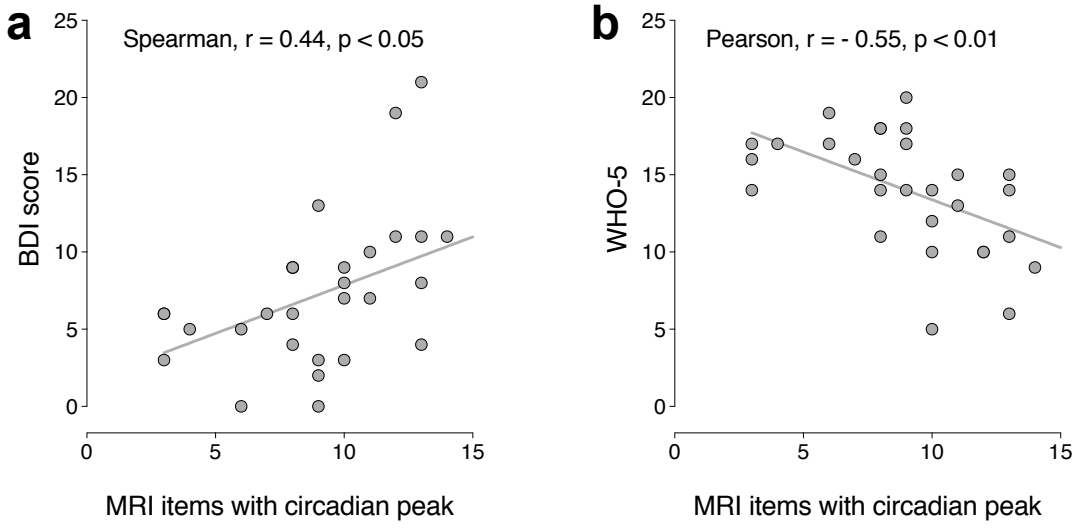

FIGURE 5 | Correlation between the number of mood symptoms with a circadian peak (MRI) and current depressive symptoms (BDI scores, a) and psychological well-being (WHO-5, b). BDI: Spearman r = 0.44, $p < 0.05$; WHO-5: Pearson r = -0.55, $p < 0.01$.

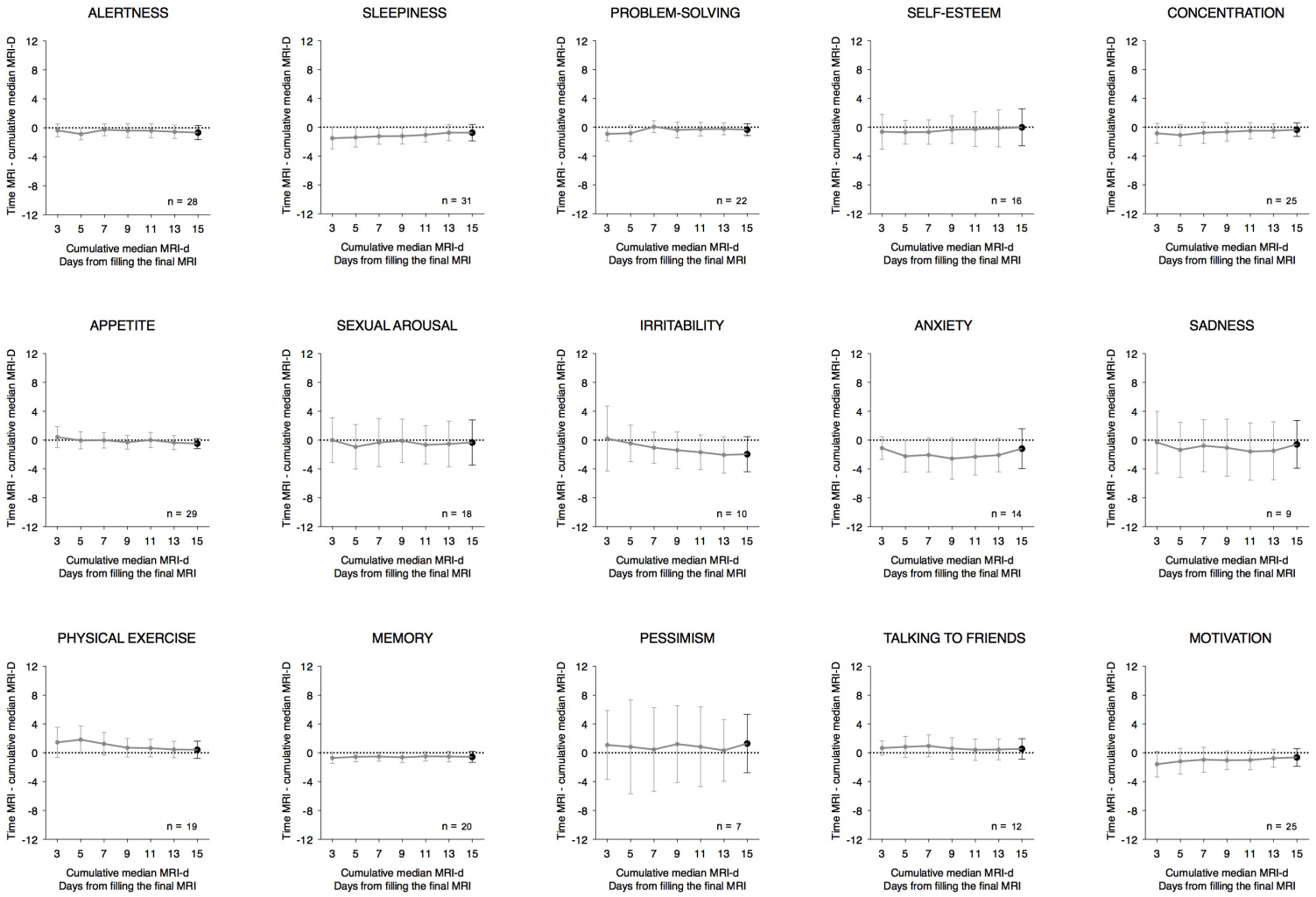

FIGURE 4 | Time differences between the Mood Rhythm Instrument (MRI) and the cumulative median of the last 3, 5, 7, 11, 13, and 15 days from the Mood Rhythm Instrument diary (MRI-d). Dots show group means and whiskers represent 95% confidence intervals. Time data on each item derived from MRI and MRI-d.

DISCUSSION

The main finding of this study is that the retrospectively-scored MRI displayed high agreement with the prospectively-recorded MRI-d, which suggests that the MRI is a valid tool to assess rhythmicity – daily patterns and timing – of mood disorders-related factors. In addition, with the exception of "irritability", none of the other MRI item scores were affected by memory bias. Regarding the dichotomous variables where participants answered whether or not they perceived MRI items to peak every 24h, we found fair to excellent agreement rates between the MRI-d and the MRI. Irritability was the only item where we observed potential memory bias and, not surprisingly, this item also had the lowest agreement rate between the MRI and MRI-d. As for the time variables, where participants reported the time when the mood-related factors typically peak, the average difference between the MRI and MRI-d was less than an hour for most items except for irritability, anxiety and pessimism. All correlations between MRI-d and MRI were ≥ 0.5, except for anxiety and pessimism. Together, these results suggest that the timing of affective/emotional symptoms such as irritability, anxiety and pessimism may be reported with somewhat less accuracy (fair-to-good agreement) as compared with cognitive/somatic mood symptoms (excellent agreement). These results are consistent with prior evidence showing that participants also tend to exaggerate the intensity of positive and negative mood on retrospectively administered scales, as compared to reports obtained daily (24). Overall, retrospective recall of past symptoms of mental illness is subject to recall bias, particularly in the long term, where underestimation of past symptoms and poor recall of previous mood episodes is very common (26). Here it is worth remembering that, contrary to most clinical questionnaires that assess the intensity/severity of symptoms over a period of time, the MRI assesses a different dimension of mood symptoms: if and what time the mood symptoms peak within the day. Our findings suggest that this type of information might be less subject to recall bias.

Interestingly, we found that higher number of mood symptoms perceived as having a daily pattern correlated with higher severity of current depressive symptoms and poorer psychological well-being. These results are consistent with previous evidence showing that depressed outpatients with diurnal mood variation present more severe symptoms and are more likely to meet criteria for melancholic depression (31). On the other hand, hospitalized depressed patients showed no association between severity of mood symptoms and diurnal mood variation (34). Although our study was conducted in the general population, our results are consistent with some of the above evidence in depressed populations suggesting that the perception of rhythmic patterns of mood symptoms is higher in individuals with more severe depressive symptoms. Notably, Wefelmeyer and Kuhs (1996) observed that the diurnal variation of mood in healthy individuals was almost exclusively attributed to external circumstances or their own activities, whereas in melancholic patients they occurred spontaneously in more than half cases. Haug and Wirz-Justice (1993) proposed that there might be an underlying circadian rhythm of mood masked by daily life events in healthy subjects (35).

The limitations of this study should be considered. We are aware that the MRI does not assess individuals' rhythmicity in a strict sense. Rather, the MRI assesses the subjective perception of recurrent daily peaks of the individual's mood symptoms. Importantly, the MRI is being developed to test its potential utility in clinical settings, such as in the investigation chronodisruption in psychiatric disorders (37), notwithstanding the limitations of self-reported questionnaires alike. Also, our sample size was relatively small, especially for some of the analyses of time variables. However, this was to be expected, because participants should only report a time variable for the items they perceive as having a peak. The fact that the sample size of the analyses of time variables will almost always be smaller should be borne in mind. Notwithstanding this, the sample size was large enough to provide an accurate estimate

of the agreement between the MRI and the MRI-d, which was the primary objective of the study. In addition, we were able to show that the MRI is not significantly subject to memory bias. Another limitation was that, due to our sampling method, our study was conducted on a relatively homogeneous sample with a high level of education, which may limit the external validity of our results. Further, we did not recruit a sample of individuals with diagnosed major depressive disorder and, therefore, we do not know if our results are also applicable to individuals with clinical depression. The fact that subjects filled out the MRI shortly after filling out the MRI-d for 15 days could have enhanced the recollection of the items assessed. However, participants were not aware that the aim of the study was to compare the agreement between these questionnaires, and had we asked the participants to fill out the MRI long after the prospective daily charting we would have lost the 15-day window.

In conclusion, we found a high agreement between the retrospectively-scored MRI and the prospectively-recorded MRI-d, indicating that the MRI is a valid tool to investigate the perceived 24h peak and timing of mood symptoms. We also found that the MRI was not influenced by recall bias. Future studies should test the usefulness of this new clinical instrument in individuals with mood disorders, as well as its ability to detect changes in the daily patterns/timing of mood symptoms before and after treatment.

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AUTHORS CONTRIBUTION STATEMENT

DS, MPH and BNF designed the study. LKP, AC, APF, MABO, RCF and MS collected and organized the data. LKP, AC, MABO, DS, MPH and BNF were involved in data analysis. LKP, AC, APF, MABO, AS, KE, BNF, and MPH wrote the first draft of the manuscript. All authors read, revised and approved the final manuscript.

CONFLICT OF INTEREST: The authors report no conflict of interest.

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CAPÍTULO 3:

Instrumento de Ritmos de Humor e risco para transtornos psiquiátricos

Artigo 2: em revisão no periódico *Scientific Reports*.

**As figuras, legendas e tabelas foram incorporadas ao texto para facilitar a avaliação da banca.

Rhythmicity of Mood Symptoms in Individuals at Risk for Psychiatric Disorders

Luísa K. Pilz[1,2], Alicia Carissimi[1,2], Melissa A. B. Oliveira[1,2], Ana Paula Francisco[1], Raul C. Fabris[1], Madeleine S. Medeiros[1,4], Marina Scop[1], Benicio N. Frey[3,+], Ana Adan[5,6,+], Maria Paz Hidalgo[1,2,+]

[1]Laboratório de Cronobiologia e Sono do Hospital de Clínicas de Porto Alegre (HCPA), Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, Rio Grande do Sul, Brasil;

[2]Programa de Pós-Graduação em Psiquiatria e Ciências do Comportamento – Faculdade de Medicina, UFRGS, Porto Alegre, Rio Grande do Sul, Brasil;

[3]Department of Psychiatry and Behavioural Neurosciences, McMaster University, Hamilton, ON, Canada; Mood Disorders Program and Women's Health Concerns Clinic, St. Joseph's Healthcare, Hamilton, ON, Canada;

[4]Hospital Materno Infantil Presidente Vargas, Porto Alegre, Rio Grande do Sul, Brasil;

[5]Department of Clinical Psychology and Psychobiology, School of Psychology, University of Barcelona, Barcelona, Spain;

[6]Institute of Neurosciences, University of Barcelona, Barcelona, Spain;

[+] contributed equally as senior authors

Abstract

Despite emerging evidence that disruption in circadian rhythms may contribute to the pathophysiology of psychiatric disorders, there is a significant knowledge gap on the rhythmicity of psychological symptoms. Here, we aimed at investigating the rhythmicity of mood symptoms in individuals at risk for psychiatric disorders. 391 Brazilian and 317 Spanish participants completed the Self-Reporting Questionnaire-20 for non-psychotic mental disorders; the Mood Rhythm Instrument was used to assess rhythmicity of mood symptoms and the Munich ChronoType Questionnaire to assess sleep patterns. We found that the rhythmicity of specific mood-related symptoms and behaviors, particularly pessimism and motivation to exercise, were associated with being at risk for psychiatric disorders, even after controlling for sleep timing, sleep deficit, and season of data collection. We also found that the peak of some mood symptoms and behaviors were different between individuals at high vs. low risk for psychiatric disorders, with specific differences between countries. These results are consistent with previous research showing that circadian misalignment is associated with higher risk for mental health conditions. These findings also suggest that lifestyle changes preventing circadian misalignment might be useful to reduce the risk of psychiatric disorders, where cultural differences must be taken into account.

Keywords: circadian rhythms, chronodisruption, chronobiology, mental health, psychiatric disorders**.**

Introduction

The large Global Burden of Disease study reported that mental health conditions are ranked amongst the leading causes of disability worldwide, with a prediction that major depression will be ranked the #1 cause of disability by 2025[1]. Consequently, there has been growing interest in the neurobiology of psychiatric disorders, which would help to identify individuals at risk, as well as improve treatment outcomes for those with established diagnoses[2,3]. Various neurobiological theories based on neurotransmitter systems, alterations in neuroendocrine and neuroimmune regulation, brain structure abnormalities, genetic and psychosocial factors, and circadian disruption have been proposed as etiological models of mental illnesses[4–6].

Research on circadian rhythms and sleep regulation has revealed that alterations in social rhythms, rest-activity and sleep-wake cycle are typically observed across common psychiatric disorders, such as depression, anxiety and psychotic disorders. In addition, several chronobiological therapeutics, such as bright light therapy, cognitive behavioral therapy for insomnia and interpersonal and social rhythm therapy, are effective in the management of mood disorders[7,8]. Despite cumulative evidence supporting the notion that circadian rhythms disruptions contribute to the pathophysiology of mood disorders, further research is warranted to better understand the underlying mechanisms and how the two are causally interconnected[6,9,10]. For instance, a significant knowledge gap is the lack of information on the rhythmicity of mood symptoms in individuals suffering from mental disorders. One of the potential reasons for this knowledge gap is that available clinical questionnaires do not take into account whether the frequency of mood symptoms follows a rhythmic pattern or the time of the day that the mood symptoms usually peak. To fill this gap, we have recently developed the Mood Rhythm Instrument (MRI), a self-reported questionnaire that evaluates the rhythmicity of mood symptoms. It includes somatic (sleep, appetite, sexual arousal), cognitive

(attention, problem-solving, alertness, concentration, memory, motivation to exercise), and affective (sadness, irritability, anxiety, self-esteem, irritability, pessimism, willingness to talk with friends in person) domains. The MRI has been validated in Brazil[11] and Spain[12,13] and is now being validated in Canada. The objective of this study was to compare the rhythmicity of mood symptoms between individuals at high vs. low risk for psychiatric disorders, as measured with the Self-Reporting Questionnaire (SRQ-20). We hypothesized that individuals at risk for psychiatric disorders would report less rhythmicity of mood symptoms.

Results

Sample Characteristics

Demographic characteristics of the study sample are described in Table 1. In total, 391 Brazilian and 317 Spanish participants completed the SRQ-20 questionnaires. There was a statistical difference between these populations in age, sex and season when the questionnaires were conducted; therefore, these variables were included as covariates in all statistical analyses.

The Brazilian sample reported significantly earlier midpoint of sleep on workdays (MSW), higher social jetlag, shorter sleep duration on workdays and work-free days, and greater sleep deficit compared to the Spanish sample. The proportion of SRQ positive (high risk for psychiatric disorder) was similar between countries.

In Brazil, the proportion of individuals who reported rhythmicity for alertness, self-esteem, anxiety, sadness, and general motivation was higher than in Spain, whereas the proportion of reported rhythmicity for sexual arousal, motivation to exercise, and talking to friends was higher in the Spanish sample (Table 1).

TABLE 1. Sample characteristics of Brazil and Spain

	Brazil (N = 391)	Spain (N = 317)	Test contrast
Sex (% female)	233 (60)	214 (68)	$\chi^2 = 4.97$, $p < 0.05$
Age, mean ± SD	21 ± 2.4	22 ± 2.5	$U = 46227$, $p < 0.001$
Season: spring/summer, n (%)	47 (12)	217 (69)	$\chi^2 = 238.43$, $p < 0.001$
SRQ-20: positive, n (%)	151 (39)	144 (45)	$\chi^2 = 3.34$, $p = 0.07$
MCTQ variables: median [IQR]			
MSW	03:19 [0:57]	04:07 [1:13]	$U = 30469$, $p < 0.001$
MSF	05:45 [2:12]	05:52 [1:42]	$U = 53435$, $p = 0.14$
Social jetlag	02:25 [1:45]	01:45 [1:18]	$U = 74008$, $p < 0.001$
SDw	06:30 [1:30]	07:25 [2:00]	$U = 34873$, $p < 0.001$
SDf	08:40 [1:40]	09:00 [1:55]	$U = 46561$, $p < 0.001$
Sleep deficit	02:00 [2:00]	01:30 [1:45]	$U = 67107$, $p < 0.001$
	Rhythmic (yes), n (%)		χ^2, p
MRI items			
Alertness	344 (88)	215 (69)	38.74, $p < 0.001$*
Sleepiness	374 (96)	305 (97)	0.65, $p = 0.42$
Problem solving	274 (70)	215 (68)	0.39, $p = 0.53$
Self-esteem	169 (43)	111 (35)	4.77, $p < 0.05$
Concentration	345 (88)	282 (89)	0.20, $p = 0.66$
Appetite	335 (86)	281 (89)	2.29, $p = 0.13$
Sexual arousal	166 (43)	165 (52)	6.43, $p < 0.05$
Irritability	243 (62)	201 (64)	0.17, $p = 0.68$
Anxiety	190 (49)	100 (32)	21.02, $p < 0.001$*
Sadness	162 (41)	95 (30)	9.74, $p < 0.01$
Motivation to exercise	274 (70)	250 (80)	8.31, $p < 0.01$
Memory	167 (43)	153 (48)	2.45, $p = 0.12$
Pessimism	135 (35)	98 (31)	1.02, $p = 0.31$
Talking to friends	156 (40)	181 (57)	21.98, $p < 0.001$*
General motivation	325 (83)	178 (57)	59.52, $p < 0.001$*

Chi-square (χ^2) or Mann-Whitney (U) was used o compare countries as appropriate. SRQ: Self-reporting questionnaire. MCTQ: Munich ChronoType Questionnaire. MSW: midpoint of sleep on workdays; MSF: midpoint of sleep on work-free days; SDw: sleep duration on workdays; SDf: sleep duration on work-free days; SDdiff: difference between sleep duration on workdays and work-free days. MRI: Mood Rhythm Instrument. *Significant after correction for multiple testing.

Rhythmicity of mood symptoms and risk for psychiatric disorders

In Brazil, among the individuals at risk for psychiatric disorders, the proportion of reported rhythmicity for the following symptoms was higher: ability to solve problems; irritability; anxiety; sadness; pessimism; and preferred time to talk to friends ($p < 0.05$; Table S1). The proportion of subjects reporting a peak for motivation to exercise was lower among individuals at risk for psychiatric disorders ($p < 0.05$). In Spain, among the individuals at risk for psychiatric disorders, the proportion of subjects who reported rhythmicity for the following symptoms was higher: anxiety; sadness; pessimism and general motivation ($p < 0.05$; Table S1).

Logistic regression models were run to ascertain the association between the MRI items and the likelihood of being SRQ positive or negative. In Brazil, the first logistic regression model showed sadness, pessimism and motivation to exercise were significant predictors of SRQ status ($R^2 = 0.30$, $p < 0.001$). After controlling for age, sex, mid-sleep on free days, sleep duration difference, and season of completion the MRI, rhythmicity for sadness and pessimism on the MRI remained independent predictors for being SRQ-positive in the Brazilian sample (Table 2). In addition, motivation to exercise was an independent predictor for being SRQ-negative. Other independent predictors of SRQ status were mid sleep on work-free days, sex and season of data collection (Table 2). This model explained 40% of the variance of the SRQ classification ($p < 0.001$). In Spain, the first logistic regression model indicated general motivation, pessimism and motivation to exercise as significant predictors of SRQ status ($R^2 = 0.17$, $p = 0.09$). Pessimism was an independent predictor for being SRQ-positive, whereas general motivation and motivation to exercise were independent predictors for being SRQ-negative in Spanish sample (Table 2). Sex was the only other predictor of SRQ status in this population (Table 2). However, the overall model was not significant ($R^2 = 0.18$, $p = 0.07$).

TABLE 2. Binary logistic regression of positive/negative SRQ for Brazil and Spain

Brazil (n = 373): Nagelkerke R^2 = .40, p < .001					
	B	S.E.	Wald	p	OR (95% CI)
Sadness (rhythmic)	1.31	0.29	20.71	< 0.001	3.71 (2.11 – 6.54)
Motivation to exercise (non-rhythmic)	0.93	0.28	10.81	< 0.01	2.53 (1.45 – 4.39)
Pessimism (rhythmic)	1.09	0.30	13.35	< 0.001	2.98 (1.66 – 5.34)
Mid-sleep on work-free days	0.22	0.09	5.42	< 0.05	1.24 (1.04 – 1.49)
Sleep deficit	0.10	0.08	1.58	0.21	1.11 (0.94 – 1.30)
Sex (female)	1.22	0.29	18.06	< 0.001	3.37 (1.93 – 5.91)
Age	-0.02	0.05	0.15	0.69	0.98 (0.88 – 1.09)
Season of data collection (winter, Mar-Sep)	-1.55	0.55	7.79	< 0.01	4.70 (1.59 – 13.92)
Constant	-4.99	1.39	12.92	<0.001	
Spain (n = 293): Nagelkerke R^2 = .18, p = .07					
	B	S.E.	Wald	p	OR (95% CI)
General motivation (rhythmic)	0.56	0.26	4.61	< 0.05	1.75 (1.05 – 2.92)
Motivation to exercise (non-rhythmic)	0.63	0.31	4.12	< 0.05	1.89 (1.02 – 3.48)
Pessimism (rhythmic)	1.13	0.28	16.45	< 0.001	3.11 (1.80 – 5.38)
Mid-sleep on work-free days	-0.02	0.10	0.06	0.80	0.98 (0.81 – 1.18)
Sleep deficit	-0.02	0.09	0.08	0.78	0.97 (0.81 – 1.17)
Sex (female)	0.87	0.30	8.61	< 0.01	2.39 (1.34 – 4.28)
Age	0.09	0.05	3.10	0.08	1.10 (0.99 – 1.22)
Season of data collection (winter, Sep-Mar)	-0.09	0.28	0.09	0.76	0.92 (0.53 – 1.60)
Constant	-3.51	1.43	6.05	0.01	

Peak time of the MRI items and risk for psychiatric disorders

Figure 1 shows the mode of the peak of each MRI item according to country and SRQ group (SRQ positive or negative). In both countries, the distribution of the peak of appetite was significantly different (Table S2). Circular distributions of the other MRI items were not different between SRQ groups; items where Mardia-Watson-Wheeler p < 0.30 are shown in Figure 2. The proportion of subjects whose sleepiness peak is in the morning (5:00 – 12:00) was higher among the individuals at risk (*SRQ negative:* 28% / *SRQ positive:* 39%, $\chi^2 = 4.42$, p < 0.05). The phase angle difference between the peak of motivation to exercise and appetite was significantly higher in the SRQ negative group (*SRQ negative*: 2.5 [8.0] / *SRQ positive:* -1.0 [8.6], U = 3782.5, p < 0.01). The proportion of individuals who reported an earlier peak of appetite compared to the peak of motivation to exercise was higher among the SRQ positive group (SRQ *negative:* 39% / *SRQ positive:* 54%, $\chi^2 = 4.45$, p <0.05).

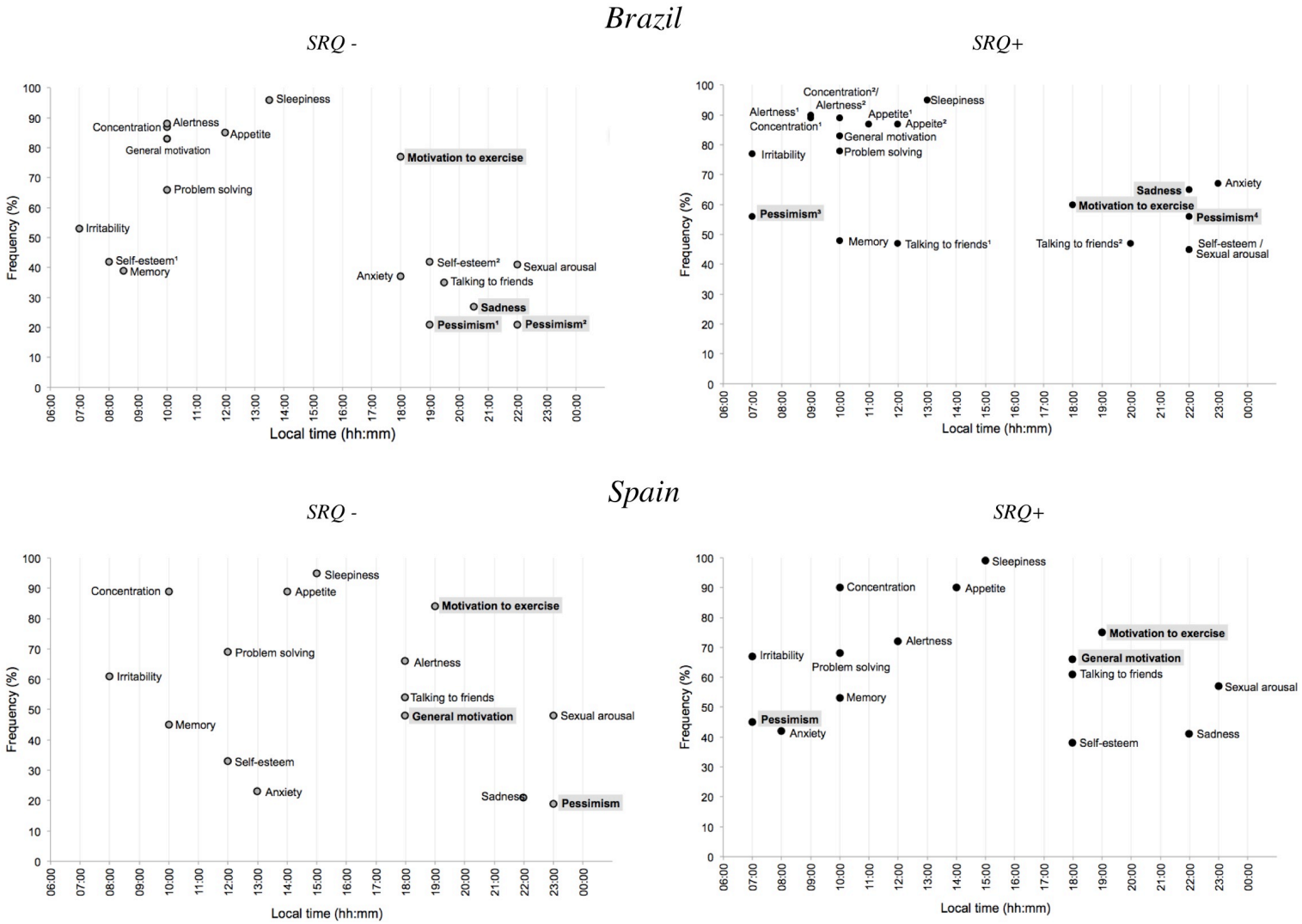

Figure 1. Frequency and peak time of each MRI item mode in Brazil and Spain according to SRQ status for psychiatric disorders (positive or negative). Time of day (h) is depicted on the x-axis and frequency (%) is depicted on the y-axis. Grey squares represent significant predictors of SRQ status in the binary logistic regression. [1,2] or [3,4]:Bimodal variables are represented twice.

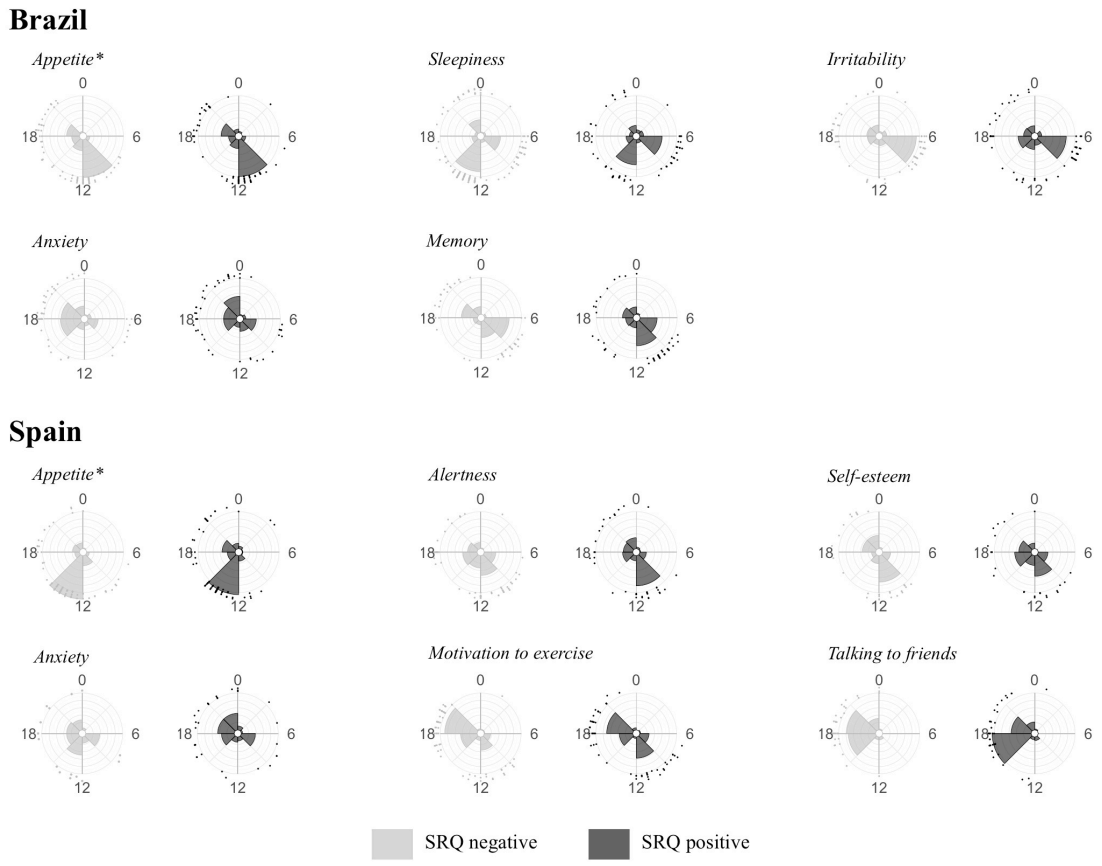

*Figure 2. Differences between Self-reporting questionnaire (SRQ) negative and SRQ positive groups in circular distributions of the Mood Rhythm Instrument (MRI) items timing in Brazil and Spain. MRI items timing distributions are shown when they differ between SRQ negative and positive at a significance level of α = 0.30 (Mardia-Watson-Wheeler test for equal distributions). Circles represent the 24h day. Dots along the outermost circumference represent individuals timing. Grey bars stand for the SRQ negative and black bars stand for the SRQ positive distribution. Each concentric circumference represents 10% of the sample. *Appetite was significantly different between SRQ status groups at the significance level of α = 0.05 in both countries (Mardia-Watson-Wheeler test for equal distributions).*

Discussion

The main finding of this study was that the rhythmicity of specific mood symptoms and behaviors were strongly associated with risk for psychiatric disorders in a large sample of young adults. The perception that pessimism tends to peak at a specific time of the day was associated with high risk for psychiatric disorders, whereas the perception that the motivation to exercise tend to peak at a specific time of the day was associated with low risk for psychiatric disorders. Notably, the rhythmicity of pessimism and motivation to exercise were

strongly associated with risk for psychiatric disorders in both Brazilian and Spanish samples. Considering that many psychiatric disorders are associated with pessimistic thoughts[14], it is noteworthy that a large proportion of individuals at risk identified that they tend to feel pessimistic at a specific time of the day. Previous studies have reported higher rates of pessimism in individuals of an evening type, being sadness and pessimism among the variables that better discriminated evening from intermediate and morning types[15,16]. Thus, it could be argued that our result may be a reflection of late-types being at higher risk, since studies show higher prevalence of depression symptoms among these types[16,17]. However, the regression models indicated that the relationship between the perception of a pessimism peak and risk for psychiatric disorders was independent of sleep timing and deficit, suggesting that chronotype was not a confounder. We are unaware of any other studies that examined the relationship between the rhythmicity of mood symptoms and mental health outcomes.

The finding that individuals reporting a peak time of motivation to exercise were more likely to be screened as low risk for psychiatric disorders is consistent with previous studies showing that there is a bidirectional relationship between the circadian system and the motivation to exercise, both of which being disrupted in individuals with psychiatric disorders[18]. Exercise is an important nonphotic cue that can phase-shift circadian rhythms[19,20] and can help re-entraining sleep-wake cycles[21]. Notably, these circadian effects are proposed mechanisms by which exercise might improve mood[22,23]. Comparing the Brazilian and Spanish samples, we found that sadness and general motivation were differentially associated with risk for psychiatric disorders. In the Brazilian sample, the frequency of rhythmicity of sadness was higher among individuals at risk for psychiatric disorders. We are unaware of previous studies that assessed the specific time of the day or the rhythmicity with which sadness may occur. It is worth noting that in our sample sadness peaked later in the day for the majority of participants. This suggests that the rhythmicity of sadness reported in this

sample of young adults is different from the concept of *melancholia*, defined as "feeling worse in the morning". Finally, feeling motivated at a specific time of the day was associated with low risk for psychiatric disorders in the Spanish sample. It has been shown that motivational behaviors have at least two components: the goal-directed or directional and the arousal or activational component[18]. Evolutionary speaking, motivational behaviors that are critical for survival such as sleeping, eating and mating cannot occur at the same time[24,25]. Thus, the circadian regulation promotes optimal timing for the various physiological needs.

We also found that the distribution of the peaks (timing) of certain mood symptoms and behaviors may differ when comparing individuals at high risk vs. low risk for psychiatric disorders. Peak of appetite, in particular, was significantly different between the two countries. Furthermore, in Brazil, the proportion of subjects whose sleepiness peak occurred in the morning was higher among individuals at risk for psychiatric disorders, which probably reflects higher rates of chronodisruption in this population. Although circular data analyses did not detect the distribution of other variables to be associated with risk for psychiatric disorders, the time relationship between items might be altered in individuals at higher risk. In Spain, the difference between the peak of appetite and motivation to exercise was significantly lower in individuals at risk, with a higher proportion of individuals at risk reporting that the peak of appetite happened earlier than the peak of motivation to exercise. This may also be a reflection of chronodisruption, since these physiological variables might have specific phase relationships[26,27], which our results show to differ between individuals at risk or not. These findings suggest that individuals at higher risk for psychiatric disorders suffer from chronodisruption characterized by a desynchrony of biological rhythms[28]. The circadian clock is a temporal system responsible for conferring circa-24 h rhythms to physiological systems, from molecular to the behavioral level[27]. In that sense, the circadian clock orchestrates bodily functions so that they peak at optimum times during the day.

Importantly, our internal rhythms need to synchronize and align with the external environment (e.g., light-dark cycle, work/school schedule, etc.), a process known as entrainment. Chronodisruption occurs when sleep/wake rhythms imposed by social schedules, such as work/school demands, result in misalignment of physiological and psychological functions. Notably, chronodisruption has been associated with a range of health issues including psychiatric disorders[5,28].

When comparing sleep patterns across cultures, Brazilians displayed more sleep deficit, higher social jetlag, and shorter sleep duration than Spaniards. It is striking that the percentage of participants reporting less than 6 hours of sleep on workdays was 32% and 13% in Brazil and Spain, respectively. These rates are comparable to a large (n = 124,517) populational study from the US, where 15% reported sleeping less than 6 hours[29], a known risk factor for psychiatric disorders and increased mortality[30,31]. These cultural differences may be related to how work schedules are organized since an earlier midpoint of sleep on workdays but not on free days was observed in Brazil. In Spain, most of the work activities start later than in Brazil (8/9 AM). Therefore, students and workers could delay their sleep phase without interfering with their labor activities. Considering how social routines can influence sleep/wake rhythms and may cause circadian misalignment, we hypothesize that cultural differences are essential modulators of the association between chronodisruption and risk for psychiatric disorders.

This research is a population-based cross-sectional study and, therefore, the direction of causality between MRI items and SRQ status cannot be determined. Another limitation is that we did not use a diagnostic tool for mental disorders. However, the SRQ-20 is a widely used screening questionnaire for psychiatric disorders that was tested and validated across several cultures, including Brazil and Spain[32–35]. Also, our study was mainly comprised of a homogeneous sample of young adults attending university. Thus, we cannot extrapolate our

results to other populations. Despite these limitations, our relatively large and homogeneous sample allowed us to assess the association between the rhythmicity of mood symptoms and risk of psychiatric disorders, a significant knowledge gap in the interface between chronobiology and mental health research.

In conclusion, our main finding showed that the rhythmicity of specific mood-related symptoms and behaviors, particularly pessimism and motivation to exercise, were strongly associated with risk for psychiatric disorders both in Brazilian and Spanish young adults. We also found that the differences in the peak of mood symptoms and behaviors between individuals at high vs. low risk for psychiatric disorders are consistent with previous studies supporting that circadian misalignment is associated with a wide range of mental health conditions. These findings also suggest that life style changes preventing circadian misalignment might help reducing the risk of psychiatric disorders, where cultural differences must be taken into account. Future studies should try to examine how MRI may be related to objective measures of circadian rhythms, such as actimetry, cortisol or melatonin levels. Also, future studies using the MRI questionnaire in individuals with psychiatric illnesses, such as major depression and bipolar disorder, will be useful to investigate the applicability of this new clinical questionnaire to enhance the understanding of the role of chronodisruption in individuals with mood disorders.

Methods

Participants

In this study, 391 Brazilian and 317 Spanish participants between 18 and 29 years old were recruited. In Brazil, a sample of university students was collected. In Spain, data were collected through snowball sampling[36]. All participants gave their informed consent. The study was approved by the Ethics Committee of Hospital de Clínicas de Porto Alegre (#15-0539 GPPG/HCPA) and was conducted in accordance with the Declaration of Helsinki.

Procedures

Mood Rhythm Instrument (MRI)

The MRI is a 15-item self-reported questionnaire developed by de Souza et al. (2016) to detect a rhythmicity of mood-related domains (affective, cognitive, somatic, physical). This scale is composed of two questions to characterize each item; the first question asks whether or not individual symptoms or behaviors had a rhythmic pattern in the last 15 days (participants report dichotomously if they had a peak for that variable - yes/no). In the second question, participants mark the exact hour in which the peak of the symptoms occurs (unimodal temporal response). The validated Portuguese[11] and Spanish[13] versions of the MRI were used in this study. The questionnaire demonstrated a satisfactory internal consistency (Cronbach's alpha of 0.70 and 0.73 for Brazil and Spain, respectively[11,12]).

Munich ChronoType Questionnaire (MCTQ)

The Munich Chronotype Questionnaire (MCTQ) assesses, separately for workdays and work-free days, the time that people go to bed and the time they are ready to go to sleep, how long it takes for them to fall asleep, at what time they wake up and get up, and if they use an alarm clock[37,38]. A number of variables can be derived from the MCTQ including: the midpoint between sleep onset time and wake up time on workdays (midpoint of sleep on workdays, MSW) and on work-free days (midpoint of sleep on work-free days, MSF); social jetlag, defined as the discrepancy between the biological and social clocks calculated by the difference between MSF and MSW (social jetlag, SJL[39]); and the sleep deficit, which is calculated by the difference between sleep duration on workdays (SDw) and sleep duration on work-free days (SDf).

Self-Reporting Questionnaire (SRQ-20)

SRQ-20 is a self-reported instrument that consists of 20 items with a yes/no answer

format. It was developed by Harding and colleagues (1980) to screen for non-psychotic psychiatric disorders and was widely used in several countries and different cultures[32,33]. In this study, we used the validated Brazilian Portuguese and Spanish versions and their corresponding validated screening cut-offs to detect psychiatric disorders: scores higher than 7 were considered SRQ positive in Brazil, while scores higher than 3 were considered SRQ positive in Spain. The Spanish and Portuguese versions of the SRQ-20 showed fair to excellent sensitivity (70% / 86%) and specificity (70% / 89%)[34,35].

Data Analysis

Chi-square tests were used to verify the relationship between dichotomous variables (i.e., SRQ positive/negative and MRI rhythmic/not rhythmic). The items that showed $p < 0.20$ in the chi-square were used in a binary logistic regression. The significant predictors in the model were tested controlling for sex, age, mid-sleep on free days and the difference of sleep duration between workdays and work-free days, and season of data collection (spring/summer or autumn/winter).

Taking into account that the variable "peak of the MRI item" is only scored when the item is considered "rhythmic", the distribution of MRI items peaks were compared between SRQ-positive and SRQ-negative groups using the Mardia-Watson-Wheeler test. Since data are not uniform or follow a Von Mises distribution (equivalent to the normal distribution for circular data), we chose this test to examine whether the variables were distributed differently between groups[40]. Distributions of items that were different at a significance level of $\alpha = 0.30$ were plotted in circular graphs.

Considering the well-established differences in circadian rhythms across cultures[41,42], all analyses were performed separately in the Brazilian and Spanish samples. Statistical significance was set at $p < 0.05$. Data analyses of linear variables were performed using SPSS

24, whereas for the analyses of circular data we used NCSS 12. Circular graphs were plotted using the R package ggplot2[43].

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Authors Contributions Statement

AC, MABO, APF, MSM, BNF, AA and MPH designed the study. AA, MABO, APF, MS, RCF collected and organized the data. LKP, AC, MABO and MPH analyzed the data. LKP, AC, MABO, APF, BNF, AA and MPH wrote the first draft of the manuscript. All authors read, revised and approved the final manuscript.

Additional information

Competing interests

The authors report no competing interests. The authors alone are responsible for the content and writing of the paper.

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TABLE S1 – Descriptive and test statistics of the proportion of rhythmic variables across SRQ + and - groups

MRI - 15 items	Brazil					Spain				
	Rhythmic - count (%)		Chi-square			Rhythmic - count (%)		Chi-square		
	SRQ -	SRQ +	χ^2 (1)	p	n	SRQ -	SRQ +	χ^2 (1)	p	n
Alertness	208 (87)	136 (90)	1.01	0.31	391	112 (66)	103 (72)	1.60	0.21	312
Sleepiness	230 (96)	144 (95)	0.05	0.82	391	164 (95)	141 (99)	2.69	0.10^{+}	315
Problem solving	157 (66)	117 (72)	6.71	< 0.05*	389	118 (69)	97 (68)	0.02	0.88	315
Self-esteem	101 (42)	68 (45)	0.29	0.60	390	57 (33)	54 (38)	0.73	0.39	315
Concentration	211 (88)	134 (89)	0.02	0.89	390	153 (89)	129 (90)	0.13	0.72	315
Appetite	203 (85)	132 (87)	0.61	0.44	391	152 (89)	129 (90)	0.14	0.70	314
Sexual arousal	99 (41)	67 (45)	0.60	0.44	388	83 (48)	82 (57)	2.21	0.14^{+}	315
Irritability	127 (53)	116 (77)	23.43	< 0.001*	390	105 (61)	96 (67)	1.25	0.26	315
Anxiety	90 (37)	100 (67)	31.43	< 0.001*	390	39 (23)	61 (42)	14.04	< 0.001*	316
Sadness	64 (27)	98 (65)	56.83	< 0.001*	390	36 (21)	59 (41)	15.32	< 0.001*	315
Motivation to exercise	185 (77)	89 (60)	14.55	< 0.001*	391	143 (84)	107 (75)	3.72	0.05^{+}	314
Memory	94 (39)	73 (48)	3.07	0.08^{+}	390	77 (45)	76 (53)	2.39	0.12^{+}	314
Pessimism	51 (21)	84 (56)	50.05	< 0.001*	389	33 (19)	65 (45)	25.14	< 0.001*	315
Talking to friends	85 (35)	71 (47)	5.20	0.02*	391	93 (54)	88 (61)	1.63	0.20	314
General motivation	200 (83)	125 (83)	0.02	0.89	391	83 (48)	95 (66)	10.16	< 0.01*	314

* $p < .05$, $^{+}$ $p < .20$ (items tested in the binary logistic regression)

TABLE S2. Mardia-Watson-Wheeler test for comparing SRQ - vs. SRQ + in Brazil and Spain

	Brazil					
	SRQ -		SRQ +		Mardia-Watson-Wheeler	
	Mode	n	Mode	n	Test stat	p
Alertness	10:00	198	09:00 / 10:00	134	0.10	0.95
Sleepiness	13:30	213	13:00	136	2.55	0.28
Problem solving	10:00	151	10:00	115	0.89	0.64
Self-esteem	08:00 / 19:00	96	22:00	67	0.22	0.90
Concentration	10:00	201	09:00 / 10:00	130	1.12	0.57
Appetite	12:00	190	11:00 / 12:00	127	**18.49**	**< 0.01**
Sexual arousal	22:00	92	22:00	64	1.18	0.55
Irritability	07:00	127	07:00	108	2.70	0.26
Anxiety	18:00	87	23:00	93	4.88	0.09
Sadness	20:30	63	22:00	93	0.29	0.87
Motivation to exercise	18:00	177	18:00	88	1.36	0.51
Memory	08:30	87	10:00	70	3.85	0.15
Pessimism	19:00 / 22:00	50	07:00 / 22:00	79	1.08	0.58
Talking to friends	19:30	82	12:00 / 20:00	69	1.55	0.46
General motivation	10:00	190	10:00	122	0.80	0.67
	Spain					
	SRQ -		SRQ +		Mardia-Watson-Wheeler	
	Mode	n	Mode	n	Test stat	p
Alertness	18:00	109	12:00	103	2.38	0.30
Sleepiness	15:00	156	15:00	137	1.38	0.50
Problem solving	12:00	116	10:00	95	1.86	0.39
Self-esteem	12:00	56	18:00	54	3.92	0.14
Concentration	10:00	144	10:00	129	1.78	0.41
Appetite	14:00	141	14:00	125	**8.33**	**< 0.05**
Sexual arousal	23:00	73	23:00	80	0.78	0.68
Irritability	08:00	103	07:00	94	0.34	0.84
Anxiety	13:00	37	08:00	58	3.68	0.16
Sadness	22:00	34	22:00	58	0.31	0.86
Motivation to exercise	19:00	130	19:00	105	3.48	0.18
Memory	10:00	73	10:00	76	0.65	0.72
Pessimism	23:00	33	07:00	64	0.49	0.78
Talking to friends	18:00	84	18:00	85	4.54	0.10
General motivation	18:00	83	18:00	94	0.56	0.76

CAPÍTULO 4:

Sono e exposição à luz em comunidades quilombolas

Artigo 3: em revisão para publicação na Scientific Reports em 2018.

**As figuras, legendas e tabelas foram incorporadas ao texto para facilitar a avaliação da banca.

Sleep and light exposure across different levels of urbanisation in Brazilian communities

Luísa K. Pilz[1,2], Rosa Levandovski[3,4], Melissa A. B. Oliveira[1,2],
Maria Paz Hidalgo[1,2,+], Till Roenneberg[5,6,+]

[1] Laboratório de Cronobiologia e Sono, HCPA/UFRGS. Porto Alegre, RS - Brazil.

[2] PPG em Psiquiatria e Ciências do Comportamento, UFRGS. Porto Alegre, RS - Brazil.

[3] PPG Avaliação e Produção de Tecnologias para o SUS, GHC. Porto Alegre, RS – Brazil.

[4] PPG Saúde Coletiva, UFRGS. Porto Alegre, RS – Brazil.

[5] Institute of Medical Psychology, LMU. Munich, BY – Germany.

[6] Visiting Professor at UFRGS/CAPES. Porto Alegre, RS – Brazil.

[+] These authors contributed equally to this work.

Abstract

Quilombos are settlements originally founded by Africans and African descendants (Quilombolas) in remote parts of Brazil to escape slavery. Due to individual histories, Quilombos nowadays exhibit different states of industrialisation, making them ideal for studying the influence of electrification on daily behaviour. In a comparative approach, we aimed to understand whether and how human sleep changes with the introduction of artificial light. We investigated daily rest-activity-rhythms and sleep-patterns in the Quilombolas' by both wrist actimetry and the Munich ChronoType Questionnaire (MCTQ; the results of these two instruments correlated highly). Seven communities (*MCTQ:* N = 213 / *actimetry:* N = 125) were compared in this study. Light exposure, phase of activity, sleep timing and duration differ across communities with various levels of urbanisation and histories of access to electricity. People living without electricity and those, who acquired it only very recently on average sleep earlier than those in more urbanised communities (mid-sleep about 1 hour earlier); sleep duration tends to be longer. Our results and those of others show that use of electricity and modern lifestyles have changed sleep behaviour. To understand the consequences of these changes for health, further studies are warranted.

Keywords: electricity, circadian rhythms, chronobiology, rest-activity, actimetry, Quilombos, urbanisation.

Introduction

The strategies that help organisms to cope with cyclic environments (daily or seasonal) include anticipating their regular changes. Practically all organisms have therefore developed biological clocks. These temporal programmes need to run in synchrony with their cyclic environment. They secure this by actively entraining to light signals, which are the source of all rhythmic changes (temperature, resources, predators, etc.). Light and darkness are therefore the predominant entraining agent (so-called zeitgeber) that biological clocks use for entrainment[1–3]. The entrainment process results in a stable phase relationships between the internal, circadian time and the external light-dark-cycle time. This 'phase of entrainment' is reflected in all aspects of physiology and behaviour (e.g., body temperature, metabolism, activity/rest, wake/sleep)[4]. It depends both on how an individual's clock responds to a zeitgeber and on how strong the zeitgeber is. In industrialised societies, the majority of people spend most of their time indoors, thereby being exposed to relatively dim light during the day and lack of darkness after sunset (due to the use of artificial light). As a result of this drastically reduced zeitgeber strength, the circadian clocks of most people delay[5], while work schedules remain similar. People therefore routinely use alarm clocks on workdays and thereby accumulate a sleep debt, which they compensate for on weekends. This weekly structure of alternating short-early and long-late sleep is called social jetlag[6] and is calculated as the difference between the respective mid-sleeps on work-free and workdays. Social jetlag is associated with several health issues, including depressed mood, obesity, and cardio-metabolic risk[7–10].

While recent studies have shown that electric lighting can influence sleep timing, its impact on sleep duration is controversial[11–16]. De la Iglesia et al. showed that indigenous communities with access to electricity sleep later and shorter when compared to communities without artificial light in the Argentinean Gran Chaco[12]. When Amazon rubber tappers have access to

electricity, their sleep duration was also shorter and their sleep onset and dim-light melatonin onset delayed[13]. On the other hand, Yetish et al. suggest that "modern humans" do not sleep shorter than pre-industrial societies[14]. However, this study investigated samples of hunter-gatherers that had no electricity, without comparisons to controls (e.g., similar lifestyle but access to electricity). A more recent study compared a Mozambican village with no access to electricity to a neighboring town and found that individuals in the former slept earlier but not longer[16], whereas a small-scale agricultural society in Madagascar was reported to sleep shorter than industrial societies[17].

Here, we report results from studying light and sleep in Quilombola communities. Originally, Africans and African descendants founded these settlements (called Quilombos) in remote areas of Brazil to escape slavery and hide from recapture. Presently, Quilombos exist in divers geographical areas of Brazil[18]. Quilombos are especially apt for studying changes in sleep across industrialisation because they exist in all states, from rural (exposed to sunlight during the day and to actual darkness at night) to urban (predominantly working indoors with access to artificial light). The communities compared here are situated in rural areas in the south of Brazil and rely predominantly on natural light (outdoor work), contrasting the industrialised urban 24/7 society. These communities have different histories in their exposure to electricity, and therefore represent a unique population for studying how light affects behaviour without potential confounders of urban lifestyles.

Methods

Participants

Quilombolas participants (all Portuguese native speakers, *MCTQ:* N = 213 / *actimetry:* N = 125) were older than 16 living in the South of Brazil distributed among seven Quilombos in nine cities and four states (Fig 1). Data were collected between March 2012 and March 2017.

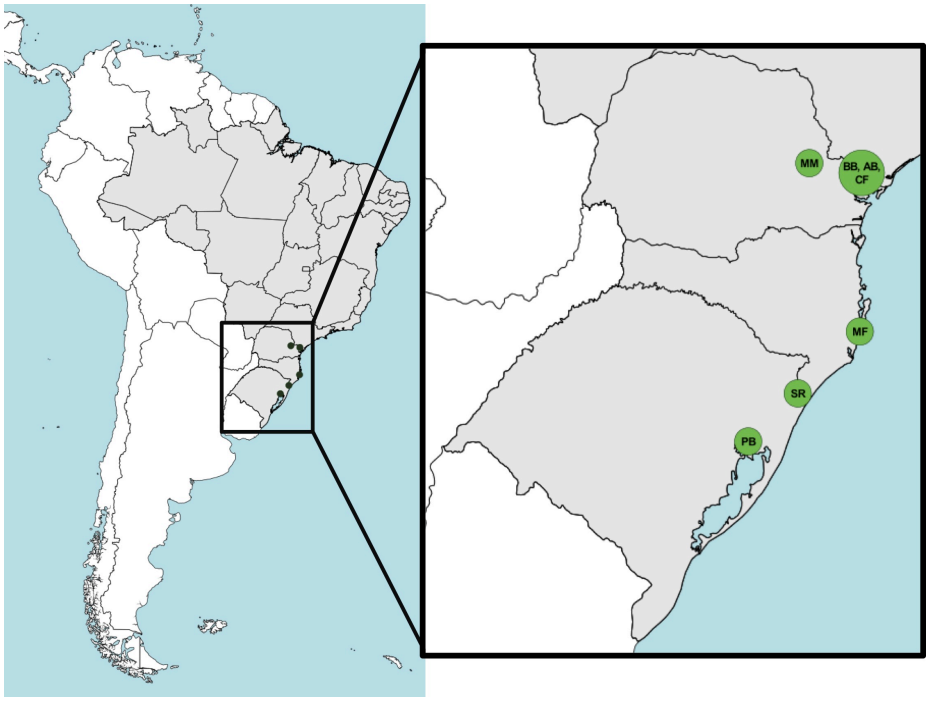

Figure 1. ***Studied Quilombos in the South of Brazil***. *Peixoto dos Botinhas (PB – Viamão, RS), São Roque (SR – Praia Grande, SC), Morro do Fortunato (MF – Garopaba, SC), Mamãs (MM – Castro, PR), Bombas (BB – Iporanga, SP), Areia Branca (AB – Bocaiúva do Sul, PR), Córrego do Franco (CF – Adrianópolis, PR). Maps created using Mapbox TileMill (v. 0.10.1).*

The study was approved by the Ethics Committee of Hospital de Clínicas de Porto Alegre (#11-0502, #15-0568) and was conducted in accordance with the Declaration of Helsinki. Participants gave written informed consent. When the participant was illiterate, an informed consent witnessed by another Quilombola was obtained. When the participant was younger than 18 years old, the parents also gave their consent.

The following paragraphs describe the communities and the areas where they are spread over.

Vale do Ribeira (PR/SP)

Vale do Ribeira comprises many traditional and local communities, including three of the seven Quilombos included in this study: Bombas, Areia Branca, and Córrego Franco. It is an area in the south of the state of São Paulo and north-east of the state of Paraná, currently with an estimated 411,500 inhabitants. It contains 21% of the remaining Atlantic forest with

protected areas; it is home for a large number and exceptionally varied endemic species, but is economically poor. The climate is humid subtropical. Evidence suggests that it was populated by native south Americans long before Europeans occupied it in the 16th and 17th century, predominantly using African labour to mine for gold. The first city was founded in 1567 (Iporanga). After the mines were abandoned the region became agricultural. In the transition, many former slaves claimed lands and developed an agriculture focused on the food market (for both local consumption and trade with other regions). Rice cultivation is an example: its cycle started in the end of the 17th century, and it was intensely commercialized to other provinces of the Empire of Brazil until mid 19th century. Many former slaves settled into the woods, became small farmers and gave rise to communities in the area19.

1. Bombas (BB – Iporanga, SP)

Bombas area is located in a natural reserve in the heart of the Atlantic forest, near to the small town Iporanga in the south of the state of São Paulo. The area was first occupied in the 19th century; the historical reference is of slaves who fled and families that were forced to leave their lands by a lead mining company. Iporanga's history is marked by gold mining and rice cultivation. Bombas is situated in a conservation area, which prevented the construction of roads until today and thus, the communities remained isolated, accessible only on a trail over hills covered with woods. The community is divided in two main parts: Bombas de Baixo (≈5 km from the main road) and Bombas de Cima (≈10 km from the main road) neither of which have yet been connected to the electricity grid. It takes 2-3 hours to reach the first houses of Bombas de Baixo on foot. This Quilombo is not a closed village, but houses are dispersed over an area of approximately 32 km2. Nevertheless, they have a strong sense of community. Quilombolas report to visit the city on average once to twice a month and some of them spend the night there. They practice an itinerant form of agriculture known as 'coivara' (i.e., opening a clearing in the forest during the dry season by cutting down trees and hoeing and

burning it to enrich nutrients before planting). This coivara is often used in forest areas and presents an essentially subsistence character, even though surpluses might be sold (mainly to buy clothes, construction materials or salt in the city). Besides subsistence agriculture and animal breeding, the community survives from social donations18. Bombas Quilombolas lead a pre-industrial life. Work activities are organised according to weather. Their definition of workdays vs. free days does not necessarily reflect the one of industrialised societies. Before retreating to bed, they use oil/gas lamps and sit close to clay ovens to eat and talk. Their houses are made of mud and/or wood and people sleep indoors on beds with mattresses. Light at night is restricted to oil/gas lamps used mainly while dinner is being prepared while the rest of the house remains in darkness. Flashlights are used but mainly to move from one room to another and for going outdoors. Data were collected in 2016 - 2017.

2. Areia Branca (AB – Bocaiúva do Sul, PR)

Areia Branca is part of the municipality of Bocaiúva do Sul, in the state of Paraná, about 115 km from this large city, and 50 km from the small town Barra do Turvo. According to the collective memory of the community's members, a recaptured slave established the settlement when given some land, which can now be accessed on dirt roads. The community installed a photovoltaic system in 2014 (our data were collected between August – December 2016), which provided electricity when weather permitted. Some members of the community have already lived in the city. The community lives mostly from agriculture. Recently, with support from the government, they also got a community kitchen abling the production of jams and bananas/manioc chips to be sold in nearby towns20,21. Their houses are made wood and/or concrete. Although they have bulbs indoors to light their night, individuals reported they still run out of electricity after extend periods of overcast days. Individuals usually have televisions at home, only a few have cell phones, internet signal was not available when data were collected, in 2016.

3. Córrego do Franco (CF – Adrianópolis, PR)

Córrego do Franco is part of the municipality of Adrianópolis, in Paraná, about 124 km from the next large city and 10 km from the nearest small town, Barra do Turvo. The community has lived in the region for about 250 years. According to the official community's anthropologic Report, the inhabitants' life is tightly connected to subsistence agriculture, with a daily work routine following climate rhythms. However, they often work for a salary in the plantations of large landowners or in the city to supplement their income20,22. Members of the community report to have had electricity for around 20 years (before our still ongoing data collection was started in 2012). People live in wood/concrete houses (many of which permanently under construction), and people's houses are relatively close to each other when compared to the two communities described above. They have light bulbs, televisions and some participants already had access to internet when data were collected, in 2016.

Praia Grande (SC)

The Quilombola community São Roque, from this municipality was included in this study. Praia Grande is a municipality in the south of Santa Catarina (≈7,364 inhabitants23. Despite meaning "large beach", the town is landlocked and is near to the canyons. The climate is temperate with a considerable forest reserve. It is predominantly an agricultural economy but tourism is a growing business.

4. São Roque (SR – Praia Grande, SC)

This Quilombolas in Praia Grande pursue agriculture and its headquarters is located about 20 km from the city centre. Slaves were usually forced to do domestic and manual works. When sent to do field work in or to deliver goods to other locations, some took to refuge when travelling through remote regions between the valleys and caves that line the hillsides and formed supporting networks in the 19th century. Agriculture has been the main mean of survival, and currently, maize, beans, bananas and manioc are among the main harvests. The

other main sources of income are working in domestic and agricultural jobs, and retirement24–27. São Roque is located in a protected area (national park), and is also engaged in touristic services. The community reports to have had electricity for about 15 years, and except for one individual, they had light bulbs at home. Most houses are made of either wood or concrete (the younger generations seek to build their houses nearer to the city, using concrete). Data were collected in 2013 - 2014.

Castro (PR)

Castro comprises four Quilombos, of which one, Mamãs, was included in this study. Castro is the third largest municipality in terms of area in the state of Paraná (estimated 71,501 inhabitants23), founded in the 18th century on the trail connecting Viamão in Rio Grande do Sul with Sorocaba in São Paulo. The climate is subtropical, and the economy is based on agriculture and dairy farming developed by Dutch colonies28.

5. Mamãs (MM – Castro, PR)

Mamãs is located about 60 km from the city centre of Castro. The community is divided in many family centres living up to 70 km distant from each other, and some of the families are located in the neighbour municipality of Cerro Azul. The history of this dispersed community goes back to a farm that was owned by Carmelite Fathers in 1749. The farm was abandoned and taken care for about a hundred years by the African descendants who were previously forced to work there. After about a century of freedom, in 1864, these descendants did not agree when the priests negotiated the farm and they were sold to a company: the new landowners wanted to take them to São Paulo as slaves. After being defeated by the military force in an uprising, the ones who could, fled to different areas forming the communities Serra do Apon e Mamãs20. The Quilombolas in Castro live from agriculture and, as in Córrego do Franco, they often work in the plantations of large landowners or in the city to complement their income. People from Mamãs reported they have had electricity for around

20 years, though some got it later, about 8 years ago. Most houses are made of wood. Mobile signal was poor to inexistent when data were collected and Internet signal was also unavailable. The majority but not all people had televisions and they did not have smartphones. Data were collected in 2014 - 2016.

Garopaba (SC)

Garopaba comprises two Quilombolos, of which one, Morro do Fortunato, was included in this study. Garopaba is a Brazilian municipality on the southern coast of the state of Santa Catarina. Its estimated 22,082 inhabitants23 are mainly engaged in tourism, construction, fishing and subsistence agriculture. The area was colonised by Portuguese from the Azores Islands in the 17th century and still retains many traces of the Azorean culture.

6. Morro do Fortunato (MF – Garopaba, SC)

Morro do Fortunato is located in Garopaba, about 8 km from the city centre. Their territory is very close to the seaside and it is told that the lands were given to Fortunato, the son of a white landowner and his slave. These lands were deep in the woods because he did not want Fortunato, with marked genetic characteristics of European and African miscegenation (e.g. dark skin and blue eyes), to draw attention from the 'white community'. According to one of the leaders, who was 55 years old in 2013, he is Fortunato's great grandson. This Quilombo, therefore, emerged as a place of making miscegenation invisible to the white society and, following that, marriages with African descendants from other communities, and consanguine marriages designed its constitution. The inhabitants lived for decades from subsistence agriculture and breeding and many of them still develop agricultural practices in rural areas, ranging from breeding livestock to growing sugar cane29,30. Women also produce jam to sell locally and the community is engaged in tourism. Inhabitants and the electricity company report electricity was installed 30 years ago. People in MF have light bulbs and television at

home, and street lighting. Most houses are made of concrete. Only a few people have access to Internet at home. Data were collected in 2013 - 2014.

Viamão (RS)

In Viamão, a Quilombola community (Peixoto dos Botinhas) was included in this study. Viamão is a city in the metropolitan region of the state of Rio Grande do Sul (estimated population: 239,38423) founded in 1741. Important commercial routes began where the municipality is located and it is the region where the first cattle ranches were established. Its economy is still based in farming and services.

7. Peixoto dos Botinhas (PB – Viamão, RS)

This Quilombo is located in the municipality of Viamão about 86 km from the city centre, close to a highway, in a rural area. Public transportation service is available throughout the day, and only few families live remote. The community origins refer to two African ancestors, who occupied vacant land after disembarking on a nearby lagoon (Lagoa dos Patos) and built ranches. The communities occupied lands isolated and considered marginal then, but that today are valued by the agribusiness for its location, with great potential for rice production and cattle breeding31. In Peixoto dos Botinhas, people are not only engaged in agriculture, but many inhabitants are also personal service workers. According to the responsible grid company, electricity was brought to the first families around 1977. People reported adhesion to the use of electricity to be a slow process. In general, people did not have smartphones when data were collected, but had light bulbs and televisions at home. Houses were made of either wood or concrete. Data were collected in 2012 - 2013.

Procedure

Communities were selected based on geographical localisation, and history of access to electricity. After first contact with Quilombo organizations and the approval of the

community leaders, meetings were organised at the Quilombo's association headquarters (located in the community territory). After participants gave their informed consent, they were interviewed and provided with the respective instruments (either right after the meeting or at their homes).

Instruments

Questionnaires were adapted to the Quilombolas cultural context and interviewers were trained to inquire in a standardised way about sleep-wake behaviour and average natural light exposure using the Munich ChronoType Questionnaire (MCTQ).

Demographic characteristics

Demographic characteristics were collected using a standard questionnaire[32]. Participants were asked about their age, educational level, occupation, as well as drinking and smoking habits. They were also asked about medical history (whether they present or not any chronic disease) and whether they take any medication.

Munich ChronoType Questionnaire (MCTQ)

The Brazilian Portuguese version of the Munich Chronotype Questionnaire (MCTQ) was applied to assess sleep-wake behaviour and self-reported natural light exposure on work and work-free days. It asks, separately for workdays and work-free days, at what time people go to bed and are ready to sleep, how long it takes them to fall asleep, at what time they wake up and get up and if they use an alarm clock. A number of variables can be derived from MCTQ data, including sleep duration, chronotype (midpoint between sleep onset and sleep offset on work-free days, corrected for oversleeping if individuals sleep longer on work-free days than on work days, MSFsc) and social jet lag (difference between mid-sleep on work and work-free days[6,33]). Self-reported outdoor light exposure is also assessed separately for work and work-free days using specific questions. 215 participants filled out the MCTQ, out of which

data from two could not be used (overly irregular patterns reported). Both the English version of the questionnaire (*English MCTQ core + time spent outdoors question from MCTQ full*) and information on the calculation of the variables can be found at: http://thewep.org/documentations/mctq.

Actimetry

For actimetry analyses, the inclusion criteria were: continuous actimeter use for at least 7 days. Days were not included in the calculation of activity phase markers and light exposure when more than 4 hours were missing. Missing episodes were identified as stretches of at least 5-10 consecutive bins (50-100 min) of no activity and were excluded from the analysis. Wrist actimeters (Actiwatch 2: Philips Respironics, ActTrust: Condor, Daqtomter: Daqtix) were distributed to 148 participants, out of which data from 125 (84%) could be used considering the excluding criteria aforementioned. Data were averaged into 10-min bins for analyses. Actimeters were shown not to differ in sleep detection (Fig S1A, B). Light intensity data (daily averages of light exposure during photoperiod and daily averages of light exposure after dusk) were normalised using the correlation slope equation from data collected over 14 days using both actimeters (Actiwatch 2 and ActTrust: 1657 bins of 10 min, representing 276 hours of recordings) at the same time (Fig S1C). The only two light sensors used from Daqtix did not work and these subjects could not be included in light analyses. Data from Bombas, Areia Branca and Córrego do Franco were collected using ActTrust. Data from São Roque, Morro do Fortunato and Peixoto dos Botinhas were collected using Actiwatch 2. Data from Mamãs were collected using both brands. Data from two subjects from Areia Branca were collected using Daqtix. The software ChronoSapiens[33] was used to asses activity phase markers (i.e., centre of gravity – acrophase – of the first harmonic fit), sleep onset, sleep end, mid-sleep, sleep duration, and light exposure patterns (average light exposure during photoperiod and after dusk). For all variables derived from actimetry, group central tendency

measures were calculated using subjects' daily averages. As previously described[33], sleep bouts in activity records were identified using stretches of relative immobility; bins with activity counts below 20% of the 24-hr centered moving average were classified as potential sleep; this retrieval was then filtered for sleep bouts with durations of at least 30 min and consolidated into longer bouts based on correlations with produced test series of different lengths. For this study, bouts with durations of 3-12 hours were included. Bouts interrupted for less than 2 h were combined (second bout length: 120-540 min). Bout-lengths were considered as sleep durations. When bouts were fused, sleep duration was calculated as the sum of their lengths, sleep onset was taken from the first bout while sleep offset was taken from the second. Mid-sleep was calculated as midpoint between sleep onset and offset. Since we focused on sleep at night, episodes that began after 8 am and ended before 3 am were checked for and manually excluded.

Data Analysis

Shapiro-Wilk was used to test continuous variables for normality. To validate the use of the MCTQ in Quilombolas communities, the correlation between data from actimetry and MCTQ was tested using Pearson. Light exposure data were analysed using Kruskal-Wallis followed by Dunn's test adjusted by Bonferroni correction. Activity phase and sleep patterns of communities were compared using ANOVA, followed by Tukey. Statistical significance was set at $p < 0.05$. SPSS 24 and GraphPad Prism 6 were used for statistical analysis. Colour codes for all graphs were RGB calculated using a Geographical Isolation Index. This index was calculated by multiplying the urbanisation rate of the municipality by 1 if there was no access to vehicles, 3 if public transport was available 1-2x/day, 5 if public transport was available more than 2x/day or many inhabitants have cars (Geographical Isolation, Table 1). The fraction of red, green and blue were then calculated proportionally to this index, with the greenest being the community more isolated, and the reddest the least.

TABLE 1. Characteristics of each Quilombo studied

	Municipality	Urbanisation rate (%)[1]	Difficulty of access[2]	Geographical Isolation[3]	Electricity history[4]	Coordinates (decimal degrees)
Bombas (BB)	Iporanga (SP)	55.8	1	55.85	No electricity*	48.6 W 24.6 S
Areia Branca (AB)	Bocaiúva do Sul (PR)	46.7	3	140.02	2 yrs*	48.6 W 25.0 S
São Roque (SR)	Praia Grande (SC)	59.1	3	177.39	15 yrs*	50.0 W 29.2 S
Córrego do Franco (CF)	Adrianópolis (PR)	32.3	5	161.54	> 20 yrs*	48.6 W 24.8 S
Mamãs (MM)	Castro (PR)	73.4	3	220.32	20 yrs / 8 yrs*	49.7 W 24.8 S
Morro do Fortunato (MF)	Garopaba (SC)	84.5	5	422.32	30 yrs*#	48.6 W 28.0 S
Peixoto dos Botinhas (PB)	Viamão (RS)	94.0	5	469.84	> 30 yrs #	51.0 W 30.1 S

[1] Referring to the entire municipality; source: last census of the Brazilian Institute of Geography and Statistics [47,48].

[2] Subjective classification: 1 no access to vehicles; 2 no public transport; 3 public transport 1-2x/day (far from the city centre); 4 (near to the city centre); 5 public transport more than 2x/day or many inhabitants have cars.

[3] Calculated by multiplying urbanisation rate by difficulty of access

[4] * reported by inhabitants; # reported by electric company.

TABLE 2. Characteristics of the Quilombolas sample (*MCTQ:* N = 213 / *actimetry:* N = 125)

	N MCTQ/acti	Age (mean ± SD)	Sex (%F)	Housekeeper or Farmer (%)	Regular work schedule (%)	Alarm clock usage (%)	Alarm clock need (%)	Season acti (S; W)
Bombas (BB)	29 / 27	33 ± 15 / 31 ± 14	48 / 52	93 / 93	3 / 4	3 / 4	0 / 0	5; 22
Areia Branca (AB)	13 / 14	55 ± 12 / 54 ± 12	46 / 50	92 / 93	38 / 36	0 / 0	0 / 0	2; 12
São Roque (SR)	25/ 18	44 ± 16 / 42 ± 15	44 / 50	64 / 61	56 / 60	28 / 33	8 / 13	11; 7
Córrego do Franco (CF)	16 / 11	33 ± 10 / 33 ± 11	50 / 54	69 / 64	56 / 56	44 / 56	6 / 11	5; 6
Mamãs (MM)	29 / 19	41 ± 19 / 43 ± 21	62 / 68	76 / 79	38 / 32	28 / 26	14 / 16	11; 8
Morro do Fortunato (MF)	60 / 18	46 ± 19 / 61 ± 19	55 / 72	30 / 22	68 / 47	45 / 29	34 / 17	4; 14
Peixoto dos Botinhas (PB)	41 / 18	57 ± 14 / 59 ± 15	63 / 61	29 / 39	68 /67	15 / 11	8 / 6	8; 10

Averages from subjects who filled the MCTQ / wore the actimeter appropriately

Regular work schedule: reported (MCTQ)

Season: actimetry data – summer; winter based on start of data collection

Results

Population characterisation

The Quilombola population (55% women) had an age range between 16 and 92 (mean: 45.2 ± 18.2 years; see demographic characteristics by community in Table 2) and lived mostly in rural areas. Most commonly reported occupations were farming (35%) and housekeeping (21%). 12% reported to be retired and 8% unemployed. The vast majority of subjects in Bombas (BB), Areia Branca (AB), São Roque (SR), Córrego do Franco (CF) and Mamãs (MM) were farmers and/or housekeepers. A small portion reported to be retired, unemployed or work as personal service/ health workers. In Morro do Fortunato (MF) and Peixoto dos Botinhas (PB), fewer participants reported to be farmers and a considerable proportion of subjects works in elementary occupations/as personal service or sales workers (Table S1).

Validation of the MCTQ in Quilombos

Subjective data from the MCTQ correlated highly with data calculated from actigraphy data (Figure S2).

Light exposure

Average light profiles of each community can be seen in Figure 2. Figure 3 shows the average light exposure for each community during photoperiod and from dusk to midnight. No statistical difference between groups but a tendency (Kruskal-Wallis, $\chi^2(6) = 11.95$, $p = 0.06$) was observed in exposure to light during photoperiod. People in PB and MM were on average exposed to higher levels of light after dusk than BB, AB and MM.

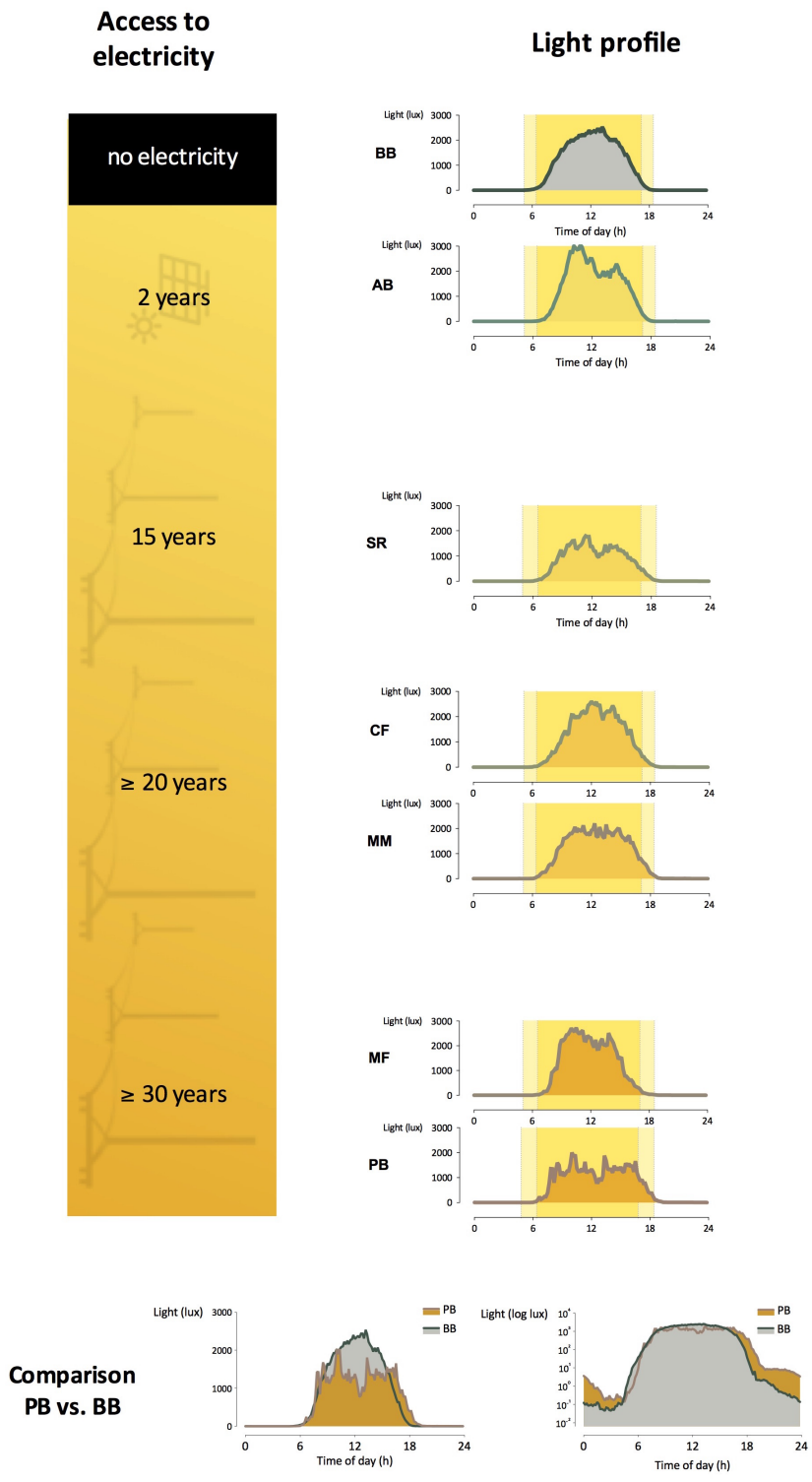

Figure 2. Average light profiles*: each graph shows the average light profile of a community, calculated using individuals actimetry data. A comparison between the extremes, Bombas (BB) and Peixoto dos Botinhas (PB) can be seen at the bottom, being the graph to the right log scaled. Backgrounds represent photoperiod length (on the longest and shortest day). n = 11-27. BB: Bombas, AB: Areia Branca, SR: São Roque, CF: Córrego do Franco, MM: Mamãs, MF: Morro do Fortunato, PB: Peixoto dos Botinhas.*

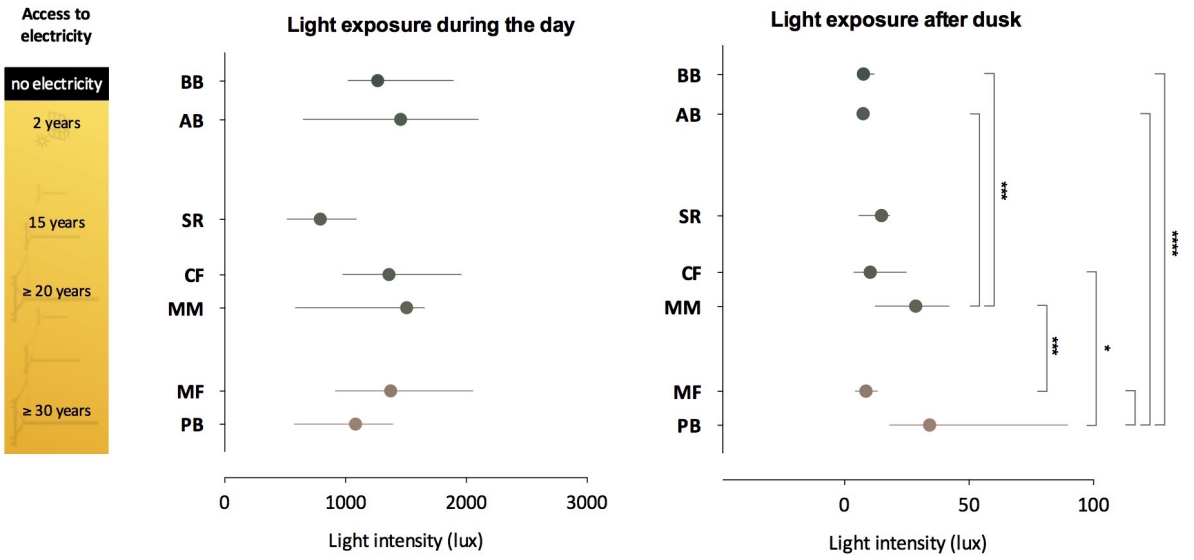

Figure 3. Light exposure: *dots represent the median, whiskers represent interquartile ranges of light exposure during photoperiod (Kruskal-Wallis, $\chi^2(6) = 11.95$, $p = 0.06$) and after dusk (Kruskal-Wallis, $\chi^2 (6) = 50.96$, $p < 0.0001$). Dots are colour coded according to the geographical isolation index. The greener the bar, the more geographically isolated the community. Dunn's test adjusted p-values: $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$; $^{****}p < 0.0001$). n = 11-27. BB: Bombas, AB: Areia Branca, SR: São Roque, CF: Córrego do Franco, MM: Mamãs, MF: Morro do Fortunato, PB: Peixoto dos Botinhas.*

Activity and sleep phase

The centre of gravity of activity (CoG_{act}) delayed systematically with longer artificial light history from BB to PB (Figure 4).

Based on the sleep-assessment of the actimetry data, sleep onset in PB and MF was statistically later than in BB and AB, and in MM than in BB. The mid-sleep also occurred later in PB and MF than in BB and AB. MF presented later wake up times than BB, SR and MM. PB, SR and MM slept shorter than AB and BB (Figure 5).

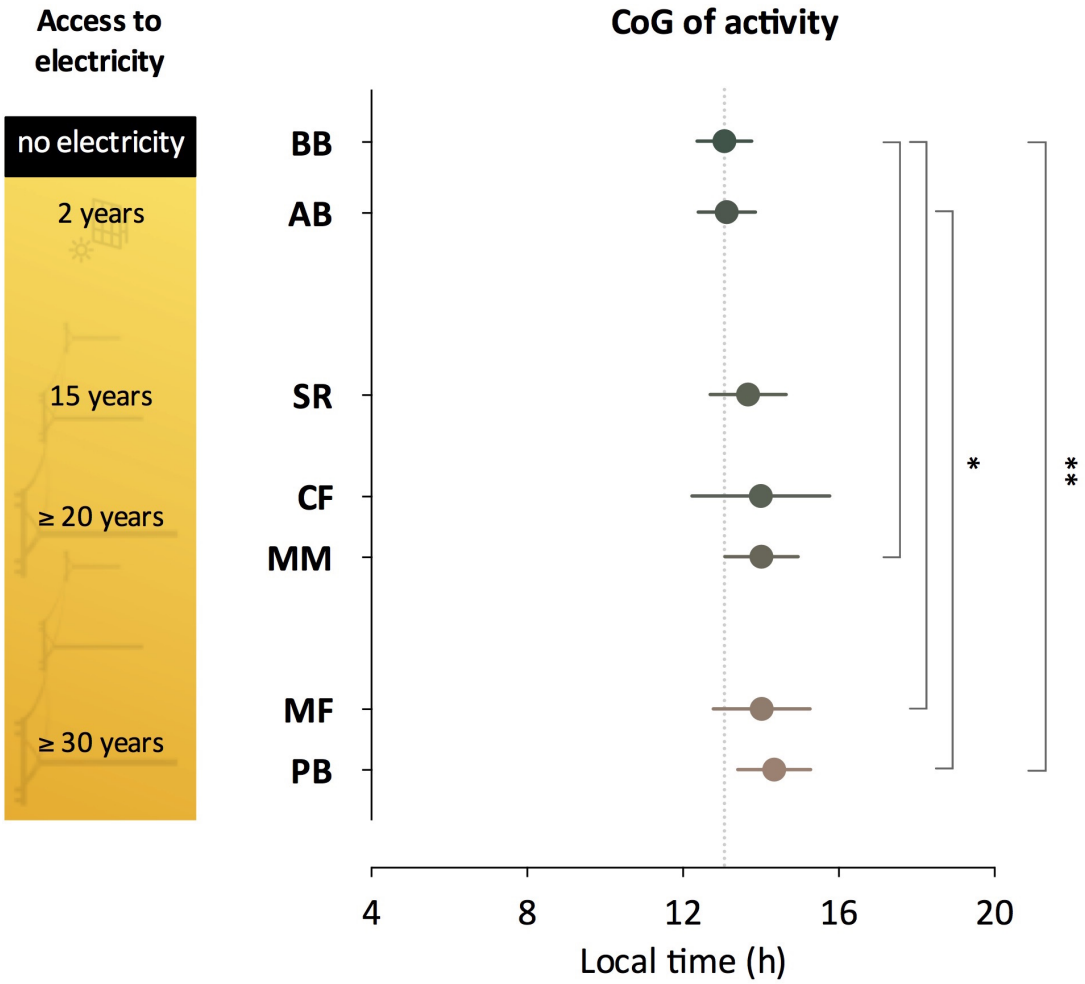

Figure 4. Centre of Gravity of Activity: *dots represent the mean, whiskers represent the standard deviation. Dots are colour coded according to the geographical isolation index. The greener the bar, the more geographically isolated the community. The dotted line marks BB mean as a reference. ANOVA,* $F_{(6,118)} = 4.40$, $p < 0.001$. *Tukey's adjusted p-values:* $^{*}p < 0.05$; $^{**}p < 0.01$. $n = 11\text{-}27$. *BB: Bombas, AB: Areia Branca, SR: São Roque, CF: Córrego do Franco, MM: Mamãs, MF: Morro do Fortunato, PB: Peixoto dos Botinhas.*

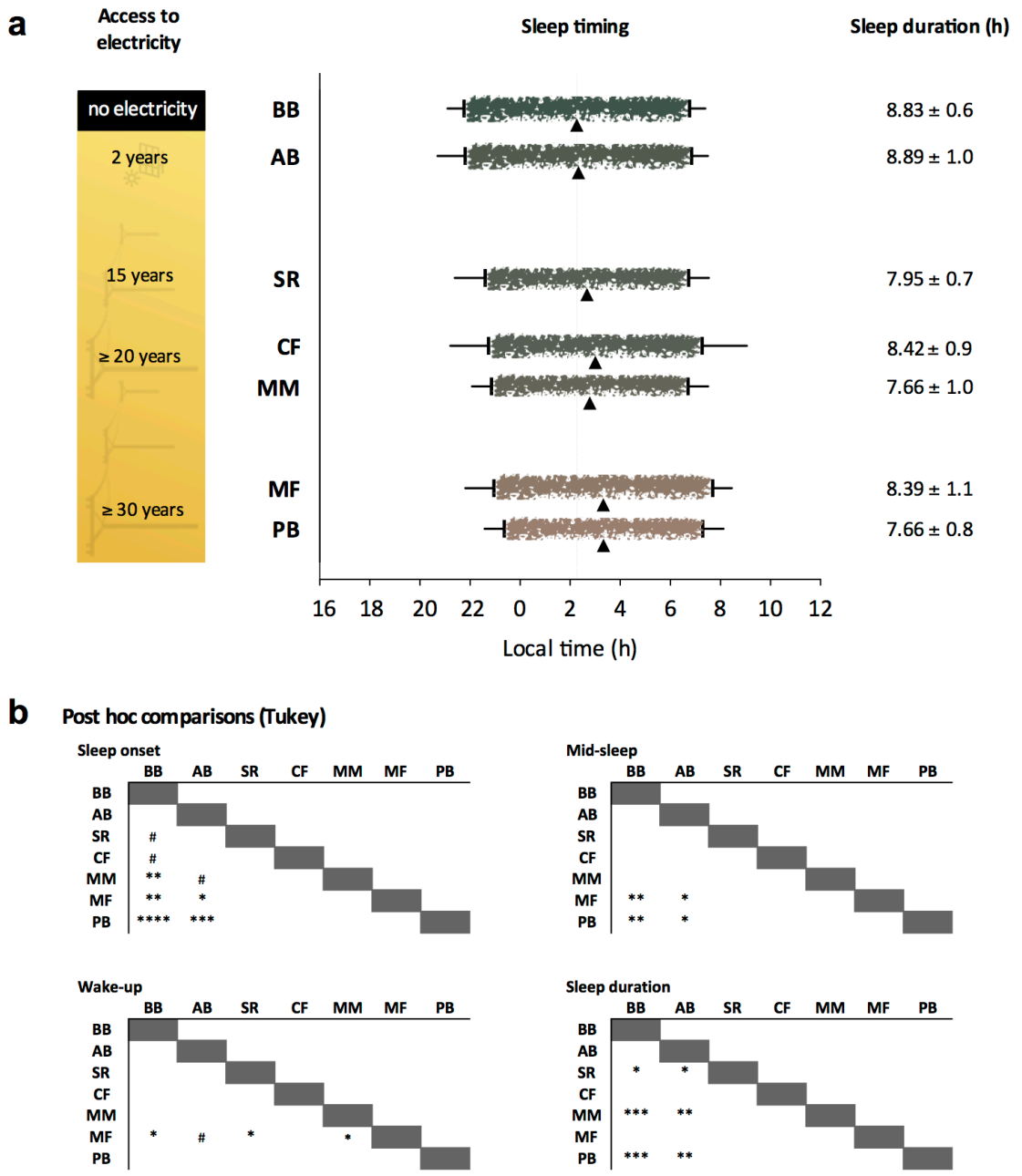

Figure 5. Sleep timing and duration: *(a) 'Brushed' lines represent average sleep episodes calculated from actimetry data. Horizontal whiskers represent standard deviation and triangles mid-sleep. Episodes are colour-coded according to the geographical isolation index. The greener the bar, the more geographically isolated the community. The dotted line marks BB mid-sleep mean as a reference. Sleep onset: ANOVA* $F_{(6,118)} = 6.88$, $p < 0.0001$, *mid-sleep: ANOVA* $F_{(6,118)} = 5.09$, $p < 0.001$, *wake-up: ANOVA* $F_{(6,118)} = 3.36$, $p < 0.01$, *sleep duration: ANOVA* $F_{(6,118)} = 6.54$, $p < 0.0001$. *(b) Tukey's adjusted p-values:* $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$; $^{****}p < 0.0001$, $^{\#}p < 0.15$). *n = 11-27. BB: Bombas, AB: Areia Branca, SR: São Roque, CF: Córrego do Franco, MM: Mamãs, MF: Morro do Fortunato, PB: Peixoto dos Botinhas.*

Considering that data were collected in different seasons, we performed the same analyses using only data from March to September and retrieved similar results (Table S2). We also found similar results comparing communities' reported sleep times on work-free days from the MCTQ (Table S2).

Average light exposure was significantly correlated with sleep onset and duration (calculated from actigraphy), both during photoperiod (Spearman, *onset:* $r = -0.28$, $p < 0.01$; *onset - no Daylight Saving Time change:* $r = -0.35$, $p < 0.001$; *duration:* $r = 0.18$, $p < 0.05$) and after dusk (Spearman, *onset:* $r = 0.29$, $p < 0.01$; *onset - no Daylight Saving Time change:* $r = 0.20$, $p < 0.05$; *duration:* $r = -0.22$, $p < 0.05$). Zeitgeber strength (ratio of light exposure during photoperiod to light exposure after dusk) also correlated with sleep onset and duration (Spearman, *onset:* $r = -0.46$, $p < 0.001$; *onset - no Daylight Saving Time change:* $r = -0.38$, $p < 0.001$; *duration:* $r = 0.36$, $p < 0.001$). Alarm clock usage was also associated with sleep duration (*use alarm:* 8.33 h ± 1.01, *do not use:* 7.88 h ± 0.86. Student's t test, $t_{116} = 1.95$, $p = 0.05$).

A hierarchical multiple linear regression was performed to assess significant predictors of midpoint of sleep and sleep duration and control for effects of age and sex. In both models, the effect of 'community' was significant when controlling for age and sex. When season of data collection and exposure to light during photoperiod were added, they were also significant predictors. Having regular work schedules was not included in the model in favour of a parsimonious model for it was not a significant predictor of sleep timing or duration. These results are detailed in Tables S3 and S4.

Discussion

The primary findings of this study corroborate the notion that phase of activity and sleep changed as a result of electricity usage and other modern lifestyles. Communities, which have not yet or very recently acquired electricity, sleep earlier than those which have been

connected to the grid long ago. These results are similar using different assessment methods (subjective questionnaires versus objective light and activity measurements) and both correlate highly. Even the Quilombolas who live without electricity have a clear concept of clock time. Many of them use clocks to communicate with the outside world, but not necessarily to organize their day. It was therefore possible to ask them the questions of the MCTQ and get meaningful answers based on the subjects' concept of local time. From knowledge about circadian entrainment[34] and experimental studies[35], our results were predictable due to varying zeitgeber strength. The combination of high daylight exposure and low light at night generates a strong zeitgeber signal while indoor work and artificial light after sunset weaken the signal. In general, the stronger the zeitgeber, the earlier the phase of entrainment (chronotype). Due to the weak zeitgebers in industrialised societies, most clocks have delayed while social schedules have remained relatively unchanged. People therefore accumulate sleep debt over the workweek determining the amount of social jetlag they suffer from[9]. Even Peixoto dos Botinhas (PB), according to the Geographical Isolation Index the closest to an urbanised community, is still a rural one, showing a considerably high average light exposure during the day. Despite the mostly rural lifestyles in the different communities they did show differences in light exposure, and light exposure during the day and at night significantly correlated with sleep timing.

Sleep duration also differed between communities. Three of the five communities that had electricity for more than a decade (SR, MM and PB) sleep shorter than those with no or more recent access to electricity (BB and AB). The other two long-term electricity communities (MF and CF) did sleep 25 to 30 min shorter than BB/AB, but the difference did not reach significance. MF also had later wake-up times and we have collected actimetry data mostly from retired participants. Although the human circadian timing is mainly influenced by light, sleep is additionally influenced by many other factors related to urbanisation and life routines,

which may explain the heterogeneous reports about sleep duration in the literature[12–14,16,17]. An important factor that impacts sleep is globalization: social interaction, commercial activities and work responsibilities are not local anymore, but often virtually connected across time zones. Although they might communicate using mobiles (rarely smartphones), the Quilombolas described here have no access to the Internet or were connected only very recently. Sleeping arrangements and strain of daytime work are certainly important factors to be considered. Despite houses (e.g., made from mud/clay, wood, or concrete) and environmental noise varying in the Quilombos, people sleep indoors and on beds with mattresses in all communities we have visited. High physical work (e.g. rubber tappers in Acre, Brazil) was associated with lower sleep quality[36]; physical inactivity was likewise reported to be a predictor of sleep complaints and depression[37]. While physical exercise is recommended to prevent or treat sleep disorders, the interrelationship between these two factors is not yet fully understood[38]. It remains to be tested in future studies how the levels of physical activity during work and leisure in Quilombos may be a factor contributing to the differences seen in sleep behaviour.

In this study, all communities mainly live from farming, but may vary in the way they see productivity and subordinate it to time. In the words of the leader of the community that has no electricity: “In the city, it is easier to get tired. It is a matter of time. During the day, everything is as soon as the clock tells. Here we work by solar time”. In the US, work is the main activity sleep is exchanged for and findings suggest that interventions aiming to increase sleep duration should focus on delaying start times of work or making them more flexible[39]. Adolescents with electric light were seen to sleep later than adolescents without electricity at home, but only those, who attended morning classes and had electricity showed a reduction of night sleep duration[15]. In line with our study presenting a relatively long sleep in Quilombos,

longer sleep and lower prevalence of short sleep duration have been reported in farmers (large cross-sectional studies in China and the United States)[40,41].

Less than 10% of people from the community that has no electricity (BB) report to have regular work schedules, whereas in PB (the less isolated community) more than half do. Differently from the other communities, people in BB and AB, the community that has had electricity for only two years, do not use alarm clocks. Despite the percentage of people, who report the usage of an alarm clock being low in all Quilombolas communities, those who use alarm clocks sleep shorter than those who do not. Early wake up might still be culturally associated to success and productivity in some Quilombos even if to a lesser degree. Supporting this rationale, a rural community in the South Brazil was shown to present similar sleep duration than an urban one[32] and in the Brazilian Southeast, a rural population with conservative lifestyles also showed relatively earlier wake up and bed times[42] than what was reported in other studies in rural areas. These findings stress the importance of raising general awareness of the consequences of insufficient sleep, since whether or not sleep duration has substantially changed from pre-electricity era to present days, sleep deprivation have well-known health consequences[43–45].

Some limitations of the study are noteworthy. The fact that we collected data over different seasons could have influenced our results[46]. However, the differences in sleep timing remained similar when comparing only data collected in the winter. Our study was conducted over 4 years, allowing for the first time to study many communities that are engaged in similar daytime activities, but differ in urbanisation and access to electricity. The differences in mean age between the communities could represent another confounder considering that elder people usually sleep shorter and earlier[47,48]. However, results were similar when controlling for age and sex. Light exposure measured at the wrist may not reflect the levels received by the eye and photoreceptors sensitivity was not taken into account. Still, light intensities should vary similarly at both the eye and the wrist.

Studying human behaviours is challenging because they are typically complex and subject to many environmental factors. However, the communities here analysed are considerably similar regarding work activities, cultural background, social organisation and they live at relatively near geographical locations with rather similar photoperiods and weather conditions (Table S5), allowing us to draw important conclusions regarding conceivable changes brought by electricity. Supporting previous studies, our findings indicate that access to electricity might have brought changes to sleep patterns. Further studies in these communities might help us to recognise the consequences of these changes for health and propose ways of minimising them.

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Author contributions

L.K.P. and R.L. collected data. L.K.P analysed data and drafted the manuscript. R.L., M.P. and T.R. designed the study. M.P. and T.R. were involved in data analysis. All authors were involved in data collection, discussed the results and revised the manuscript.

Additional information

Competing interests statement

The authors declare no competing interests.

TABLE S1: Quilombolas occupation: MCTQ and actimetry samples (total %)

		Farmer	Housekeeper	Farmer AND housekeeper	Retired	Elementary occupations* and personal service workers	Unemployed	Sales workers	Personal care and health workers	Teacher	Student	Craft and related trade workers	Machine operators (drivers)	Not reported
Bombas (BB)	MCTQ	72.4	17.2	3.4	3.4	3.4								
	Actimetry	70.4	18.5	3.7		3.7								3.7
Areia Branca (AB)	MCTQ	69.2	-	23.1	7.7									
	Actimetry	71.4	-	21.4	7.1									
São Roque (SR)	MCTQ	44.0	20.0		8.0	8.0					8.0	8.0		4.0
	Actimetry	38.9	22.2			5.6					5.6	11.2		16.7
Córrego do Franco (CF)	MCTQ	37.5	25	6.3	6.3	12.6	6.3				6.3			
	Actimetry	36.4	18.2		9.1	9.1					9.1			18.2
Mamãs (MM)	MCTQ	58.6	10.3	6.8	10.3	6.8			6.8					
	Actimetry	57.9	10.5	10.5	10.5				10.5					
Morro do Fortunato (MF)	MCTQ	10	20		21.7	28.5	1.7	1.7	1.7		3.3	3.3	3.3	5.0
	Actimetry	5.6	16.7		44.4	11.2		5.6						16.7
Peixoto dos Botinhas (PB)	MCTQ		29.3		17.1	31.6	7.3	9.8		2.2		2.2		
	Actimetry		38.3		16.7	22.3	5.6	16.7						

*cleaners, helpers, construction
According to the International Standard Classification of Occupation
Percentages might not sum up to 100 % due to rounding.

TABLE S2: Sleep timing and duration

Data from actimetry collected in the winter (March - September, n = 7 - 23)

	Quilombola communities							ANOVA results			
	BB	AB	SR	CF	MM	MF	PB	F	df	p	n^2
Sleep onset*	21:40 ± 0:38[a]	21:37 ± 1:04[a]	22:25 ± 1:06[a,b]	22:27 ± 1:50[a,b]	22:39 ± 1:03[a,b]	22:35 ± 0:59[a,b]	23:16 ± 0:57[b]	4.20	6, 76	< 0.01	0.25
Mid-sleep**	02:15 ± 0:31	02:17 ± 0:48	02:30 ± 0:45	02:54 ± 2:11	02:41 ± 0:56	03:06 ± 0:43	03:13 ± 0:44	2.36	6, 76	< 0.05	0.16
Sleep end**	06:49 ± 0:34	06:57 ± 0:35	06:34 ± 0:42	07:20 ± 2:37	06:43 ± 1:07	07:37 ± 0:41	07:12 ± 0:39	1.50	6, 76	0.19	0.11
Sleep duration*	08:58 ± 0:38[a]	09:10 ± 0:38[a]	07:55 ± 0:58[a,b]	08:46 ± 1:06[a,b]	07:54 ± 1:06[b]	08:39 ± 1:00[a,b]	07:40 ± 0:55[b]	5.25	6, 76	< 0.001	0.29

Data from the MCTQ on work-free days (n = 13 - 59)

	Quilombola communities							ANOVA results			
	BB	AB	SR	CF	MM	MF	PB	F	df	p	n^2
Sleep onset*	21:42 ± 1:22[a]	21:57 ± 1:06[a,b]	23:24 ± 1:53[b,c]	22:27 ± 1:10[a,b]	22:48 ± 1:31[a,b,c]	23:46 ± 1:34[c]	23:54 ± 1:35[c]	10.09	6, 205	< 0.0001	0.22
Mid-sleep*	02:06 ± 1:05[a]	02:31 ± 0:44[a,b]	03:01 ± 1:31[a,b,c]	02:46 ± 1:22[a,b,c]	02:36 ± 1:10[a]	03:40 ± 1:09[c]	03:36 ± 1:25[b,c]	7.60	6, 205	< 0.0001	0.18
Sleep end*	06:30 ± 1:13[a]	07:06 ± 0:55[a,b]	06:39 ± 1:36[a,b]	07:05 ± 1:48[a,b]	06:24 ± 1:27[a]	07:40 ± 1:27[b]	07:18 ± 1:38[a,b]	3.77	6, 205	< 0.01	0.10
Sleep duration*	08:48 ± 1:25[a]	09:07 ± 1:24[a]	07:15 ± 1:45[b]	08:39 ± 1:16[a,b]	07:36 ± 1:51[a,b]	08:00 ± 1:34[a,b]	07:24 ± 1:31[b]	5.07	6, 205	< 0.0001	0.13

Columns show the communities mean ± standard deviation.

* different letters represent statistically significant differences detected by the post hoc (Tukey).

** differences detected by ANOVA, despite not having enough power for the post hoc comparisons to detect where.

*** for MCTQ comparisons: one outlier removed (group MF, ROUT method)

Table S3: Hierarchical regression - predictors of midpoint of sleep (N = 123)

		Model 1			**Model 2**			**Model 3**		
		Adjusted R^2 = -0.010			Adjusted R^2 = 0.190			Adjusted R^2 = 0.302		
		F(2, 120) = 0.39, p = 0.68			F(8,114) = 4.58, p < 0.0001			F(10,112) = 6.27, p < 0.0001		
Variables		B	β	p	B	β	p	B	β	p
Age		0.00	0.04	0.66	-0.01	-0.21	< 0.05	-0.01	-0.27	< 0.01
Sex (male)		-0.13	-0.07	0.46	-0.01	0.00	0.96	0.33	0.17	0.05
Quilombo (Bombas)	Areia Branca (AB)				0.21	0.07	0.49	0.30	0.10	0.31
	São Roque (SR)				0.52	0.20	< 0.05	0.13	0.05	0.61
	Córrego do Franco (CF)				0.76	0.24	< 0.05	0.65	0.20	< 0.05
	Mamãs (MM)				0.64	0.25	< 0.05	0.51	0.20	< 0.05
	Morro do Fortunato (MF)				1.36	0.52	< 0.0001	1.51	0.58	< 0.0001
	Peixoto dos Botinhas (PB)				1.34	0.52	< 0.0001	1.17	0.45	< 0.0001
Season of data collection (winter)								-0.41	-0.21	< 0.05
Average light exposure during the day								0.00	-0.35	< 0.001

The model was carried out with midpoint of sleep derived from actimetry as dependent variable. Quilombo represents participant's community. Season of data collection refers to when actimetry data were collected. Average light exposure during the day as measured by actimetry.

Table S4: Hierarchical regression - predictors of sleep duration (N = 123)

		Model 1			**Model 2**			**Model 3**		
		Adjusted R^2 = 0.018			Adjusted R^2 = 0.218			Adjusted R^2 = 0.307		
		F(2, 120) = 2.13 p = 0.12			F(8,114) = 5.24, p < 0.0001			F(10,112) = 6.41, p < 0.0001		
Variables		B	β	p	B	β	p	B	β	p
Age		-0.01	-0.18	<0.05	-0.01	-0.12	0.22	-0.01	-0.10	0.30
Sex (male)		-0.10	-0.05	0.56	-0.17	-0.09	0.29	-0.41	-0.21	< 0.05
Quilombo (Bombas)	Areia Branca (AB)				0.28	0.08	0.40	0.23	0.07	0.46
	São Roque (SR)				-0.80	-0.29	< 0.01	-0.41	-0.15	0.14
	Córrego do Franco (CF)				-0.40	-0.12	0.20	-0.25	-0.07	0.41
	Mamãs (MM)				-1.12	-0.41	< 0.0001	-0.91	-0.33	< 0.001
	Morro do Fortunato (MF)				-0.29	-0.10	0.36	-0.36	-0.13	0.23
	Peixoto dos Botinhas (PB)				-1.01	-0.36	< 0.01	-0.81	-0.29	< 0.01
Season of data collection (winter)								0.58	0.29	< 0.001
Average light exposure during the day								0.00	0.21	< 0.05

The model was carried out with midpoint of sleep derived from actimetry as dependent variable. Quilombo represents participant's community. Season of data collection refers to when actimetry data were collected. Average light exposure during the day as measured by actimetry.

TABLE S5: Photoperiod and climate

	Winter solstice	Summer solstice*	Closest meteorological station	Monthly insolation (h)	Relative humidity (%)	Nebulosity**
Bombas (BB)	06:23 - 17:11	05:10 - 18:24	Curitiba (PR)	151.80	80.41	7.21
Areia Branca (AB)	06:25 - 17:09	05:08 - 18:26	Curitiba (PR)	151.80	80.41	7.21
São Roque (SR)	06:29 - 16:57	04:56 - 18:30	Torres (SC)	176.33	83.91	6.12
Córrego do Franco (CF)	06:25 - 17:09	05:08 - 18:26	Curitiba (PR)	151.80	80.41	7.21
Mamãs (MM)	06:21 - 17:05	05:04 - 18:22	Castro (PR)	117.03	83.59	7.04
Morro do Fortunato (MF)	06:31 - 17:03	05:02 - 18:32	Florianópolis (SC)	168.46	79.04	6.71
Peixoto dos Botinhas (PB)	06:23 - 16:47	04:47 - 18:24	Porto Alegre (RS)	178.30	77.57	5.64

Photoperiod as calculated using ChronoSapiens

Meteorological data available from Instituto Nacional de Metereologia (INMET). Monthly averages from 2013-2016.

*no Daylight Saving Time advance

**Fraction of sky covered by clouds (1-10)

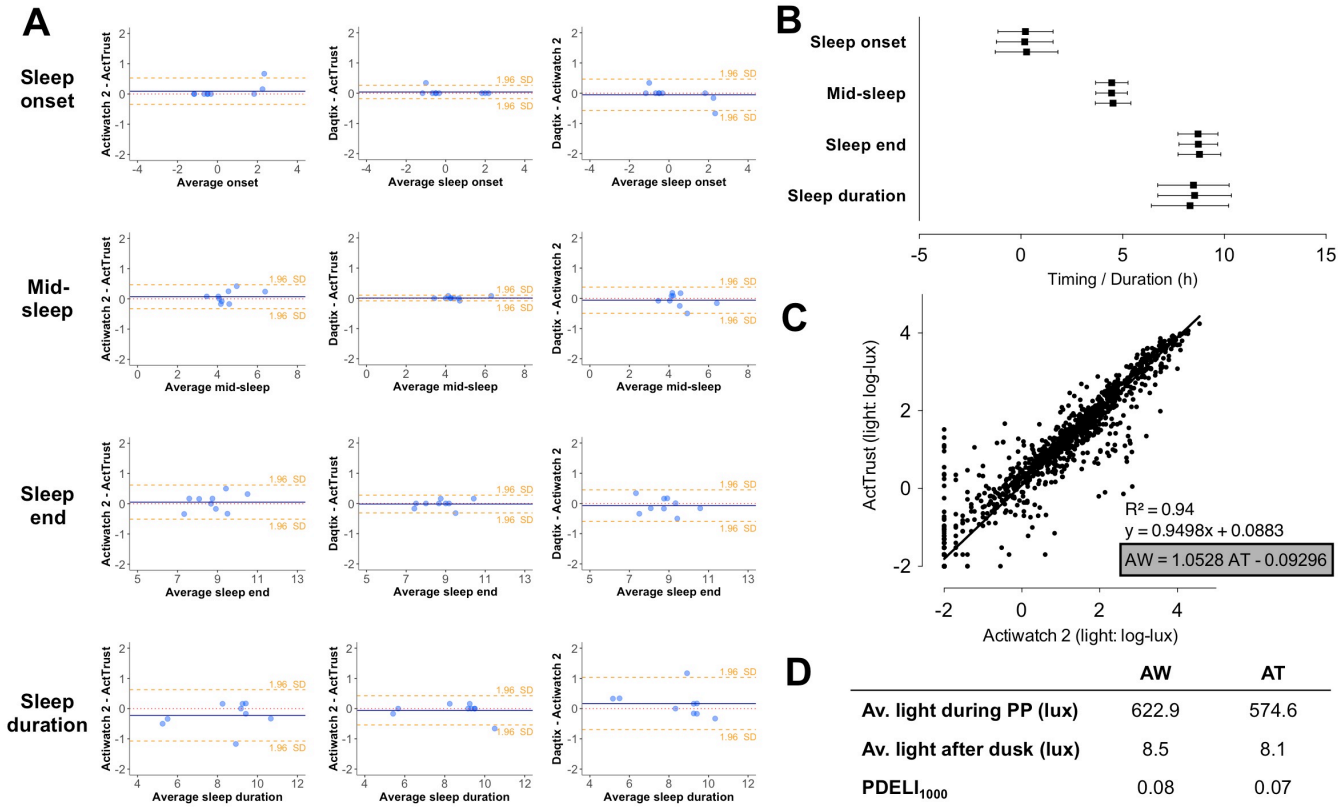

Figure S1. Comparison between Actiwatch 2 (Philips Respironics) and ActTrust (Condor). *(a) Bland-Altman plots for comparing Actiwatch 2 and ActTrust, Daqtix and ActTrust, and Daqtix and Actiwatch. The blue line represents the mean difference between all days (n = 9) recorded with the two different devices worn at the same time on the same wrist. The orange dashed lines represent 1.96 standard deviation. (b) Squares and whiskers represent the calculated average and standard deviation of recorded days for each variable (sleep onset, mid-sleep, sleep end and sleep duration) and each actimeter. Top: Actiwatch 2; Middle: Daqtix; Bottom: ActTrust. (c) The grey square shows the slope equation from data collected over 14 days using Actiwatch 2 and ActTrust: 1657 bins of 10 min, representing 276 hours of recordings shown on the scatterplot. This was used to normalise daily averages derived from ActTrust. (d) Daily averages of light exposure during photoperiod (PP), daily averages of light after dusk and* $PDELI_{1000}$ *calculated from data from both actimeters were not significantly different (Wilcoxon Matched-pairs signed rank test, average light during photoperiod: Z = - 22, p = 0.37; light after dusk: Z = 12, p =0.64).*

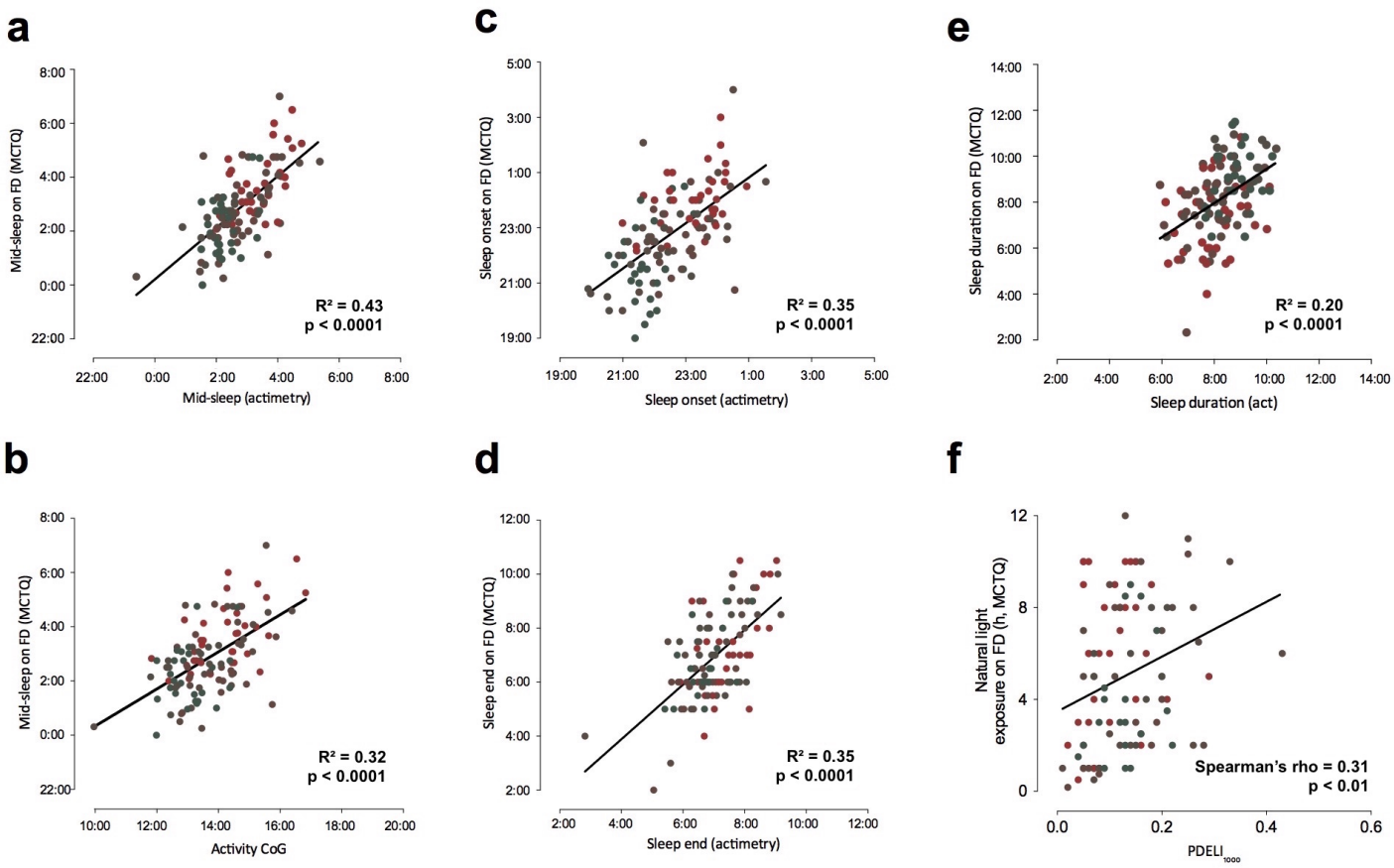

Figure S2. Correlation between subjective data from the MCTQ and actimetry. *MCTQ-derived mid-sleep on free days correlated significantly with mid-sleep calculated from actigraphy data and the centre of gravity of activity (Pearson: a, mid-sleep: $r = 0.66$, $p < 0.0001$; b, activity CoG: Pearson, $r = 0.57$, $p < 0.0001$, $N = 117$). The same was true for sleep onset, end and duration (Pearson, c, onset: $r = 0.60$, $p < 0.0001$; d, end: $r = 0.59$, $p < 0.0001$; e, duration: $r = 0.45$, $p < 0.0001$, $N = 117$). MCTQ assessed natural light exposure correlated with the proportion of day exposed to more than 1000 lux ($PDELI_{1000}$, Spearman, rho = 0.31, $p < 0.01$, $N = 99$). Dots are colour coded according to the geographical isolation index. The greener the dots, the more geographically isolated the community.*

CAPÍTULO 5:

Discussão dos achados da tese.

DISCUSSÃO

"Clocks slay time… time is dead as long as it is being clicked off by little wheels; only when the clock stops does time come to life." – W. Faulkner, 1929.

O relógio biológico faz com que a organização temporal tenha um sentido e um ritmo individual. Com a utilização da luz elétrica, as diferenças na nossa relação com o tempo externo se tornam ainda mais acentuadas. Incoerentemente, a maneira como nossa sociedade se organiza ignora este tempo vivo e segue um tempo mecânico, que desconsidera os aspectos singulares da cadência da vida. Nesta tese, a aplicabilidade e a conveniência da avaliação de ritmos em estudos epidemiológicos foi testada.

A prevalência de transtornos psiquiátricos cresceu junto à inquietude dos estilos de vida atuais. Os resultados apresentados nos capítulos 2 e 3 apontam para o potencial de estudar associações entre alterações de ritmos e manifestações clínicas. A presença de picos em determinados sinais e comportamentos que estão alterados em transtornos de humor apresentou diferentes prevalências em indivíduos com risco para transtornos psiquiátricos. Além disso, em uma amostra brasileira com altos níveis de desalinhamento circadiano, mais indivíduos em risco reportaram que seu pico de sonolência acontecia pela manhã. Em uma amostra espanhola, por outro lado, a diferença entre o pico de apetite e motivação para se exercitar foi significativamente menor em indivíduos em risco, tendo uma maior proporção destes reportado que seu pico de apetite ocorre antes do pico de motivação para o exercício. Tais achados sugerem que eles podem estar em condição de cronorruptura. Estudos adicionais que avaliem as diferenças de fase entre os itens do Instrumento de Ritmos de Humor podem contribuir no entendimento desta condição. Da mesma forma, estudos futuros em pacientes deprimidos podem comprovar a aplicabilidade do instrumento na clínica.

As evidências apresentadas no capítulo 4 reforçam a hipótese de que o sono humano vem se transformando com a industrialização e expansão da utilização de luz artificial. Neste estudo, a actimetria e o MCTQ demonstraram correspondência entre si e, no contexto da pesquisa de campo, se mostraram como instrumentos ideais para medida contínua e retrospectiva de ritmos de atividade-repouso e sono-vigília. Apesar de nosso estudo ser transversal, inferimos que a utilização da luz elétrica tem efeitos tanto na fase, quanto na duração do sono. O fato de nem todas as comunidades que têm acesso à eletricidade há mais tempo apresentaram sono mais curto ilustra como uma série de fatores estão envolvidos na regulação deste estado. Considerando as características destas comunidades, a maneira como elas organizam seus horários de trabalho e associam o conceito de produtividade ao tempo

podem ser alguns destes fatores. O trabalho parece ser a principal atividade pela qual o sono é substituído em sociedades industrializadas; intervenções com o objetivo de atrasar o início dos horários de trabalho ou torná-los mais flexíveis poderiam contribuir para diminuir a prevalência de sono insuficiente (BASNER; SPAETH; DINGES, 2014). Tanto duração quanto qualidade subjetiva de sono são diferentes quando comparados dias de trabalhos e dias livres (ROENNEBERG et al., 2007; PILZ et al., 2018).

Muitas vezes se pensa que trabalhar mais tempo significa ser mais produtivo, noção que entendemos estar errada (HAFNER et al., 2017). Neste contexto, um entendimento da associação entre alterações da organização circadiana e de sono e suas uma implicações na saúde torna-se essencial. Já havia sido observada uma associação entre desalinhamento circadiano e sintomas depressivos em moradores do Vale do Taquari (LEVANDOVSKI et al., 2011). Em comunidades quilombolas, tal associação também parece estar presente, sugerindo que o jetlag social, aferido pelo MCTQ, é uma medida interessante em estudos que investiguem as consequências dos estilos de vida atuais para a saúde. Estudos longitudinais que demonstrem como o desalinhamento circadiano em diferentes níveis pode estar envolvido na etiologia de estados patológicos são essenciais para que possamos propor estratégias de prevenção e desenvolver tratamentos que atentem para estes aspectos.

Alterações no estilo de vida que previnam o desalinhamento circadiano podem representar uma estratégia de diminuição do risco para transtornos psiquiátricos. Neste sentido, a avaliação de ritmos apresenta potencial para auxiliar no diagnóstico e tratamento. Estudos futuros podem agregar ao conhecimento sobre os efeitos de alterações da organização temporal e sua relação com sintomas de transtornos de humor.

CONCLUSÃO

Os resultados aqui apresentados demonstram a relevância e aplicabilidade do estudo de ritmos biológicos e do sono. Vivemos em um tempo em que se buscam resultados a qualquer custo, em que seguimos um relógio mecânico em oposição ao nosso relógio vivo. Entender as consequências dos estilos de vida atuais para a saúde e como o desalinhamento circadiano em diferentes níveis pode estar envolvido na etiologia de estados patológicos é essencial para que possamos propor, além de uma reflexão sobre a relação do ser humano com o tempo, estratégias de prevenção e o desenvolvimento de tratamentos que atentem para estes aspectos.

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APÊDICE 1:

Aplicabilidade do estudo de ritmos: sintomas depressivos em comunidades quilombolas

Resultados preliminares do estudo da associação entre alterações do ritmo de atividade/repouso e depressão em comunidades com diferentes níveis de urbanização

Luísa K. Pilz[1,2], Nicóli B. Xavier[1,2], Rosa Levandovski[3,4], Melissa A. B. Oliveira[1,2], Debora B. Constantino[1], André C. Tonon[1,2], Till Roenneberg[5,+], Maria Paz Hidalgo[1,2,+]

1 Laboratório de Cronobiologia e Sono, HCPA/UFRGS. Porto Alegre, RS - Brazil.

2 PPG em Psiquiatria e Ciências do Comportamento, UFRGS. Porto Alegre, RS - Brazil.

3 PPG Avaliação e Produção de Tecnologias para o SUS, GHC. Porto Alegre, RS – Brazil.

4 PPG Saúde Coletiva, UFRGS. Porto Alegre, RS – Brazil.

5 Institute of Medical Psychology, LMU. Munich, BY – Germany.

6 Visiting Professor at UFRGS/CAPES. Porto Alegre, RS – Brazil.

[+] These authors contributed equally to this work.

Sintomas de depressão e ritmos biológicos em comunidades quilombolas

Os resultados deste capítulo são acrescentados como resultados complementares à tese. Estes são os primeiros resultados de um manuscrito em construção em que investigamos a relação entre sintomas depressivos e alterações/características de ritmos de sono-vigília em comunidades quilombolas do sul do Brasil. Para isso, utilizamos o Inventário de Depressão de Beck (Beck Depression Inventory, BDI) e o Questionário de Cronotipos de Munique (MCTQ). em comunidades quilombolas do sul do país. As comunidades apresentam-se como essencialmente rurais com históricos de acesso a eletricidade variados. Para estas análises, utilizamos dados coletados em 12 comunidades (N = 221, por comunidade n = 2 - 50).

Tabela 1 – características da amostra (N = 221)	
Idade: mediana [IQR]	46 [27]
Sexo - mulher: n (%)	132 (58%)
Escolaridade: n (%) N = 221	
Analfabeto	27 (13)
Até 4a série	93 (46)
Até 7a série	35 (17)
Ensino Fundamental completo	16 (7)
Ensino Médio incompleto	11 (5)
Ensino Médio completo	15 (7)
Ensino Superior	4 (2)
Pós-Graduação	1 (.5)
Escore de BDI:	
mediana [IQR]	4 ± 7
Variáveis do MCTQ	
Ponto médio de sono em dias livres: mediana [IQR]	3.04 [1.86]
Ponto médio de sono em dias de trabalho: mediana [IQR]	2.54 [1.40]
Jetlag social: mediana [IQR]	0.25 [1.00]
Exposição à luz em dias livres: mediana [IQR]	5.00 [6.75]

Inicialmente, verificamos as características do BDI nesta população. A figura 1 representa a matriz de correlação entre itens. Linhas mais espessas indicam associações mais fortes entre os itens e entre os itens e o escore total.

A escala apresentou boa consistência, com um alfa de Cronbach de 0.84. A tabela 2 mostra, entre outros, a correlação ajustada entre itens e o escore total. Podemos ver que questões relacionadas a humor deprimido, auto-rancor, auto-acusação, pessimismo, sensação de fracasso e sentimentos de culpa são as mais relacionadas ao escore.

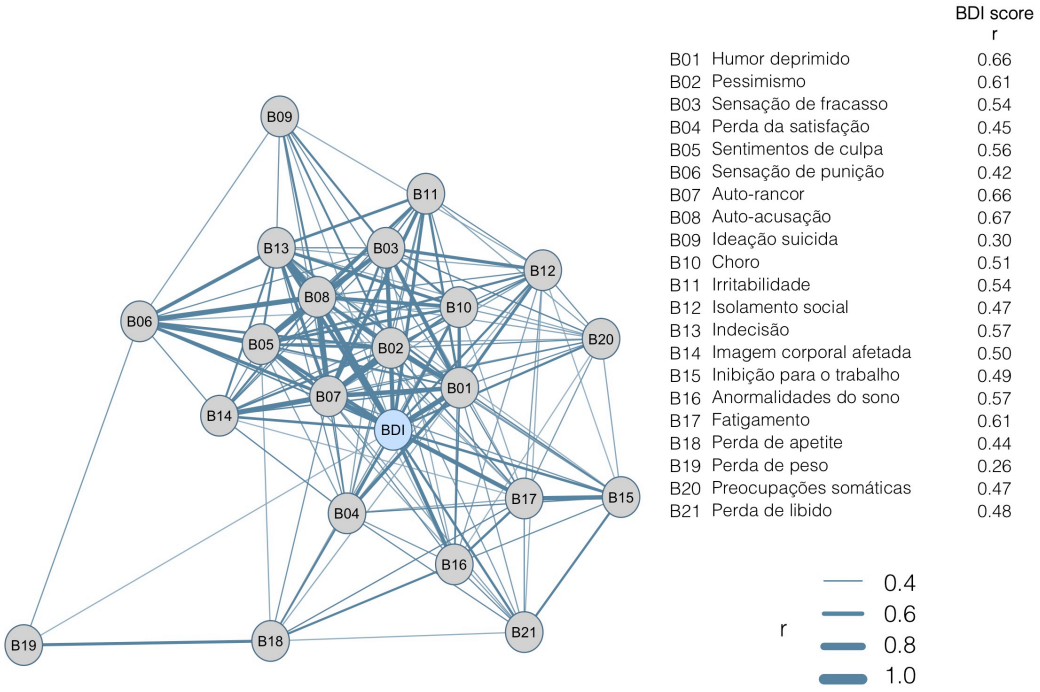

Figura 1. Rede de correlações dos itens do Inventário de Depressão de Beck (BDI, Beck Depression Inventory). Representação da correlação entre itens e com o escore total. Linhas mais espessa indicam correlações mais fortes. BDI: escore total. Os valores ao lado da legenda de cada item representam seu coeficiente de correlação com o escore total.

Tabela 2 - Inventário de Depressão de Beck em comunidades quilombolas

	Correlação Item-Total Ajustada	Média da escala se o item fosse excluído	Variância da escala se o item fosse excluído	Correlação Quadrática Múltipla	Alfa de Cronbach se o item for excluído
Humor deprimido	0.60	6.22	38.69	0.44	0.82
Pessimismo	0.55	6.23	39.12	0.47	0.82
Sensação de fracasso	0.50	6.35	40.22	0.42	0.83
Perda da satisfação	0.33	6.31	40.96	0.20	0.83
Sentimentos de culpa	0.50	6.22	39.69	0.41	0.83
Sensação de punição	0.25	6.33	41.10	0.37	0.84
Auto-rancor	0.59	6.33	39.60	0.49	0.82
Auto-acusação	0.57	6.25	38.25	0.56	0.82
Ideação suicida	0.16	6.47	42.96	0.14	0.84
Choro	0.46	6.25	39.29	0.34	0.83
Irritabilidade	0.44	6.1	39.07	0.31	0.83
Isolamento social	0.41	6.33	40.52	0.28	0.83
Indecisão	0.50	6.12	38.49	0.45	0.82
Imagem corporal afetada	0.44	6.3	40.12	0.31	0.83
Inibição para o trabalho	0.39	6	38.81	0.37	0.83
Anormalidades do sono	0.41	5.9	37.48	0.34	0.83
Fatigamento	0.49	5.9	38.15	0.38	0.82
Perda de apetite	0.35	6.18	40.24	0.32	0.83
Perda de peso	0.08	6.28	42.32	0.22	0.84
Preocupações somáticas	0.40	6.04	39.66	0.20	0.83
Perda de libido	0.32	5.95	38.67	0.23	0.84

Após caracterizar a manifestação de sintomas depressivos nessas comunidades, avaliamos quais variáveis aferidas pelo MCTQ são capazes de prever um escore de Beck maior que 10, limiar sugerido para detectar depressão em amostras não clínicas[1]. Para isso, utilizamos um modelo hierárquico de regressão logística (tabela 2). O jetlag social, controlando-se para idade e sexo está significativamente associado a sintomas depressivos (BDI >10).

Tabela 2: Regressão hierárquica - preditores de BDI > 10 (N = 221)

	Modelo 1			**Modelo 2**			**Modelo 3**		
	Nagelkerke R^2 = 0.125			Nagelkerke R^2 = 0.125			Nagelkerke R^2 = 0.181		
	p < 0.001			p < 0.001			p < 0.001		
Variáveis	β	p	OR (95% IC)	β	p	OR (95% IC)	β	p	OR (95% IC)
Idade	0.01	0.15	1.01 (0.99 - 1.03)	0.01	0.18	1.01 (0.99 - 1.03)	0.02	< 0.05	1.03 (1.00 - 1.05)
Sexo (feminino)	1.53	<.001	4.63 (2.03 - 10.52)	1.54	< 0.001	4.67 (2.02 - 10.79)	1.82	< 0.001	6.20 (2.53 – 15.21)
Luz em dias livres				0.01	0.91	1.01 (0.91 - 1.11)	0.03	0.62	1.02 (0.92 - 1.14)
MSF							0.10	0.52	0.90 (0.67 - 1. 23)
SJL							0.78	< 0.01	2.19 (1.24 - 3.87)

Luz em dias livres: horas de exposição à luz natural por dia; MSF: ponto médio de sono em dias livres; SJL: jetlag social.

Estes resultados indicam que o desalinhamento circadiano e os tempos impostos pela sociedade podem ser um fator de risco para desenvolver depressão. Estudos longitudinais poderão revelar se a redução de níveis de jetlag social pode ser uma estratégia de prevenção da depressão.

[1] GORENSTEIN, C.; WANG, Y.-P.; HUNGERBÜHLER, I. **Instrumentos de Avaliação em Saúde Mental**: Artmed Editora, 2015.

APÊNDICE 2

Apresentações dos trabalhos desenvolvidos na tese em eventos científicos

Pôster: Sleep in women and men from Quilombolas communities. **Lisbon Sleep Summit.** Lisboa, 2018.

Pôster: Human sleep and activity in Quilombolas communities at different levels of electrification and urbanisation. **Gordon Research Conference - Sleep Regulation and Function.** Galveston, 2018.

Apresentação Oral: Human sleep and activity in Quilombolas communities at different levels of electrification and urbanisation. **Gordon Research Seminar - Sleep Regulation and Function.** Galveston, 2018.

Pôster: Estudo da associação entre exposição à luz, sono e depressão em comunidades quilombolas. **69ª Reunião Anual da SBPC.** Belo Horizonte, 2017.

Simposista: Ciclo circadiano. **II Semana do Cérebro - FSG.** Caxias do Sul, 2017.

Pôster: What we learned from Quilombolas about light, sleep and depression. **23rd Congress of the European Sleep Research Society.** Bologna, 2016.

Simposista: Enlightening the effects of artificial light on biological rhythms. **XIV European Biological Rhythms Society (EBRS) and IV World Congress of Chronobiology (WCC)**. Manchester, 2015.

Apresentação Oral: Enlightening the effects of artificial light on biological rhythms. **LMU-Harvard Young Scientists Forum**. Munich, 2015.

Simposista: Licht, Schlaf und die innere Uhr: Sleep, light and depression in the wilderness. **XXXIV Deutsche Gesellschaft für Kinder- und Jugendpsychiatrie, Psychosomatic und Psychotherapie e.V.** Munich, 2015.

Apresentação oral: Luz Artificial, Ritmos Biológicos e Transtornos Psiquiátricos (Artificial light, biological rhythms and psychiatric disorders). **27º Ciclo de Avanços em Clínica Psiquiátrica**. Porto Alegre, 2014.

Outros artigos publicados/produzidos durante o período de doutoramento

DE LA IGLESIA, HORACIO O; MORENO, CLAUDIA; LOWDEN, ARNE; LOUZADA, FERNANDO; MARQUEZE, ELAINE; LEVANDOVSKI, ROSA; **PILZ, LUISA K**; VALEGGIA, CLAUDIA; FERNANDEZ-DUQUE, EDUARDO; GOLOMBEK, DIEGO A; CZEISLER, CHARLES A; SKENE, DEBRA J; DUFFY, JEANNE F; ROENNEBERG, TILL. Ancestral sleep. Current Biology, v. 26, p. R271-R272, 2016.

PILZ, LUÍSA K; KELLER, LENA KATHARINA; LENSSEN, DAVID; ROENNEBERG, TILL. Time to rethink sleep quality: PSQI scores reflect sleep quality on workdays. SLEEP, *in press*, 2018.

BEAUVALET, JULIANA C; **PILZ, LUÍSA K;** HIDALGO, MARIA PAZ; ELISABETSKY, ELAINE. **Is chronodisruption a vulnerability factor to stress?** *Under revision*, Behavioral Brain Research.

ROSA, GABRIEL S; ANDRADES, GUSTAVO S; CAYE, ARTHUR; HIDALGO, MARIA PAZ; OLIVEIRA, MELISSA A B; **PILZ, LUÍSA K**. Thirteen Reasons Why: the impact of suicide portrayal on adolescents' mental health**.** *Under revision,* Journal of Psychiatric Research.

Prêmios

Carl Storm International Diversity (CSID). Award to attend the 2018 Sleep Regulation and Function GRC, Gordon Research Conferences. 2017.

Destaque XXIX Salão de Iniciação Científica. Bolsista: Debora Constantino. Estudo do Processo Evolutivo do Sono em Ambientes de Luz Natural e Artificial. UFRGS, 2017.

Jovem Pesquisador XXVIII Salão de Iniciação Científica. Bolsista: Valdomiro Machado. Estudo da associação entre exposição à luz, sono e depressão em comunidades quilombolas. UFRGS, 2016.

Prêmio Sony Santos. Bolsista: Valdomiro Machado. Prevalência de hipertensão arterial sistêmica em comunidades quilombolas. I Simpósio Internacional de Saúde da População Negra. Prefeitura Municipal de Porto Alegre, UFRGS, CONASEMS, ONU AMERICAS, UNFPA, OPAS, ONU MULHERES, MS, 2016.

Destaque XXVII Salão de Iniciação Científica. Bolsista: Juliana Beauvalet. Estudo do efeito do estresse e do fotoperíodo sobre ritmos de temperatura central e de atividade e repouso, UFRGS, 2015.

Difusão da ciência

Organização de blog e página em rede social:

PILZ, LUÍSA K; CARISSIMI, ALICIA; ILGENFRITZ, CARLOS A; OLIVEIRA, MELISSA A B; TONON, ANDRÉ C; BEAUVALET, J; HIDALGO, MARIA PAZ. Seu corpo, seu tempo. Tema: Cronobiologia. 2014 – 2017.

Textos produzidos para o blog:

PILZ, LUÍSA K. Bridging the science gap. 2017. https://seucorposeutempo.wordpress.com/2017/03/24/bridging-the-science-gap/

PILZ, LUÍSA K. Ainda? 2015. https://seucorposeutempo.wordpress.com/2015/05/09/ainda/

PILZ, LUÍSA K. Por que você PRECISA dormir? 2015. https://seucorposeutempo.wordpress.com/2015/07/29/por-que-voce-precisa-dormir/

ILGENFRITZ, CARLOS A; PILZ, LUÍSA K. 2015: o ano internacional da luz. 2015. https://seucorposeutempo.wordpress.com/2015/03/18/2015-o-ano-internacional-da-luz/

PILZ, LUÍSA K. Já inventaram um aplicativo para isso... 2014. https://seucorposeutempo.wordpress.com/2014/10/30/ja-inventaram-um-aplicativo-para-isso/

PILZ, LUÍSA K. Sobre o seu tempo interno e o que são cronotipos. 2014. https://seucorposeutempo.wordpress.com/2014/09/16/sobre-o-seu-tempo-interno-e-o-que-sao-cronotipos-2/

Apresentações em eventos relacionados à difusão da ciência:

Participação na mesa "Cronobiologia e alterações do comportamento". V Semana Nacional do Cérebro - UFRGS. 2016.

Palestra no minicurso "Alterações de sono e ritmo: fatores associados e o que fazer". XXIII Semana Acadêmica da Medicina da UFRGS. 2017.

Aula na liga de Cronobiologia "Qualidade do sono: o que é dormir bem" – UFRGS. 2017.