UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
FACULDADE DE MEDICINA
PROGRAMA DE PÓS-GRADUAÇÃO EM PSIQUIATRIA E CIÊNCIAS DO COMPORTAMENTO

MELISSA ALVES BRAGA DE OLIVEIRA

ABRINDO CAMINHOS PARA A CRONOMEDICINA: DE ESTUDOS PRÉ-CLÍNICOS E EPIDEMIOLÓGICOS À PRÁTICA CLÍNICA

Porto Alegre

2023

MELISSA ALVES BRAGA DE OLIVEIRA

ABRINDO CAMINHOS PARA A CRONOMEDICINA: DE ESTUDOS PRÉ-CLÍNICOS E EPIDEMIOLÓGICOS À PRÁTICA CLÍNICA

Tese apresentada como requisito parcial para a obtenção do título de Doutora em Psiquiatria e Ciências do Comportamento, à Universidade Federal do Rio Grande do Sul, Programa de Pós-Graduação em Psiquiatria e Ciências do Comportamento.

Orientadora: Professora Dra Maria Paz Loayza Hidalgo

Co-orientadora: Professora Dra Maria Elisa Calcagnotto

Porto Alegre

2023

CIP - Catalogação na Publicação

Alves Braga de Oliveira, Melissa
ABRINDO CAMINHOS PARA A CRONOMEDICINA: DE ESTUDOS PRÉ-CLÍNICOS E EPIDEMIOLÓGICOS À PRÁTICA CLÍNICA / Melissa Alves Braga de Oliveira. -- 2023.
242 f.
Orientadora: Maria Paz Loayza Hidalgo.

Coorientadora: Maria Elisa Calcagnotto.

Tese (Doutorado) -- Universidade Federal do Rio Grande do Sul, Faculdade de Medicina, Programa de Pós-Graduação em Psiquiatria e Ciências do Comportamento, Porto Alegre, BR-RS, 2023.

1. Cronobiologia. 2. Ritmos biológicos. 3. Eletrofisiologia. 4. Iluminação. 5. Transtornos de Humor. I. Loayza Hidalgo, Maria Paz, orient. II. Calcagnotto, Maria Elisa, coorient. III. Título.

Elaborada pelo Sistema de Geração Automática de Ficha Catalográfica da UFRGS com os dados fornecidos pelo(a) autor(a).

AGRADECIMENTOS

À minha família pelo amor, zelo e apoio incondicionais. Em especial aos meus pais, Tania e Solano, que investiram na minha educação, respeitarem as minhas escolhas e a quem eu devo grande parte das minhas conquistas e por quem eu sinto muito orgulho e gratidão; à minha irmã Maíra, pela paciência gigantesca de irmã mais velha e pelo exemplo de ser humano incrível e generoso; às minhas avós Marieta e Darcy, as matriarcas da minha família, por todas as muitas e necessárias orações e por serem exemplos de resiliência e dedicação; e à minha tia Talita (in memoriam), que nos deixou antes de poder se gabar da sobrinha doutora, que sempre me fez dar boas risadas e segue olhando por nós na paz eterna.

À minha orientadora, Maria Paz Hidalgo - gracias a la vida que me a dado tu – pelos ensinamentos do fazer ciência e da vida, pelas inúmeras e maravilhosas oportunidades, pela confiança no meu trabalho, pela paciência diante das minhas limitações, por investir no meu desenvolvimento e sempre me incentivar a seguir carreira científica, por todo o tempo disponível para me orientar e por se fazer tão presente na minha vida até nos momentos mais delicados. Muito já aprendi e sigo aprendendo contigo. Gracias por todo.

À minha co-orientadora, Maria Elisa Calcagnotto, por me abrir as portas do NNNESP Lab, por me confiar equipamentos preciosos e acreditar na minha capacidade, pela paciência e sensibilidade diante das minhas dificuldades, pela disponibilidade e por todo conhecimento compartilhado.

Aos meus queridos colegas do Laboratório de Cronobiologia e Sono, que tornam o lab a minha segunda casa, um local onde trabalho com prazer e cuja convivência sadia e divertida me torna uma pessoa melhor. Obrigada por todas as trocas, pela torcida e incentivos, por vibrarem com cada conquista individual como uma grande conquista do grupo. É sempre um prazer lotar a sala do laboratório e partilhar um café recém passado ao lado de vocês. Em especial, agradeço aos colegas "eméritos" do lab Caroline, Alicia, Felipe, Carlos, Luciene, Ju Freitas, Danilo, Alicia, Alê, Débora, Aninha e Luísa; e aos atuais Laura, Pedro, Fernanda, Gui, Manu, Pato, André e Nicóli.

Aos colegas do NNNESP Lab, pelas discussões enriquecedoras; em especial à Maiara, Letícia e Natividade pelo auxilio nos experimentos, e à Querusche pela genialidade e domínio do MATLAB para análise de dados.

Aos profissionais da Unidade de Experimentação Animal do HCPA, especialmente à enfermeira Marta, às veterinárias Daniela e Tuane, aos queridos Ignácio, Rosa, Sônia e Vera por todo auxílio e suporte durante a realização dos experimentos, pelas boas conversas e por me darem toda segurança para condução do projeto prezando pelo bem-estar animal e qualidade técnica.

Aos profissionais da Unidade de Estatística do HCPA, Vânia Hirakata e Rogério Boff, pela inúmeras consultorias e boa disposição em ajudar.

Aos meus amigos do CMPA, da dança, da UFRGS e da vida, que me apoiam e entendem minhas ausências, que deixam meus dias mais leves e divertidos e que se fazem presentes apesar da distância. Perto de vocês sempre me sinto especial e amada.

À Cláudia Grabinski e ao Everaldo Almeida, pela solicitude e disposição para resolver os imprevistos do dia-a-dia.

Ao Programa de Pós-Graduação em Psiquiatria e Ciências do Comportamento da UFRGS, às agências de fomento à pesquisa CNPq e FIPE-HCPA, e à empresa Luxion Iluminações pelo incentivo fundamental à pesquisa científica e por oportunizarem a realização desta tese.

És um senhor tão bonito
Quanto a cara do meu filho
Tempo, tempo, tempo, tempo
Vou te fazer um pedido
Tempo, tempo, tempo, tempo

Compositor de destinos
Tambor de todos os ritmos
Tempo, tempo, tempo, tempo
Entro num acordo contigo
Tempo, tempo, tempo, tempo

Por seres tão inventivo
E pareceres contínuo
Tempo, tempo, tempo, tempo
És um dos deuses mais lindos
Tempo, tempo, tempo, tempo

Oração ao Tempo – Caetano Veloso,
A Outra Banda da Terra

RESUMO

Introdução: A existência de sistemas temporizadores, ou relógios biológicos, desde os organismos mais simples aos mais complexos, permite antecipar-se a determinados acontecimentos cíclicos gerando ritmicidade nos processos biológicos. Consequentemente, esse processo permite que o ser vivo funcione de forma paralela ao ambiente, sincronizando ritmos internos de processos fisiológicos e comportamentais aos ritmos externos e, portanto, confere melhor adaptação e manutenção da saúde ao organismo. Com o advento da eletricidade e mudança no estilo de vida, o ser humano deixou de organizar suas rotinas de acordo com as transições naturais do ambiente onde está inserido. As consequências para a saúde física e mental vem sendo cada vez mais estudadas, e a cronobiologia vem ganhando crescente reconhecimento através da cronomedicina. Diante disso, faz-se necessário ter à disposição ferramentas de fácil uso e aplicação para avaliação de ritmos bem como de suas perturbações. **Objetivo:** desenvolver e aprimorar ferramentas de avaliação e intervenção em ritmos biológicos para aplicabilidade na área da cronomedicina. **Metodologia:** *Estudo 1:* Desenvolvemos uma ferramenta para auxiliar pesquisadores que não são da área de Cronobiologia durante a elaboração do desenho do estudo, bem como da coleta, análise, interpretação e divulgação dos resultados levando em consideração ritmos biológicos. *Estudo 2:* Investigamos o impacto da exposição a diferentes sistemas de iluminação nas oscilações cerebrais de roedores. *Estudo 3*: Avaliamos o impacto do uso de equipamentos individuais de proteção contra luz durante a noite no desenvolvimento e crescimento de neonatos prematuros. *Estudo 4:* Validamos escala desenvolvida para avaliação dos ritmos de humor (Instrumento de Ritmo de Humor - MRhI) para as línguas espanhola e inglesa, e realizamos uma análise transcultural do MRhI para compreender quais itens mantinham boas propriedades psicométricas entre amostras coletadas no Brasil, na Espanha e no Canadá. **Resultados:** *Estudo 1:* Criamos um fluxograma e um checklist destacando os principais aspectos que devem ser considerados e documentados no planejamento, desenvolvimento e comunicação dos estudos que visam analisar ritmos biológicos ou não, com objetivo de aumentar a validade, reprodutibilidade e qualidade geral das investigações científicas. Estudo 2: Diferentes regimes de claro-escuro afetam significativamente as oscilações cerebrais em ratos jovens e adultos. A luz constante aumenta a potência da oscilação delta em ambas as idades, a luz dinâmica aumenta a potência de beta nos ratos juvenis, e a luz dinâmica e a luz constante aumentam a potência em oscilações de alta frequência nas idades juvenil e adulta. Em relação à assimetria, a luz afeta a assimetria inter-hemisférica e a rede neural ântero-posterior, e possivelmente impacta o comportamento sono-vigília, o que ainda pretendemos compreender em análises futuras. *Estudo 3:* Os diferentes regimes de claro/escuro afetam o desenvolvimento dos neonatos. Os neonatos incluídos no grupo de uso de máscara para proteção contra luz receberam alta em média 4 dias antes daqueles que não utilizaram máscara, sugerindo que o ambiente escuro durante a noite associado ao uso de máscaras cria melhores condições para o desenvolvimento dos neonatos prematuros na unidade de tratamento intensivo. *Estudo 4*: Em humanos, faltam instrumentos para avaliar a percepção da ritmicidade. Portanto criamos um instrumento para uso na ligua Portuguesa, Inglesa e espanhol. Tanto a versão em espanhol, quanto a versão em inglês do MRhI demonstraram ter consistência interna, com alfa de Cronbach de 0.70 e 0.75 respectivamente. Além disso, em ambos estudos os participantes identificaram picos de manifestação das variáveis somáticas e cognitivas com mais frequência que as variáveis afetivas. Na amostra espanhola, as diferenças de acordo com gênero foram observadas apenas para libido, com as mulheres relatando a existência de pico para libido com maior frequência que homens, enquanto na amostra canadense, mulheres relataram maior frequência de picos diários de irritabilidade, ansiedade, tristeza e falar com amigos, enquanto os homens apresentaram picos com maior frequência para resolver problemas, libido e motivação para

praticar exercícios. Por fim, a avaliação psicométrica do MRhI nas amostras brasileira, espanhola e canadense permitiu um refinamento do instrumento, onde 11 dos 15 itens, que apresentaram boas propriedades entre culturas, foram mantidos em uma versão mais curta e revisada do MRhI (MRhI-r). Além disso, a análise da rede revelou uma estrutura onde o item relacionado ao sono desempenha um papel central na ligação dos domínios cognitivo, somático e afetivo. Desta forma, o MRhI-r é uma nova ferramenta para investigar a autopercepção da ritmicidade de sintomas e comportamentos relacionados ao humor, com boas propriedades psicométricas em múltiplas culturas. **Conclusões:** Nesta tese, a relevância de intervenções e aplicabilidade de instrumentos de avaliação em ritmos biológicos foi demonstrada através de estudos pré-clínicos, clínicos e epidemiológicos. O primeiro estudo fornece um guia útil para instruir projetos de pesquisa que possam servir de base para ações de prevenção, diagnóstico e tratamento de doenças levando ritmos biológicos em consideração. Tanto a exposição à luz exerce impacto nos ritmos cerebrais de roedores, como o uso de máscara contra exposição à luz durante a noite é capaz de diminuir o tempo até a alta hospitar em neonatos humanos. Estudos longitudinais que busquem associar a exposição à luz com características dinâmicas durante o dia e o escuro durante a noite para reforçar a sincronização dos ritmos e melhorar desfechos em saúde são de extrema importância como potenciais estratégias de prevenção e tratamento dentro da Cronomedicina. Por fim, o MRhI demonstrou ser um instrumento confiável e de fácil aplicação que pode ser útil em estudos transculturais sobre a associação entre padrões diários de variabilidade do humor e saúde mental. É uma ferramenta inovadora no estudo de transtorno de humor, na medida em que leva em consideração as possíveis percepções das oscilações de comportamentos que até agora não são avaliados pelos instrumentos que medem intensidade de humor ou presença de transtorno de humor. Estudos adicionais que avaliem a percepção de mudanças diárias do humor em amostras clínicas podem contribuir para a aplicação deste instrumento no diagnóstico e tratamento de transtornos psiquiátricos.

PALAVRAS-CHAVE: Cronobiologia; exposição à luz; eletrofisiologia; ritmos de humor; psicometria.

ABSTRACT

Introduction: The existence of temporal systems, or biological clocks, in organisms of all levels of complexity, enables anticipating certain cyclical events, generating rhythmicity in biological processes. Consequently, this process allows the living being to function in parallel to the environment, synchronizing internal rhythms of physiological and behavioral processes with external rhythms and, therefore, providing better adaptation and health maintenance. With the advent of electricity and lifestyle changes, human beings no longer organize their routines according to the natural transitions of the environment. The consequences for physical and mental health have been studied, and chronobiology has been gaining increasing recognition through chronomedicine. Therefore, it is imperative to have available easy-to-use tools to evaluate rhythms and its disturbances. **Objective**: to develop and improve tools for assessment and intervention in biological rhythms for applicability in Chronomedicine. **Methodology**: *Study 1*: We developed a tool to assist researchers who are not from the Chronobiology field during the study design, as well as the collection, analysis, interpretation and dissemination of results taking into account biological rhythms. *Study 2*: We investigated the impact of exposure to different lighting systems on brain oscillations in rodents. *Study 3*: We evaluated the impact of using individual light protection equipment at night on the development and growth of preterm infants. *Study 4*: We validated a scale developed to evaluate mood rhythms (Mood Rhythm Instrument - MRhI) for the Spanish and English languages, and carried out a cross-cultural analysis of the MRhI to understand which items maintained good psychometric properties among Brazilian, Spanish and Canadian samples. **Results**: *Study 1*: We created a flowchart and checklist highlighting the main aspects that must be considered and documented in the planning, development and communication of studies that aim to analyze biological rhythms or not, to increase the validity, reproducibility and general quality of the scientific investigations. *Study 2:* Different light-dark regimes significantly affect brain oscillations in juvenile and adult rats. Constant light increases delta oscillation power at both ages, dynamic light increases beta power in juvenile rats, and dynamic light and constant light increase power in high-frequency oscillations in juvenile and adult ages. Regarding asymmetry, light affects inter-hemispheric asymmetry and the antero-posterior neural network, and possibly impacts sleep-wake behavior, which we still intend to understand in future analyses. *Study 3*: Different light/dark regimes affect the development of newborns. The preterm infants included in the group using masks to light protection were discharged on average 4 days before the control group, suggesting that the dark environment at night associated with the use of masks creates better conditions for the development of newborns in the intensive care unit. *Study 4*: In humans, there is a lack of instruments to assess the perception of rhythmicity. Therefore, we criated an instrument for use in Portuguese, English and Spanish. Both the Spanish and English versions of the MRhI demonstrated adequate internal consistency, with Cronbach's alpha of 0.70 and 0.75 respectively. Furthermore, in both studies participants identified peaks of somatic and cognitive variables more frequently than affective variables. In the Spanish sample, differences according to gender were observed only for sexual arousal, with women reporting the existence of a peak in sexual arousal more frequently than men, while in the Canadian sample, women reported a higher frequency of daily peaks in irritability, anxiety, sadness and

talking to friends, while men showed peaks more frequently in solving problems, sexual arousal and motivation to exercise. Finally, the psychometric evaluation of the MRhI in the Brazilian, Spanish and Canadian samples allowed a refinement of the instrument, where 11 of the 15 items showed good cross-cultural properties and were maintained in a shorter and revised version of the MRhI (MRhI-r). Furthermore, the network analysis revealed a structure where the sleep-related item plays a central role in linking the cognitive, somatic and affective domains. Thus, the MRhI-r is a new tool to investigate the self-perception of the rhythmicity of mood-related symptoms and behaviors, with good psychometric properties across multiple cultures. **Conclusions**: In this thesis, we demonstrated the relevance of interventions and the applicability of assessment instruments in biological rhythms through pre-clinical, clinical and epidemiological studies. The first study provides a useful guide to instruct research projects that can serve as a basis for actions to prevent, diagnose and treat diseases, taking biological rhythms into account. Both exposure to light has an impact on the rodents' brain rhythms, and the use of a mask against exposure to light at night is capable of reducing the time until hospital discharge in human neonates. Longitudinal studies that seek to associate exposure to light with dynamic characteristics during the day and darkness during the night to reinforce the synchronization of rhythms and improve health outcomes are extremely important as potential prevention and treatment strategies within Chronomedicine. Finally, the MRhI demonstrated to be a reliable and easy-to-use instrument that can be applied in cross-cultural studies on the association between daily patterns of mood variability and mental health. It is an innovative tool in the study of mood disorders, as it takes into account possible perceptions of behavioral fluctuations that have not yet been assessed by instruments that measure mood intensity or the presence of a mood disorder. Additional studies that evaluate the perception of daily mood changes in clinical samples may contribute to the application of this instrument in the diagnosis and treatment of psychiatric disorders.

Keywords: Chronobiology; light exposure; electrophysiology; mood rhythms; psychometrics.

LISTA DE ABREVIATURAS E SIGLAS

aMT6s – 6-sulfatoximelatonina

BMAL1 – do inglês, *Brain and Muscle ARNT like protein 1*

CLOCK – do inglês, *Circadian Locomotor Output Cycle Kaput*

Cry – do inglês, *Cryptochromes*

DLMO - do inglês, *dim light melatonin onset* – início da secreção de melatonina

ipRGCs – células ganglionares intrinsicamente fotossensíveis da retina

NSQ – Núcleo supraquiasmático

Per – do inglês, *Period*

SUMÁRIO

CAPÍTULO 1 ï INTRODUÇÃO, REFERENCIAL TEÓRICO E OBJETIVOS

1. INTRODUÇÃO

Cada vez mais se valoriza o caráter translacional dos estudos científicos, buscando encurtar e facilitar a aplicação dos achados do laboratório na prática clínica. Esse movimento é desafiador na medida em que a grande demanda de dedicação por parte do pesquisador acaba gerando distanciamento do leito, ao mesmo tempo que o clínico se aprofunda na sua prática e se afasta da produção de conhecimento científico. Diante deste hiato que se apresenta no cuidado com o paciente, a Cronobiologia, ciência que estuda os ritmos biológicos, vem buscando estabelecer um caminho entre a ciência básica e a Medicina (1). A literatura atual mostra que disfunções do sistema temporizador circadiano estão presentes em uma variedade de condições clínicas como obesidade, diabetes, doenças cardiovasculares, câncer e transtornos de humor (2). Essa relação pode ser bidirecional, com as próprias patologias levando a comportamento e fisiologia circadiana alterados (3). Diante disso, desenvolver e aprimorar ferramentas de avaliação e intervenção em ritmos biológicos apresenta grande potencial para incorporar o conhecimento acerca dos ritmos na prevenção, diagnóstico e tratamento de doenças, ao qual damos o nome de Cronomedicina (4).

A Cronobiologia nos faz repensar o ser humano como um organismo inserido em um meio que é cíclico e interage com a nossa biologia em diferentes níveis de complexidade (5). A nível sistêmico, dispomos de uma estrutura chamada núcleo supraquiasmático (NSQ), que ocupa posição central no sistema temporizador circadiano, e que é responsável por sincronizar nossos ritmos internos ao ambiente onde estamos inseridos a partir da informação fótica dos ciclos claro/escuro (6). O NSQ tem projeções para diversas estruturas de regulação como em relógios periféricos localizados nos diferentes órgãos do corpo e que também estão sujeitos a pistas temporais como horários de alimentação e atividade física (7). A nível celular, como bem desvendado pelos ganhadores do prêmio Nobel de Medicina de 2017, Jeffrey C. Hall, Michael Rosbash e Michael W. Young, existem mecanismos moleculares de regulação circadiana compostos por alças de feedback positivo e negativo que regulam a expressão de genes relógio (8,9). Essa complexa maquinaria que sustenta os ritmos do organismo em sincronia aos ritmos do ambiente, pode ser afetada, por exemplo, pela exposição inadequada à luz, e as consequências para a saúde vem sendo associadas a diversas condições patológicas (10,11).

Com base no novo paradigma apresentado pela Cronomedicina, além de repensar o processo saúde-doença, ainda existe a necessidade de ampliar o conhecimento acerca do impacto dos ritmos na prática da pesquisa com vistas a aumentar a confiabilidade e reprodutibilidade dos estudos, propiciando maior utilização dos instrumentos atualmente disponíveis na prática clínica.

Esta tese busca fortalecer o caráter translacional da pesquisa que tem como base a fisiologia circadiana, com o objetivo final de promover a Cronomedicina através de estudos experimentais com foco na exposição à luz e estudos epidemiológicos com desenvolvimento de ferramentas para estudo do ritmo de humor. Primeiramente, descreve-se um estudo de revisão que busca fornecer orientações acerca do design, da análise e interpretação de dados, bem como da divulgação dos achados do estudo tendo em vista os ritmos biológicos. A seguir, é descrito um estudo experimental que objetivou compreender os efeitos da iluminação nas oscilações cerebrais de roedores, seguido por um estudo que verificou o impacto da utilização de máscaras de proteção contra iluminação em neonatos prematuros de uma unidade de terapia intensiva. Por fim, trazemos os estudos que auxiliaram na criação e validação do Instrumento de Ritmo de Humor, uma ferramenta que vem demonstrando potencial para auxílio no diagnóstico e tratamento de transtornos de humor.

2. CRONOBIOLOGIA E O SISTEMA TEMPORIZADOR CIRCADIANO

Tudo tem o seu tempo determinado, e há tempo para todo o propósito debaixo do céu.
Há tempo de nascer, e tempo de morrer; tempo de plantar, e tempo de arrancar o que se plantou;
Tempo de matar, e tempo de curar; tempo de derrubar, e tempo de edificar;
Tempo de chorar, e tempo de rir; tempo de prantear, e tempo de dançar;
Tempo de espalhar pedras, e tempo de ajuntar pedras; tempo de abraçar, e tempo de afastar-se de abraçar;
Tempo de buscar, e tempo de perder; tempo de guardar, e tempo de lançar fora;
Tempo de rasgar, e tempo de coser; tempo de estar calado, e tempo de falar;
Tempo de amar, e tempo de odiar; tempo de guerra, e tempo de paz.

Eclesiastes 3:1-8

Cronobiologia é a ciência que estuda os ritmos biológicos, que são identificados na imensa maioria dos seres vivos, podendo apresentar durações e frequências diversos. É multidisciplinar por natureza e capaz de fornecer instrumentos de estudo que podem ser aplicados a diversos campos de conhecimento (12). A existência da ritmicidade é inerente a vida na Terra, e a vida, por sua vez, se submete ao tempo.

O desenvolvimento de sistemas temporais e ritmos biológicos é resultado de uma necessidade evolutiva. É bem entendido que os organismos vivos na Terra desenvolveram relógios biológicos porque há uma vantagem evolutiva em poder prever variações cíclicas no ambiente (e.g. transições claro/escuro, variação de temperatura, aridez/chuvas, disponibilidade de alimento), o que otimiza a homeostase energética e aumenta as chances de sobrevivência (12). A existência de sistemas temporizadores, ou relógios biológicos, desde os organismos mais simples aos mais complexos, permite antecipar-se a determinados acontecimentos cíclicos gerando ritmicidade nos processos biológicos. Consequentemente, esse processo permite que o organismo funcione de forma paralela ao ambiente, sincronizando ritmos internos de processos fisiológicos e comportamentais aos ritmos externos (13,14).

O primeiro indício de que a existência de ritmos não se dá apenas como uma reação aos ciclos do ambiente, surgiu com as observações do astrônomo francês Jean Jacques d'Ortous de Mairan diante da planta *Mimosa pudica* em 1729. Mesmo quando submetida a uma situação de escuro constante, a planta permanecia abrindo suas folhas durante o dia e fechando-as durante à noite, demonstrando a existência de ritmo interno de padrão circadiano, ou seja, que durava cerca de um dia (≅ 24h) (15). Ainda não se sabia qual estrutura era responsável por regular esse comportamento, mas com os avanços na pesquisa circadiana descobriu-se uma natureza altamente conservada no que se refere ao relógio biológico de diversos organismos, desde cianobactérias, fungos, e plantas, até *Drosophilas* e mamíferos (Figura 1)(16).

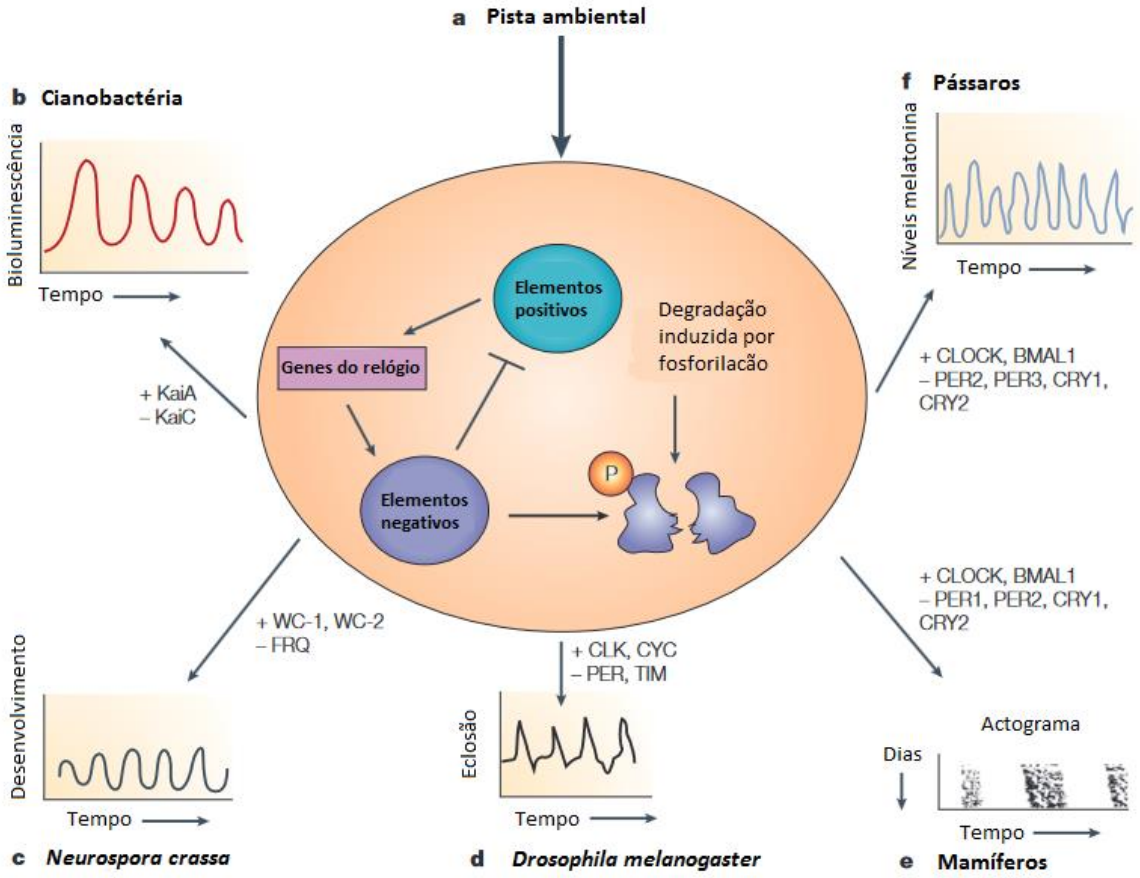


Figura 1. Mecanismo comum de controle de osciladores circadianos. (a) A maior parte dos sistemas circadianos usa um mecanismo temporal que envolve osciladores formados por elementos positivos e negativos, que formam ciclos de retroalimentação. Os elementos positivos ativam a expressão dos genes do relógio, enquanto estes, além de produzirem outputs biológicos de padrão rítmico, codificam elementos negativos que inibem as atividades dos elementos positivos. A fosforilação dos elementos negativos leva à sua eventual degradação, permitindo que os elementos positivos reiniciem o ciclo. Por vezes, os genes do relógio também podem funcionar positivamente para aumentar a expressão dos elementos positivos. Embora o mesmo mecanismo básico esteja presente, os componentes variam em diferentes organismos (b-f). Brain and muscle Arnt-like protein 1 (BMAL1), CLOCK (CLK na *Drosophila melanogaster*), CYCLE (CYC), cryptochrome (CRY), FREQUENCY (FRQ), KaiA, KaiC, period (PER), timeless (TIM), WHITE COLLAR-1 (WC-1) e WHITE COLLAR-2 (WC-2). Adaptada de Bell-Pedersen et al, 2005.

Em mamíferos, o sistema temporizador circadiano se organiza de forma hierárquica, tendo o núcleo supraquiasmático (NSQ) na posição central, sendo também denomindado relógio ou marca-passo central. O papel dessa estrutura na gênese dos ritmos foi descoberto em 1972 (17), após roedores serem submetidos a lesões restritas aos NSQs, possibilitando a visualização de alterações em ritmos biológicos como atividade motora e níveis de corticosterona (18,19). Trata-se de uma estrutura anatômica formada por dois núcleos simétricos e ovais situados na zona periventricular ventral, dorsal ao quiasma óptico, lateral ao terceiro ventrículo e medial à área hipotalâmica anterior (Figura 2, Figura 3a). O motivo pelo

qual, na maioria das vezes, esses núcleos são denominados como uma única estrutura (NSQ), se dá em razão de estarem interconectados através de diversos circuitos locais e funcionalmente atuarem como um só (6).

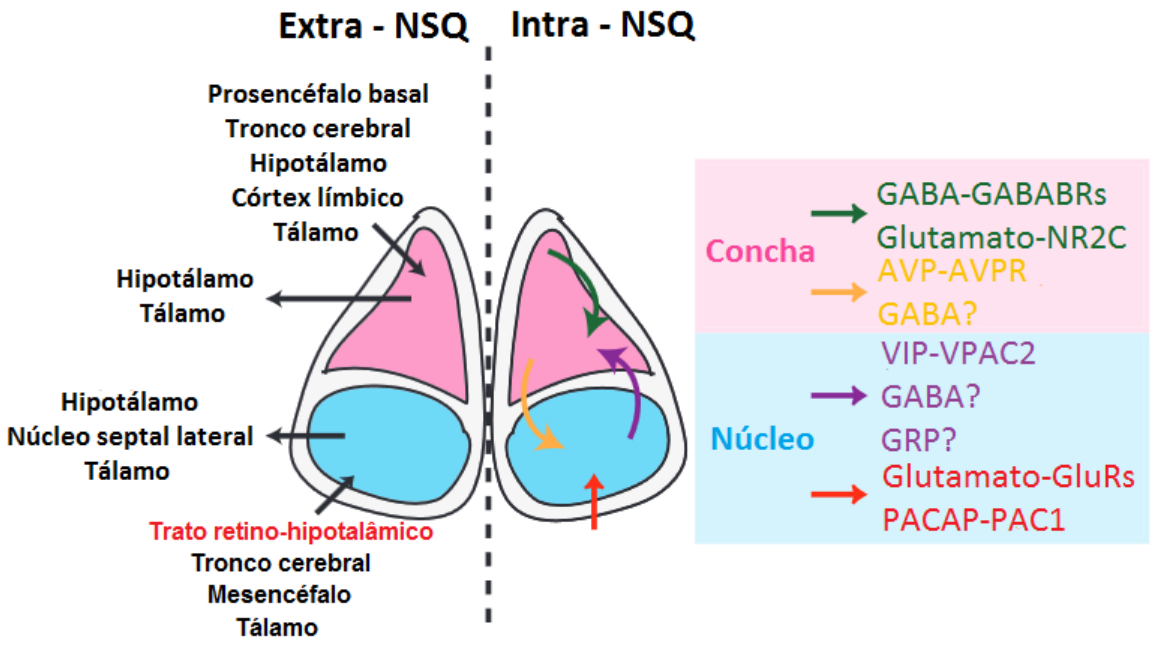


Figura 2. Sinalizações do núcleo supraquiasmático. Esquema mostrando as projeções aferentes e eferentes do núcleo supraquiasmático (NSQ), juntamente com sinais neuroquímicos e subdivisões neuroanatômicas desta estrutura. Adaptado de Patton e Hastings, 2018.

Além de receber aferências com origem na retina via trato retino-hipotalâmico, o NSQ apresenta projeções para outras regiões encefálicas como prosencéfalo basal, mesencéfalo, hipotálamo e tálamo envolvidas na regulação de aprendizado, memória, comportamento, funções endócrinas, ciclos do sono, apetite entre outras funções (Figura 3b). No entanto, apesar de toda a sua complexidade neuroquímica, o NSQ parece simplesmente ligar o comportamento e a fisiologia do organismo ao meio externo, criando e transmitindo uma representação interna do tempo solar, que se dá a partir das variações claro/escuro do ambiente. A literatura atual sugere que o controle exercido pelo NSQ vai muito além de apenas programação de padrões de atividade comportamental, visto que também regula ingesta de água, qualidade de sono e temperatura corporal e humor (20).

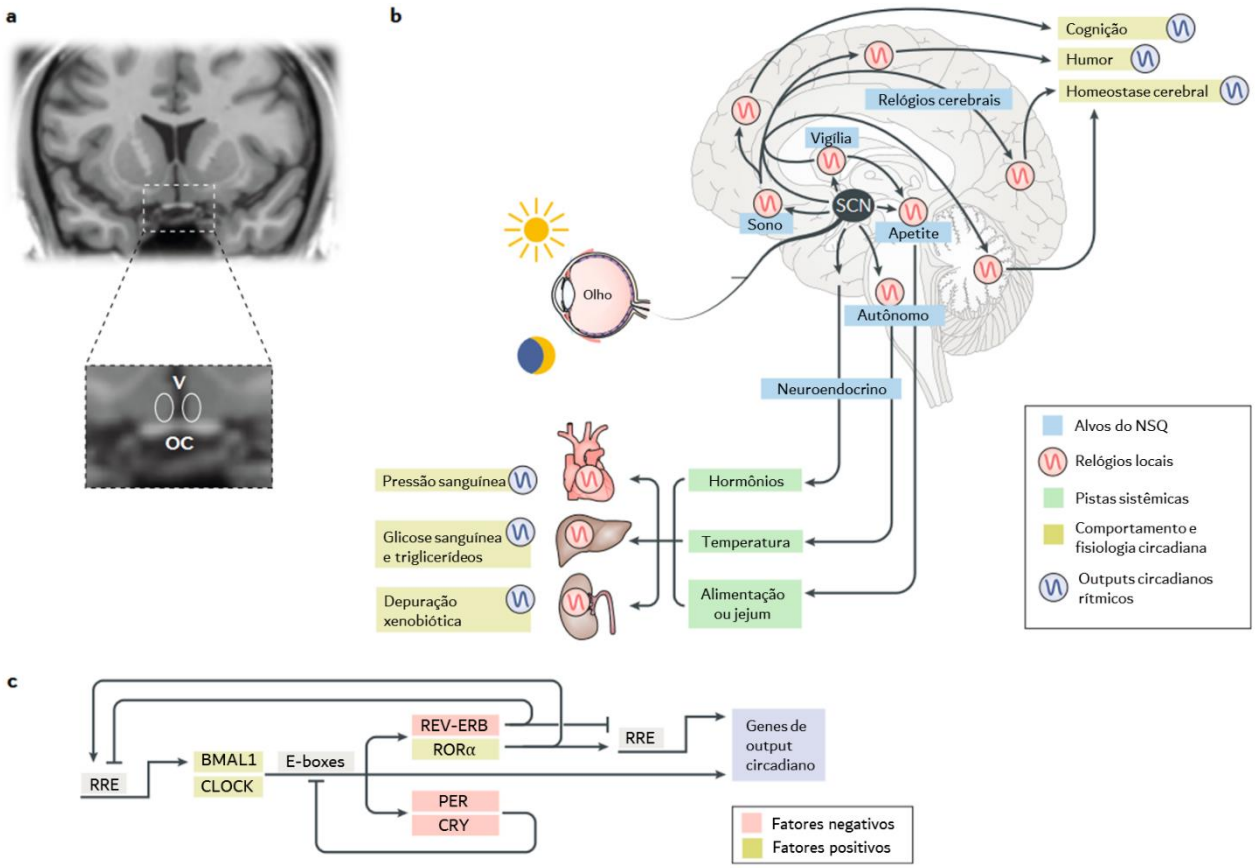


Figura 3. Sistema temporizador circadiano de mamíferos. (a) O NSQ localiza-se no hipotálamo, em destaque na imagem de ressonância magnética coronal de um cérebro humano. (b) O NSQ recebe inervação da retina através do trato retino-hipotalâmico a fim de garantir a sincronização aos ciclos claro-escuro e se projeta a regiões cerebrais, muitas das quais contêm relógios circadianos locais que coordenam ritmos circadianos comportamentais, autonômicos e neuroendócrinos. Estas pistas sistêmicas sincronizam os relógios moleculares locais dos tecidos periféricos, que, por sua vez, regulam a expressão genética local associada a ritmos fisiológicos críticos para a saúde. (c) Esquema simplificado da maquinaria molecular do relógio circadiano de mamíferos composta por alças de feedback positivo e negativo. Núcleo supraquiasmático (NSQ); Circadian locomoter output cycles protein kaput (CLOCK); Brain and muscle ARNT-like 1 (BMAL1); enhancer box (E-box); period (PER); cryptochrome (CRY); receptores nucleares (REV-ERB e RORα); REV response elements (RREs). Adaptada de Hastings et al, 2018.

A nível molecular, o sistema temporizador circadiano apresenta mecanismos de regulação circadiana compostos por alças de feedback positivo e negativo que regulam a expressão de genes do relógio nos diversos tecidos pelo corpo (Figura 3c)(21,22). No entanto, esses ritmos observados nos relógios periférico, ainda que independentes, são sincronizados pelo NSQ, como um regente que, a partir do sinal fótico do meio externo, organiza a atividade dos diferentes instrumentos em uma orquestra. Os mecanismos intracelulares dos circuitos de retroalimentação incluem os genes do relógio como Clock, Bmal1, Per1, Per2, Cry1 e Cry2,

essenciais para a geração de oscilações circadianas. Estas oscilações moleculares ocorrem tanto dentro do NSQ quanto em células periféricas, sendo redefinidas por sincronizadores externos ou internos (23).

Aos sincronizadores (ou encarrilhadores) externos, damos o nome de *zeitgebers*, que em alemão significa "o que doa ou marca o tempo". Os *zeitgebers* podem ser sinais ambientais e sociais que fornecem informações para o sistema circadiano e auxiliam na sincronização dos ritmos biológicos (24). Além da luz, que é considerado o principal e mais robusto *zeitgeber*, os estímulos não-fóticos, como a ingestão de alimentos e a atividade física, também podem influenciar os ritmos circadianos (25). Na figura 4, podemos observar a representação gráfica dos padrões oscilatórios de algumas variáveis biológicas que apresentam ritmicidade circadiana. O sistema temporizador circadiano auxilia na manutenção da saúde quando atua sem a interferência de fatores que possam causar disrupção nos ritmos, como observado em alguma doença ou comportamento que leve ao enfraquecimendo de pistas ambientais (e.g. trabalho noturno). Contudo, mudanças nos padrões de certos ritmos biológicos, como diminuição de amplitude e mudança de fase, são esperados durante a vida com o avanço da idade (26). Assim como o tempo, nosso corpo está em constante processo de mudança.

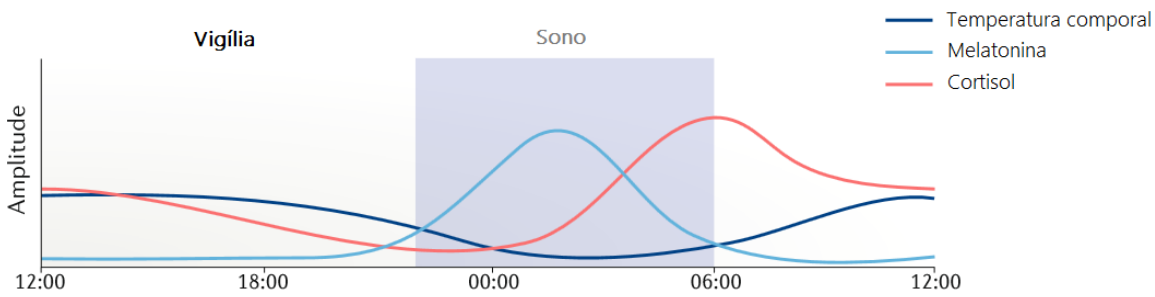


Figura 4. Ritmos circadianos durante a fase adulta. Os ritmos circadianos apresentam diferenças naturais em relação à amplitude e à fase, como podemos ver através dos padrões oscilatórios dos ritmos de temperatura corporal, melatonina e cortisol ao longo de 24h. O sistema temporizador circadiano organiza essas características temporais para que o organismo esteja sincronizado ao ambiente e para manutenção da saúde. Adaptada de Logan & McClung, 2019.

Os ritmos de sono-vigília, ou de atividade-repouso, também figuram entre os principais ritmos circadianos em mamíferos. No entanto, associados a eles, existem outros ritmos, de diferentes frequências, mas de grande relevância pois, nos informam sobre o comportamento e os estados de consciência de um organismo: os ritmos cerebrais.

3. RITMOS CEREBRAIS

Os ritmos biológicos podem ser classificados de acordo com sua frequência em três grandes grupos: ritmos circadianos, ultradianos e infradianos. O termo *circadiano* foi cunhado por Franz Halberg e, de acordo com o seu significado, os ritmos circadianos têm períodos de cerca de um dia (27). Os ritmos cujos períodos duram menos de um dia, ou melhor, menos de 20 horas são ditos *ultradianos*, e os que duram mais de 28 horas são chamados *infradianos*. Os ritmos cerebrais são considerados, portanto, ritmos ultradianos (12).

Os ritmos cerebrais são resultantes do fluxo de cargas iônicas que ocorre durante as sinapses entre as células neurais. Esse fluxo gera um campo elétrico extracelular que caracteriza as oscilações cerebrais (28). A diferença de potencial elétrico extracelular pode ser medida através de um eletrodo de registro e um eletrodo de referência. O eletroencefalograma (EEG) é uma das técnicas utilizadas para registro de atividade cerebral que envolve a colocação de eletrodos no escalpo, sendo usada em ambientes clínicos e de pesquisa (29). O EEG foi descoberto por Hans Berger em 1920 e representa uma técnica menos invasiva que a eletrocorticografia, cujos registros são feitos a partir de eletrodos subdurais na superfície do córtex, e cujas medidas de potencial de campo local são obtidas por eletrodos de profundidade intracerebrais (Figura 5a) (30).

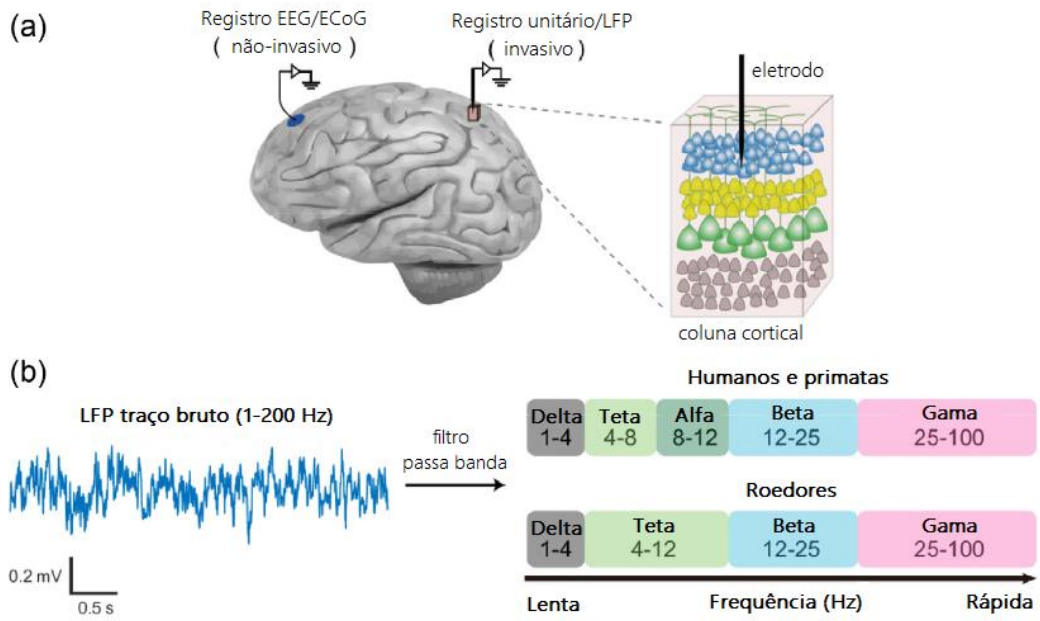


Figura 5. Registro dos ritmos cerebrais. (a) Os métodos para aquisição de sinais elétricos. (b) Decomposição do eletroencefalograma em diferentes oscilações. Eletroencefalograma (EEG); Eletrocorticograma (ECoG); *local field potential* (LFP). Figura adaptada de Luo & Guan, 2018.

As oscilações cerebrais apresentam bandas de frequências distintas dependendo dos tipos de células envolvidas, do número de sinapses e da localização espacial dentro da rede neural. Em roedores, estas frequências variam de 1-150 Hz e são classificadas em ondas delta, teta, beta e gama (Figura 5b) (31). Para melhor compreender a o processo de decomposição do sinal eletroencefálico, podemos estabelecer relação com o fenômeno que ocorre quando a luz branca incide sobre um prisma e é decomposta em ondas de distintas frequências. O mesmo ocorre com o sinal elétrico que é registrado no EEG e submetido a ferramentas matemáticas (Figura 6).

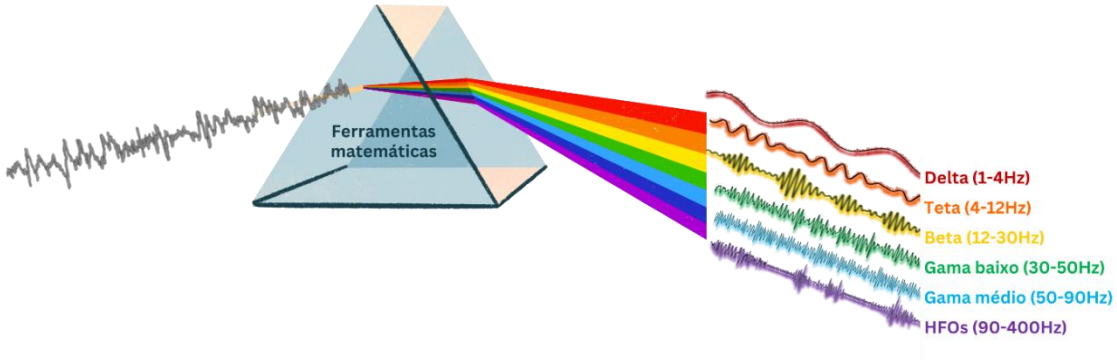


Figura 6. Decomposição do eletroencefalograma em diferentes oscilações.

As diferentes oscilações, ou ondas cerebrais, podem ser correlacionadas a diferentes estados de consciência e comportamento (28). Os ritmos considerados fisiológicos estão descritos na tabela 1 juntamente com suas manifestações comportamentais (31). Já ritmos cerebrais anormais são identificados em condições patalógicas como doenças neuropsiquiátricas e epilepsias (32).

Tabela 1. Oscilações corticais e comportamentos em humanos e roedores.

	Oscilação cortical	Estados de consciência e comportamentos
Humano	Delta (1-4 Hz)	Sono de ondas lentas
	Teta (4-8 Hz)	Meditação, sonolência
	Alfa (8-12 Hz)	Descanso acordado com olhos fechados
	Beta (12-32 Hz)	Consciente e desperto, processamento motor
	Gama (25 -140 Hz)	Sono REM, percepção sensorial
Roedor	Delta (1-4 Hz)	Sono de ondas lentas
	Teta 2 (4-7 Hz)	Imóvel, mas alerta
	Teta 1 (8-12 Hz)	Sono REM, explorando
	Beta (12-30 Hz)	Processamento motor, percepção sensorial
	Gama (30-100 Hz)	Percepção sensorial

Tabela adaptada de Luo & Guan, 2018.

A atividade da rede neural que muda de acordo com diferentes comportamentos e estados de consciência, como durante o sono e a vigília, também pode ser afetada por diferentes regimes de iluminação (33,34). Estudos com diferentes durações de fotoperíodos, exposição à luz durante a noite, e condições constantes de luz, como claro constante ou escuro contante, demonstraram impacto na potência de oscilações cerebrais e nas distintas fases do sono (35–37).

4. LUZ: PISTA MODULADORA DOS RITMOS BIOLÓGICOS

A luz é a principal pista temporal que sincroniza os nossos ritmos internos ao meio onde estamos inseridos, sendo considerada um modulador central dos ritmos circadianos, do sono e do humor (14). O caminho que o sinal fótico percorre no nosso organismo vem sendo elucidado ao longo dos anos. A luz detectada por cones e bastonetes é processada e sinaliza para as células ganglionares da retina que são os outputs neuronais da retina para o cérebro. Atualmente, sabe-se que na retina existe um subconjunto das células ganglionares que é intrinsecamente fotossensível (Figura 7a), pois contêm melanopsina - as células ganglionares da retina intrinsecamente fotossensíveis (ipRGCs) (38). Estas se projetam principalmente para o NSQ, mas também para outras regiões no cérebro envolvidas na depressão e/ou ansiedade, como a amígdala medial, a habênula lateral e a zona subparaventricular, indicando um possível papel direto da luz no humor (Figura 7b). Vários dos alvos das ipRGC também recebem inervação do NSQ, aumentando a possibilidade de que, além da função do relógio central, o NSQ possa também atuar como um condutor para informações sobre ciclos claro/escuro. Curiosamente, a amígdala medial e a habênula lateral também são relógios periféricos do cérebro que recebem inervação direta da retina, ou seja, as áreas envolvidas na regulação do humor e cognição podem ser influenciadas pela luz tanto através do NSQ ou em paralelo através destas duas outras estruturas (Figura 7c) (39). Diante disso, acredita-se que existam diferentes mecanismos através dos quais a luz pode afetar o humor, sendo um deles através de alterações nos ritmos circadianos e outro em que o sinal fótico atinge diretamente áreas cerebrais que regulam humor independende da regulação circadiana (40,41).

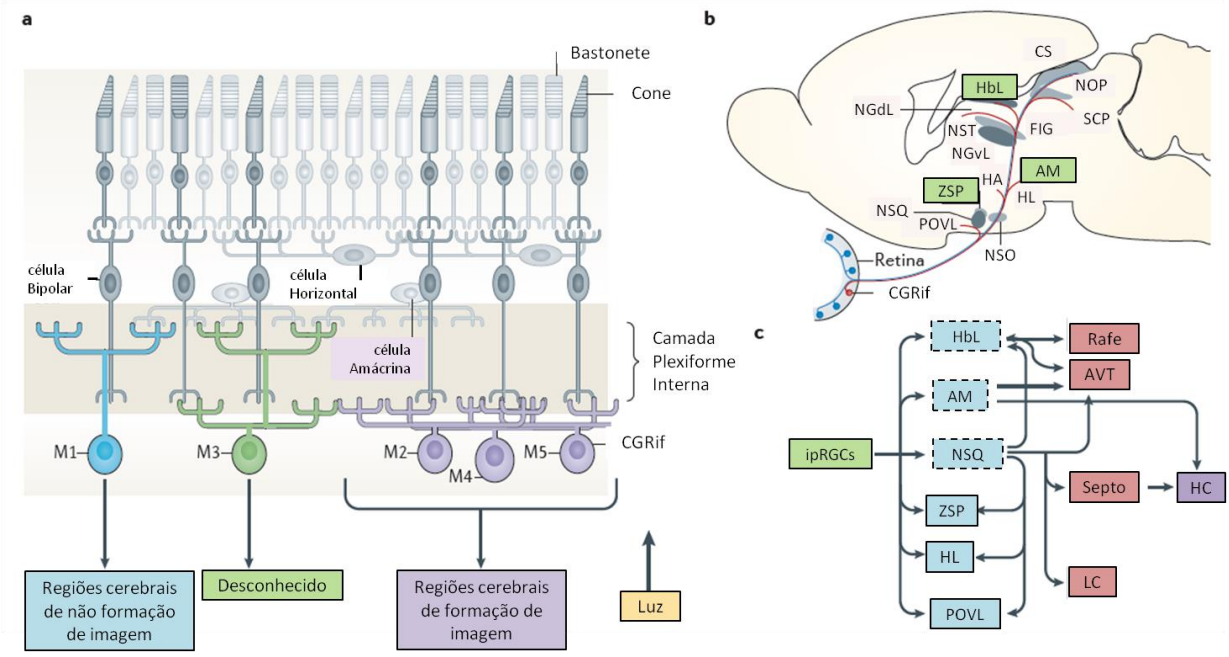


Figura 7. Circuitos cerebrais e da retina subjacentes aos efeitos não visuais da luz. (a) A luz detectada por cones e bastonetes é processada e sinaliza para as células ganglionares da retina (CGRs). Um subconjunto das CGRs é intrinsecamente fotossensível (ipRGCs) e existem pelo menos cinco subtipos de ipRGCs (M1-M5) com diferentes propriedades morfológicas e eletrofisiológicas. (b) As IpRGCs possuem projeções para inúmeras regiões cerebrais e também inervam amígdala medial (AM), a habênula lateral (HbL) e a zona subparaventricular (ZSP) (em verde), indicando um possível papel direto da luz na regulação do humor. c | Vários dos alvos das ipRGC, incluindo a ZSP, a área pré-ótica ventrolateral (POVL), hipotálamo lateral (HL) e HbL, também recebem inervação do núcleo supraquiasmático (NSQ). Tanto AM quanto a HbL são relógios periféricos do cérebro (os relógios centrais e periféricos são indicados por linhas tracejadas) que recebem inervação direta da retina. As áreas envolvidas na regulação do humor (área tegmental ventral (ATV) e rafe) e cognição (o hipocampo (HC)) podem ser influenciadas pela luz via NSQ ou via AM e HbL. HA, hipotálamo anterior; NST, núcleo da estria terminal; FIG, folheto intergeniculado; LC, locus coeruleus; NGdL, núcleo geniculado dorso-lateral; NGvL, núcleo geniculado ventro-lateral; NOP, núcleo olivar pré-tectal; SCP, substancia cinzenta periaquedutal; NSO, núcleo supra-ótico; CS, colículo superior. Adaptado de LeGates, Fernandez, e Hattar 2014.

A luz exerce uma influência potente na fisiologia e no comportamento com grande impacto na saúde. Como fatores negativos relacionados à iluminação podemos citar à exposição à luz durante a noite, invernos rigorosos com dias muito curtos (42), e pouco contato com luz natural durante o dia. Estas condições constumam estar associadas a um aumento da prevalência de perturbações do humor ou de sintomas relacionados ao humor, e de várias outras condições patológicas como câncer e doenças cardiovasculares (10,43). Recentemente, recomendações acerca da iluminação em locais fechados foram elaboradas com objetivo de evitar prejuízos à fisiologia, ao sono e à vigília na população saudável (44). E ainda, a luz também pode ser usada

como ferramenta de tratamento como a terapia de luz, que é aplicada em casos de depressão e transtorno afetivo sazonal (45,46).

Em 2015, o grupo de pesquisa em Cronobiologia e Sono Hospital de Clínicas de Porto Alegre demonstrou a associação entre a falta de janelas no ambiente de trabalho de funcionários deste mesmo hospital e altos níveis de cortisol e baixo níveis de melatonina à noite, e essas variáveis biológicas estavam relacionadas à sintomas depressivos e pior qualidade de sono (47). Posteriormente, em estudos realizados em comunidades de imigração italiana e alemã, observamos que pessoas vivendo em areas rurais apresentam maior exposição à luz natural, menor social jetlag e uma fase mais adiantada em relação aos que vive na zona mais urbanizada (48). Já em comunidades quilombolas na região Sul com diferentes níveis de urbanização e, portanto, de acesso à energia elétrica, investigou-se o impacto da exposição à luz artificial no sono e em sintomas depressivos. Verificou-se que as pessoas vivendo sem electricidade e aquelas que a adquiriram muito recentemente dormem, em média, mais cedo e a duração do sono tende a ser mais longa do que as que vivem em comunidades mais urbanizadas (49). Além disso, a exposição à luz natural, durante a manhã, está associada a menores níveis de sintomas depressivos (50). Em estudo incluindo amostras clínicas, adolescentes com depressão maior exibiram, menor duração de sono e menor amplitude de atividade, maior exposição à luz artificial durante a noite quando comparados a jovens do grupo controle ou do grupo com risco para desenvolvimento de transtorno depressivo (51).

Se a literatura que busca compreender os mecanismos pelos quais a luz influencia os mais diversos processos fisiológicos é bastante rica em humanos, em diferentes modelos animais é ainda maior. Nosso grupo inclusive já mostrou que, a partir de simulações de mudanças de fotoperíodo que ocorrem entre as estações do ano, ratos da linhagem Wistar demonstraram alteração tanto no ritmo de temperatura, quanto de atividade de acordo com o fotoperíodo, mostrando melhor adaptabilidade na transição do fotoperíodo longo para o curto do que a transição contrária (52). Diferentes linhagens de camundongos também parecem apresentar susceptibilidade distinta para ruptura dos ritmos circadianos diante de mudanças no comprimento do ciclo de claro-escuro, o que pode impactar na escolha de modelo animal ideal para estudo de disrupção de ritmo e transtornos de humor (53).

É importante considerar também as possíveis limitações dos estudos experimentais que utilizam condições laboratoriais extremamente controladas, utilizando ambientes claros e escuros com iluminação artificial temporizada, o que muitas vezes não se assemelha às condições naturalistas sob as quais se deu a evolução dos relógios circadianos (54). Já se sabe, por exemplo, que mudanças espectrais que ocorrem durante o crepúsculo são necessárias para

o encarrilhamento circadiano apropriado em condições naturais (55,56). Com base nisso, em um estudo anterior publicado durante o mestrado, buscamos compreender os efeitos da utilização de uma iluminação de LED que mimetizasse as variações naturais com alterações no espectro de cor nos ritmos de atividade-repouso de roedores (57). Durante o seu desenvolvimento, os animais expostos ao sistema de iluminação com mudanças na temperatura de cor durante o fotoperíodo sincronizaram-se antes ao ritmo de 24h do ambiente, e pareceram prever melhor as transições entre as fases claro-escura pela ausência de pico reativo de atividade observada no grupo controle com iluminação padrão da unidade.

5. RITMOS BIOLÓGICOS E O PROCESSO SAÚDE-DOENÇA

O adequado funcionamento do sistema temporizador circadiano em todos os seus níveis hierárquicos confere ao organismo as condições ideais para manutenção da saúde. No entanto, são diversos os possíveis perturbadores deste sistema e eles podem ser classificados em diferentes níveis: ambiental, como o trabalho de turno e as viagens trasmeridionais que provocam *jetlag*; comportamental, como horários de alimentação, interação social, atividade física e ciclos sono-vigília; tecidual, como em casos de cegueira e câncer; e molecular, no caso de, por exemplo, polimorfismos dos genes do relógio (11). As evidências apontam para a existência de uma relação bidirecional, na medida em que a perturbação circadiana aumenta a gravidade da doença e muitas doenças podem perturbar os ritmos circadianos (3). A perturbação circadiana pode aumentar o risco para desenvolvimento e expressão de distúrbios neurológicos, psiquiátricos, cardiometabólicos, imunológicos entre outros (10).

Interessante também considerar que a disrupção circadiana pode se manifestar de diferentes formas ao longo da vida de um indivíduo. Enquanto na primeira infância, a perturbação do ritmo pode causar irritabilidade, alterações de humor e fadiga, podendo levar inclusive ao comprometimento do crescimento e desenvolvimento, à medida que se envelhe, a perturbação crônica pode resultar em sono fragmentado e comprometimento na aprendizagem, ou até em risco aumentado para uma série de doenças crónicas, incluindo diabetes e doença de Alzheimer (Figura 8) (58). Portanto, é importante a adequada quantificação e caracterização dos ritmos levando em consideração os aspectos individuais, como idade e condições de saúde para auxiliar no correto diagnóstico e tratamento.

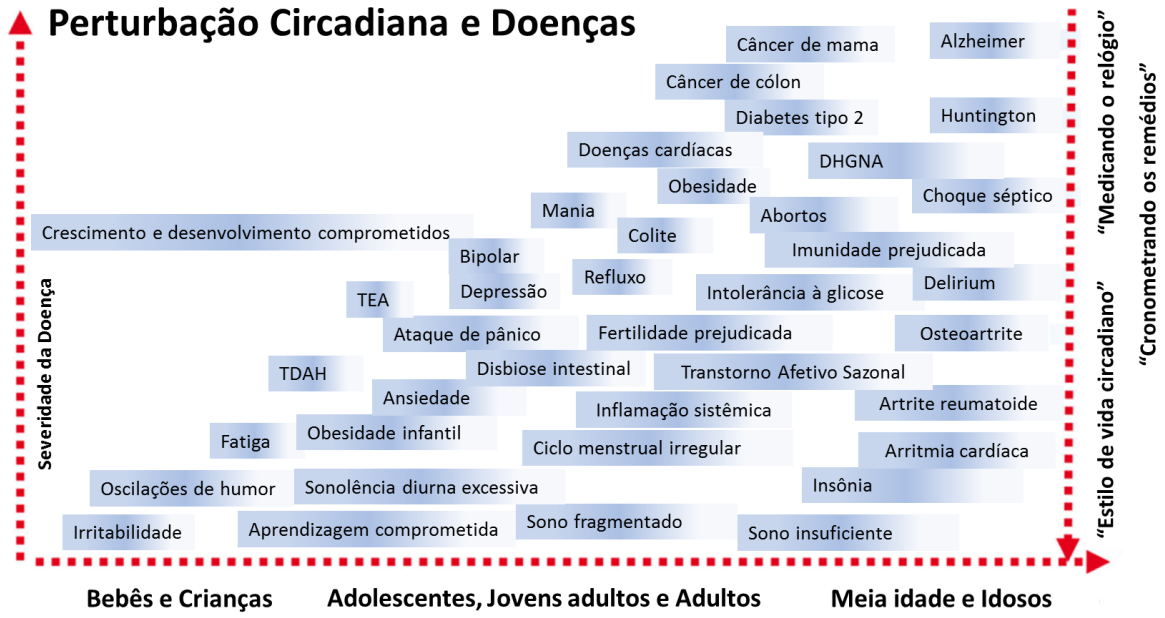


Figura 8. Disrupção circadiana e doenças associadas ao longo da vida. Adaptado de Sulli et al., 2018.

6. FERRAMENTAS PARA AVALIAÇÃO DOS RITMOS EM SAÚDE MENTAL

A literatura existente sobre ritmos circadianos e sono revela que alterações nos ritmos sociais, e nos ciclos de atividade-repouso e sono-vigília são tipicamente observadas em transtornos psiquiátricos, como depressão, ansiedade e transtornos psicóticos (59–61). Conhecendo a extensão do impacto regulatório do sistema temporizador no organismo, caracterizar os ciclos que se manifestam através de variáveis fisiológicas e comportamentais torna-se imprescindível. Diante disso, não apenas o momento do dia de coleta do dado, mas também as variações observadas ao longo do tempo podem fornecer informações importantes no estudo dos processos de saúde e doença. No contexto de saúde mental, poderíamos classificar as ferramentas para avaliação dos ritmos em objetivas e subjetivas. Dentre as objetivas, podemos destacar a actimetria (62), o início da secreção de melatonina (DLMO) (63) e as dosagens de 6-sulfatoximelatonina (aMT6s) (64), entre as subjetivas, os questionários para avaliação de comportamento e sintomas de humor.

Como anteriormente mencionado, a actimetria é responsável pela caracterização dos ritmos de atividade e repouso, temperatura corporal e exposição à luz ao longo de períodos prolongados. A literatura científica vem demonstrando a robusta relação entre transtornos mentais, sono e ritmos de atividade/repouso tanto em seres humanos como em modelos animais

(65). Dentre as variáveis já descritas que podem ser calculadas a partir dos dados de actimetria, com especial relevância para saúde mental, destacamos a amplitude relativa de atividade, que pode diferenciar indivíduos com depressão aguda de crônica, e adolescentes com depressão maior daqueles com risco para desenvolver a doença (51,66). Valores reduzidos desta variável podem ser preditores do desenvolvimento de transtornos mentais como depressão maior e transtorno bipolar ao longo da vida (67). Além disso, a quantidade de atividade noturna também já se mostrou importante para diferenciar subtipos de depressão, como a menor quantidade de atividade noturna observada em quadros de depressão melancólica quando comparados à depressão não melancólica (68). Em modelos animais, camundongos submetidos ao protocolo de estresse crônico imprevisível apresentaram amplitude de atividade e temperatura corporal reduzidas, semelhante aos achados em pacientes com transtorno depressivo maior (69).

A partir da tipologia circadiana, pode-se classificar os indíviduos conforme diferentes cronotipos, que variam de matutino a verspertino, e estão associados a características fisiológicas, genéticas e comportamentais distintas (70). Como consequência, indivíduos podem apresentar, por exemplo, tendências individuais para alocar seus períodos de atividade e repouso, o que se relaciona com DMLO, que é considerada uma medida de fase circadiana. Através do DLMO, avanços e atrasos de fase do ritmo de melatonina podem ser caracterizados, também permitindo diagnósticar indivíduos sincronizados ao ritmo claro-escuro de 24h ou em livre curso. Desta forma, o DLMO se constitui um teste útil para avaliação de perturbações do sono, para medir a alteração de fase na avaliação psiquiátrica de transtornos do humor, além de ser capaz de fornecer orientação sobre o momento ideal para terapia de luz ou para administração de melatonina exógena (63). Já o nível urinário de aMT6s, que é um metabólito da melatonina, é um método não invasivo para estimar as concentrações circulantes de melatonina. As alterações dos ritmos de excreção de aMT6 têm sido associadas à fisiopatologia da depressão, já que estudos mostraram o potencial da avaliação da aMT6s urinária como biomarcador da resposta a diferentes antidepressivos em indivíduos com transtorno depressivo maior no primeiro episódio, para resposta a diferentes antidepressivos (71–73).

Em relação as ferramentas subjetivas, como escalas e questionários, existem vários instrumentos que acessam humor ou ritmos biológicos. A maioria das escalas como a *Measure of Sleep, Circadian Rhythms, and Mood* (SCRAM) (74), a *Mood and Feelings Questionnaire* (MFQ) (75) e ainda *Profile of Mood States* (POMS) (76) têm como foco a intensidade do humor deprimido. Além disso, a Positive and Negative Affect Schedule (PANAS) (77) apesar de avaliar a intensidade de afetos positivos e negativos bem como a sua duração levando em consideração um carácter temporal como “atualmente”, “nos últimos dias”, “nas últimas

semanas", também não é capaz de caracterizar variação diária. Outro instrumento, a *Biological Rhythms Interview of Assessment in Neuropsychiatry* (BRIAN) é uma entrevista para avaliação clínica de sono e do comprometimento do ritmo em pacientes com transtornos mentais com perguntas divididas em 4 áreas específicas como sono, atividades, ritmo social e padrão alimentar. Apesar de avaliar a dificultade observada em comportamentos associados a ritmos biológicos, também não acessa possíveis variações destes comportamentos ao longo do dia, focando, portanto, em tipologias (78). Em resumo, a maioria não considera as flutuações diárias de sintomas de humor que podem, inclusive, influenciar a forma como o indivíduo responde o instrumento dependendo do momento do dia. A falta de instrumentos deste tipo também pode ser a justificativa para a controvérsia que existe na literatura acerca das variações diárias de humor (79).

O Instrumento de Ritmo de Humor, do inglês Mood Rhythm Instrument (MRhI), foi desenvolvido para suprir essa demanda, sendo questionário autoaplicável que avalia a percepção do indivíduo sobre a variação diária de sintomas relacionados ao humor (80). Esse instrumento demonstrou não ser afetado por viés de memória (81) e a percepção de ritmicidade de certos comportamentos foi relacionada a maior risco para desenvolvimento de transtornos psiquiátricos (82).

7. QUANTIFICANDO RITMOS: ANÁLISE DE SÉRIES TEMPORAIS

"*Measure what is measurable and render measurable in time what as yet is not*"
Franz Halberg's motto also attributed to Galileo

NATUREZA DAS SÉRIES TEMPORAIS

Estudos nas ciências da terra, meteorologia, biologia, física, psicologia, medicina e astronomia comumente geram dados com características circulares. Esse tipo de dado se origina principalmente de duas formas, que correspondem aos dois principais instrumentos de medição circulares: a bússola e o relógio. Considerando o viés cronobiológico desta tese, o foco será dado às medidas circulares de carácter temporal, ou seja, aquelas observações que foram feitas ao longo do tempo.

No estudo de séries temporais biológicas, o ritmo é um importante componente que pode ser observado. Dados relativos a ritmos biológicos podem, com frequência, ser aproximados a curvas senoidas, ou sinusoidais, que matematicamente descrevem uma oscilação repetitiva suave e contínua (Figura 9) (83).

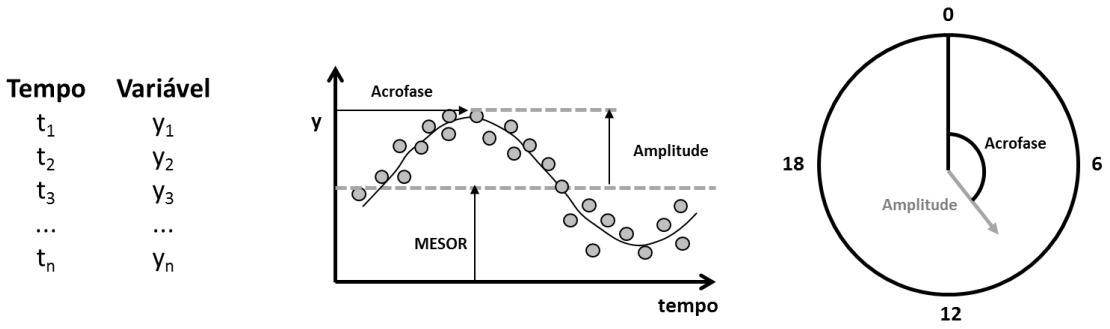


Figura 9. Representação de série temporal de dados, sua aproximação a curva sinusoidal bem como sua distribuição circular.

INSPEÇÃO VISUAL

O primeiro passo para análise de uma série de dados temporais é a inspeção visual dos dados em função do tempo (84). Esse procedimento possibilita o bom planejamento das análises, identificação de dados faltantes, bem como visualização do padrão oscilatório. Dentre as representações gráficas mais utilizadas estão os cronogramas e os actogramas. Enquanto nos cronogramas os dados são plotados em coordenadas cartesianas em função do tempo, nos actogramas o segmento de dados para cada dia assume a largura total de uma página e é posicionado abaixo dos dados do dia anterior. Abaixo um actograma obtido através de actimetria de punho (Figura 10), cada vez mais usada na pesquisa e na clínica para estudo dos ritmos de atividade-repouso, bem como de padrões de sono.

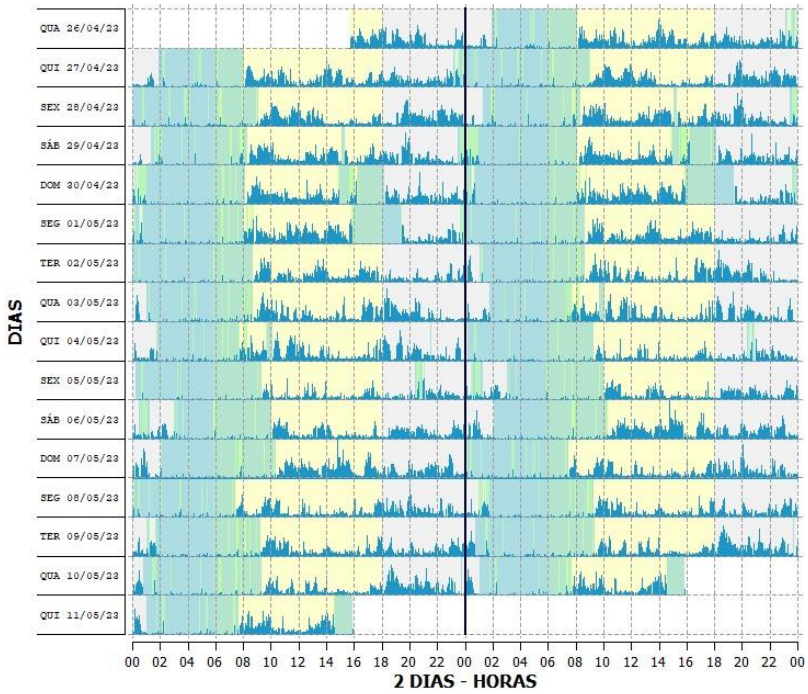


Figura 10. Actograma. Representação gráfica de dados de atividade e repouso coletados por meio de actímetro de punho em uma mulher de 30 anos durante 15 dias. Cada linha representa um dia de registro, sendo que cada dia de registro é representado duas vezes para facilitar a visualização e comparação dos dados ao longo dos dias. Atividade: barras em azul escuro; estimativa de sono: trechos em azul claro; dia (fase clara): fundo amarelo; noite (fase escura): fundo cinza.

PERIODOGRAMA

Uma vez observado o padrão de distribuição dos dados através da inspeção visual, já sendo possível inclusive inferir a existência de um padrão rítmico em muitos casos, a utilização da análise de periodograma torna possível confirmar a identificação de ritmicidade em uma série temporal. Além disso, possibilita quantificar o período do ritmo observado (e.g. ritmo circadiano, período de cerca de 24h ou 1440min).

Em 1965, James Enright publicou seu artigo propondo um procedimento de periodograma que seria uma generalização da técnica desenvolvida pelo químico e meteorologista holandês Buys Ballot para tabular observações e investigar a periodicidade em séries temporais (Tabela 2). Em 1847, Buys Ballot usou a tabela para determinar o período de rotação do sol a partir de observações diárias de temperatura na Holanda de 1729 a 1846. Normalmente, cada linha representa um ciclo/periodo e cada coluna é uma observação feita durante este ciclo. As médias representadas por Y são simplesmente os valores de U divididos

pelo número de linhas m. A partir desta técnica, o procedimento proposto por Enright consiste em fragmentar a série de dados em trechos de diferentes períodos e calcular um índice de variabilidade para cada um deles. A estatística adotada para expressar a "importância" da estimativa é a raiz quadrada média da amplitude, que pode ser vista como uma espécie de ANOVA adaptada para teste de ritmicidade. A significância de cada período é testada com testes F para identificar o período significativo (85). Uma das condições para aplicação desta análise é de que os dados sejam equidistantes, o que é mais comum em estudos de laboratório onde as condições são controladas e menos sujeitas a interrupções.

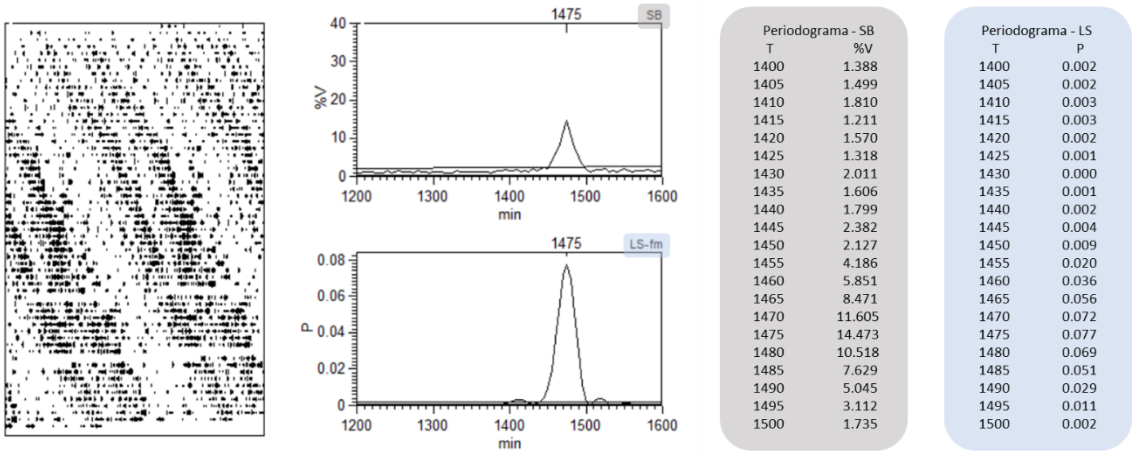
Tabela 2. Tabela de Buys-Ballot

Linha 1	X_1	X_2	...	X_P
Linha 2	X_{P+1}	X_{P+2}	...	X_{2P}
...	...	...	...	...
Linha m	$X_{P(m-1)+1}$	$X_{P(m-1)+2}$	...	X_{Pm}
Totais	$U_{P,1}$	$U_{P,2}$	...	$U_{P,P}$
Médias	$Y_{P,1}$	$Y_{P,2}$	...	$Y_{P,P}$

X = observação; P = período; m = total de linhas; U = soma das observações; Y = soma das observações /m.

O periodograma de Enright foi então refinado por Sokolove-Bushell (86), que utiliza a distribuição chi-quadrada como método estatístico, e desta maneira, o periodograma de Sokolove-Bushell também é conhecido como periodograma chi-quadrado (Figura 11). Tem como principal característica ser bastante sensível à repetibilidade, ciclo a ciclo, de qualquer padrão rítmico, ainda que não apresente padrão sinusoidal. Além disso, para confiabilidade estatística recomenda-se, no mínimo, 10 ciclos (ou dias no caso de ritmos circadianos) de registro de dados.

O periodograma Lomb-Scargle, por sua vez, permite inferir o período da série temporal com menos de 8 ciclos e consiste em uma modificação da análise de Fourier para acomodar séries temporais irregulares, ou seja, tem a vantagem de poder ser aplicado em dados não equidistantes (87). Este método é útil para séries com dados faltantes, o que é bastante frequente em pesquisas fora do ambiente controlado de laboratório. Além disso, é muito sensível à presença de qualquer ritmicidade e não é afetado por componentes harmônicos do período principal. Isso quer dizer que, para uma série temporal com período de 1440 min, o periodogram de Lomb-Scargle, ao contrário do Sokolove-Bushell, não mostra os subharmônicos 1440/2 = 720, ou 1440/3 = 480 min, nem os superharmônicos 1440 x 2 = 2880 e 1440 x 4 = 5760 min (88).



Periodograma - SB

T	%V
1400	1.388
1405	1.499
1410	1.810
1415	1.211
1420	1.570
1425	1.318
1430	2.011
1435	1.606
1440	1.799
1445	2.382
1450	2.127
1455	4.186
1460	5.851
1465	8.471
1470	11.605
1475	14.473
1480	10.518
1485	7.629
1490	5.045
1495	3.112
1500	1.735

Periodograma - LS

T	P
1400	0.002
1405	0.002
1410	0.003
1415	0.003
1420	0.002
1425	0.001
1430	0.000
1435	0.001
1440	0.002
1445	0.004
1450	0.009
1455	0.020
1460	0.036
1465	0.056
1470	0.072
1475	0.077
1480	0.069
1485	0.051
1490	0.029
1495	0.011
1500	0.002

Figura 11. Periodogramas. Representação gráfica dos periodogramas de Sokolove-Bushell (SB) e Lomb-Scargle (LS). Os gráficos mostram os valores de %V para os vários períodos de um intervalo desejado. A menos que a série temporal contenha mais de um componente rítmico, o pico mais alto corresponde ao período real estimado da série temporal. Importante notar que os valores apresentados em um periodograma são obtidos independentemente uns dos outros, a cada teste para um período específico, um valor de % da variância total é calculado por vez. Portanto, cada período testado é um teste independente, realizado sobre o mesmo conjunto de dados. Podemos observar a lista de valores obtidos para cada período em minutos testado para o periodograma de Sokolove-Bushell.

ANÁLISE DE COSINOR

O método de cosinor foi desenvolvido, para aplicação a fenomenos biológicos, no início da década de 60 por Franz Halberg e colaboradores. Em seu primeiro artigo aplicando esta metodologia de análise, Halberg justifica a utilização da nomenclatura “cosinor” como um termo análogo ao “sinor” proposto por LePage em 1949 (89,90). Este último, questionava a utilização de certas nomenclaturas, como por exemplo, o uso do termo "vetor" para descrever as linhas direcionadas usadas no tratamento de funções sinusoidais, e sugeriu que o termo "sinor" seria mais apropriado (Figura 12). Isto é, LePage argumenta que a linha direcionada que representa a função sinusoidal é um número complexo e não um vetor, e que seria melhor denominada “representante de uma onde sinusoidal” ou melhor, “sinor”.

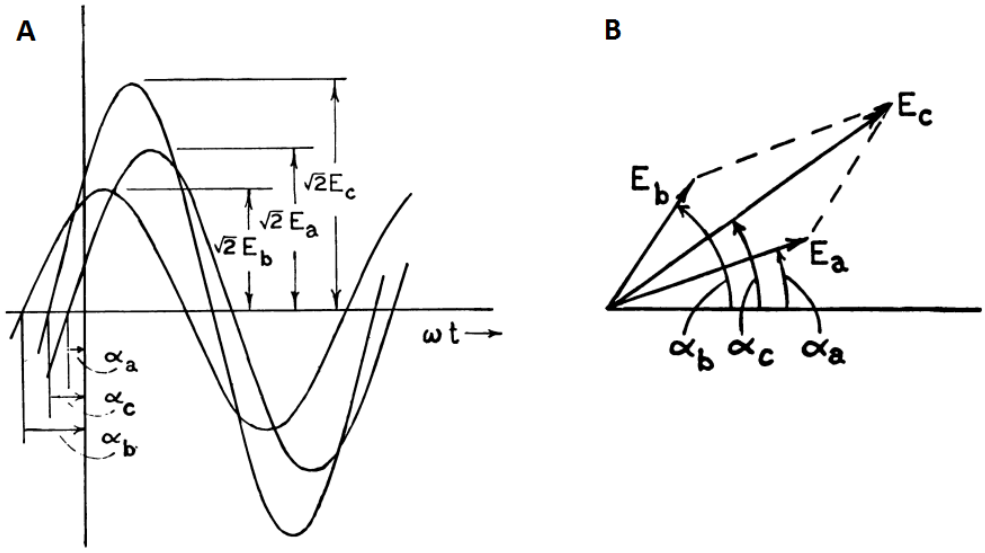


Figura 12. Sinusoides, suas representações e nomenclatura. As funções sinusoidais (A) são representadas por linhas direcionadas (B), sendo uma representação útil, pois a soma vetorial das linhas originais é ela própria uma linha, que representa, da mesma forma, a sinusoide. Imagem adaptada de LePage W. R., 1949.

Enquanto LePage focava na descriçao de certas relações de fase e amplitude para tensões e correntes, Halberg mantendo seu viés biológico, utilizou o termo “cosinor” para se referir ao método de ajuste de curvas cossenos (sinusoidais) com períodos conhecidos (e.g temperatura corporal apresenta ritmicidade circadiana, logo a análise de cosinor será feita considerando um período de 24h) aos dados pelo método de mínimos quadrados produzindo gráficos polares de amplitude-fase (Figura 13).

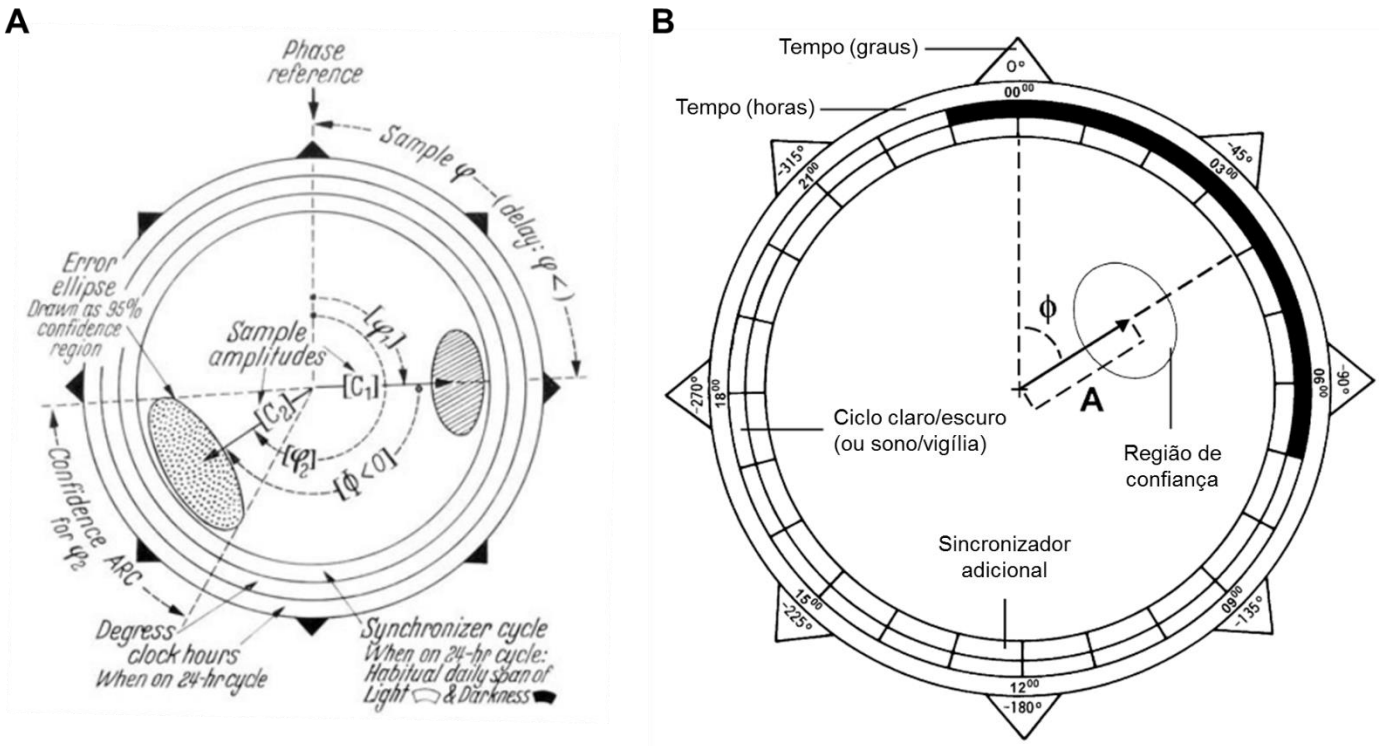


Figura 13. Representação gráfica do método de cosinor. A primeira ilustração representando os aspectos chave do método apresentada à comunidade científica com a publicação de 1967 (A) e depois uma versão atualizada publicada em 2007 (B). Em ambas representações polares, pode-se observar a acrofase (φ) indicada pelo ângulo do vetor cujo comprimento corresponde à amplitude (A) de um ritmo. A elipse representa a região de confiança de 95% para a estimativa conjunta de A e φ. Estimativas para A e φ individualmente podem ser obtidas desenhando círculos concêntricos tangentes à elipse de erro para A, determinando sua interseção com o comprimento do vetor, ou desenhando tangentes à elipse para φ. Imagem adaptada de Refinetti et al, 2007 e Halberg et al, 1967.

O método de mínimos quadrados para ajuste da curva cosseno é um procedimento matemático, ou uma regressão periódica, que encontra a curva de melhor ajuste para o conjunto de dados minimizando a soma dos quadrados dos desvios (resíduos) entre os dados e a curva cosseno ajustada (91). Uma vantagem da sua utilização também se deve ao fato de não necessitar que os dados sejam equidistantes. Abaixo, a equação do modelo proposta por Halberg (90) e depois atualizada por Cornelissen (91):

1. $Y = C_0 + C\cos(\omega t + \varphi)$ (Halberg)

Onde C_0 é o nível médio, C amplitude, ω frequência angular, t tempo e φ é a fase.

2. $Y(t) = M + A\cos(2\pi/\tau + \varphi) + e(t)$ (Cornelissen)

Onde M é MESOR (Midline Statistic Of Rhythm - valor médio do ritmo ajustado), A é a amplitude, φ é a acrofase, τ é o periodo (duração de um ciclo), e e(t) é o erro de cada horário.

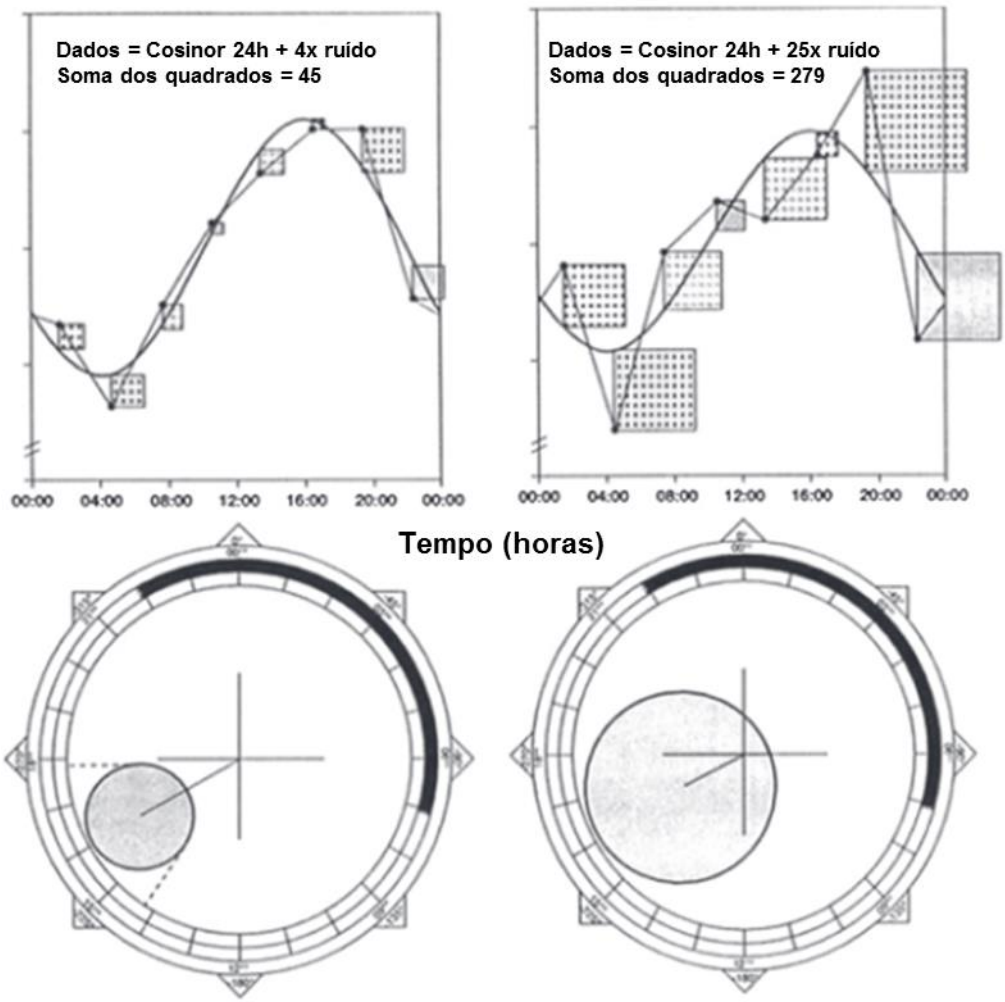


Figura 14. Cosinor. Ajuste da curva cosseno aos dados (topo) por mínimos quadrados e representação polar da dupla acrofase-amplitude, bem como da elipse de confiança de 95%. Imagem adaptada de Cornelissen Germaine, 2014.

Na figura 14, vemos dois exemplos referentes a curvas cosseno ajustadas aos grupos de dados pelo método de mínimos quadrados. Quanto maior a soma dos quadrados dos resíduos, maior a imprecisão em relação à estimativa dos parâmetros, que pode ser visualizada pela região da elipse de confiança de 95%. Quando a elipse não cobre o centro do gráfico polar, a hipótese nula de que não existe ritmo é rejeitada, e logo, existe ritmicidade presente nos dados. Contudo, quando a elipse se sobrepõe ao centro, a hipótese nula de que não há ritmo, ou seja, a amplitude é igual a zero, pode ser aceita.

A análise de cosinor pode ser aplicada quando assumimos que o período da oscilação dos dados é conhecido. No caso de dados que seguem um ritmo circadiano, o componente único seria de 24h. No entanto, podem existir ritmos que resultem da interação de mais de um componente, como por exemplo de 24h e de 12h e, desta forma, deve-se ajustar a equação. Além disso, quando os dados são coletados em função do tempo em 3 ou mais sujeitos, o

cosinor populacional médio pode ser aplicado (91) e os parâmetros de mesor, acrofase e amplitude derivados podem ser comparados entre grupos.

ÍNDICES NÃO-PARAMÉTRICOS

Enquanto para a análise de cosinor parte-se do princípio que os dados podem ser ajustados a uma sinusoide pelas características de uma oscilação repetitiva suave e contínua, quando não assumimos o pressuposto acerca da distribuição dos dados, podemos aplicar análises ditas "não-paramétricas".

Em 1990, Witting e colaboradores propuseram variáveis não-paramétricas para estudo do ritmo de atividade/repouso em pacientes com Alzheimer (92). Dentre as variáveis propostas estão variabilidade intradiária, estabilidade interdiária, atividade total nas 10 mais e 5 menos horas ativas. Posteriormente, Van Someren (1999) também introduziu a variável amplitude relativa. Abaixo, as variáveis não-parametricas são descritas juntamente com os respectivos achados clínicos descritos na literatura.

Variabilidade Intradiária (IV - intradaily variability)

Este parâmetro informa sobre a fragmentação do ritmo com base na frequência e extensão das transições entre horas ativas e inativas. É calculada como a razão dos quadrados das diferenças entre dados sucessivos e a variância geral. Isso significa que quanto maior a variabilidade nas transições, maior o valor de de IV. Valores altos indicam presença de cochilos diurnos bem como de despertares noturnos. Maiores valores de IV estão correlacionados com pior qualidade de sono, menor amplitude do ritmo de atividade/repouso, pior performance cognitiva e motora (93). Pacientes com Alzheimer e hipersonolência apresentam valores altos para IV.

$$I\ \ V = \frac{N\ \sum_{i=2}^{N}(X_i - X_{i-1})^2}{(N-1)\sum_{i=1}^{N}(\bar{X} - X_i)^2}$$

Estabilidade Interdiária (IS – interdaily stability)

Este parâmetro informa sobre a sincronização do ritmo de atividade/repouso ao ciclo claro-escuro de 24h ao longo dos dias, ou também a "força" do padrão circadiano de um ritmo. Pacientes com transtorno bipolar e demência apresentam menor níveis de IS que controles (94,95). Além disso, trabalhadores de turno, que acabam apresentando períodos de atividade durante a noite e vice-versa, apresentam menor sincronização que indivíduos com rotinas

regulares de trabalho diurno (96). Outro exemplo interessante é de indivíduos que apresentam transtorno de ritmo circadiano diferente de 24h, onde a cada dia, a fase de sono apresenta mudança e, portanto, há instabilidade do ritmo de atividade/repouso ao longo dos dias. Abaixo, o registro actigráfico de um indivíduo diagnosticado com transtorno de ritmo diferente de 24h, onde o valor de IS, se calculado, seria muito inferior ao de um indivíduo sem esse transtorno (Figura 15).

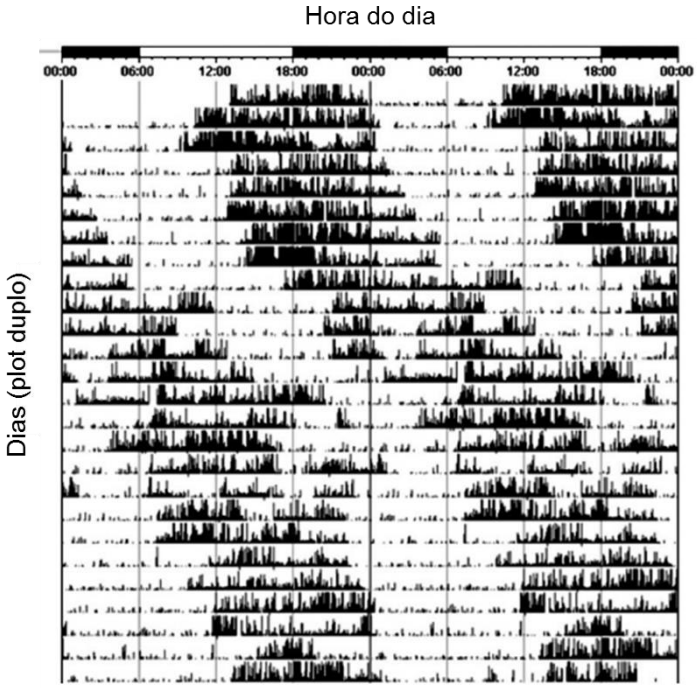


Figura 15. Ritmo de atividade/repouso de paciente com transtorno de ritmo de sono-vigília diferente de 24h antes do tratamento. Actograma com plot duplo mostra que há avanço na fase de atividade a cada dia e que o padrão de ciclagem é de 26h. Imagem adaptada de Dagan & Ayalon, 2005 (97).

$$IS = \frac{N \sum_{h=1}^{p} (\bar{X}_h - \bar{X})^2}{p \sum_{i=1}^{N} (X_i - \bar{X})^2}$$

Atividade total durante 10 horas mais ativas (M10)

A quantidade de atividade nas 10 horas mais ativas nos informa sobre o quão ativo é um indivíduo, e esta informação será, posteriormente, utilizada para cálculo da amplitude do ritmo de atividade. Espera-se que pessoas saudáveis e com estilo de vida ativo apresentem altos níveis de atividade durante este período de 10 horas.

Atividade total durante 5 horas menos ativas (L5)

A quantidade de atividade durante as 5 horas menos ativas nos informa sobre a atividade noturna, pois em geral este período equivale a fase de escuro, durante a qual espera-se que o indivíduo esteja em repouso e dormindo. Este parâmetro também é utilizado para cálculo da amplitude da atividade do indivíduo.

Amplitude Relativa (RA – relative amplitude)

Esta variável é calculada a partir da relação entre as variáveis M10 e L5, e nos informa sobre a relação entre os períodos de maior e menor atividade. Um valor de amplitude relativa elevado, pode traduzir que durante o dia o indivíduo está bastante ativo e durante à noite em repouso. Valores elevados de amplitude relativa relacionam-se positivamente com saúde. Em um estudo de coorte que incluiu mais de 90 000 mil participantes, a redução na amplitude relativa foi associada a aumento no risco para transtorno depressivo maior, transtorno bipolar, instabilidade de humor e outros desfechos negativos em saúde mental (67). Amplitude relativa também se encontra reduzida em indivíduos com depressão (51).

$$R\ A = \frac{M_{1\ 0} - L_5}{M_{1\ 0} + L_5}$$

ANÁLISE DE FOURIER

Também conhecida como análise espectral, esta ferramenta de análise desenvolvida por Joseph Fourier parte do princípio de que qualquer série temporal, independentemente de sua forma ou regularidade, pode ser descrita por uma série de ondas sinusoidais e cossenoidais de várias frequências (84). Desta forma, a análise espectral decompõe uma forma de onda em seus componentes harmônicos, e no caso de identificado um componente espectral/harmônico principal que esteja dentro de uma faixa de frequência circadiana, pode-se inferir que o fenômeno estudado exibe ritmicidade circadiana.

A análise de Fourier também pode ser realizada de forma seriada, quando dividimos a série temporal em trechos com comprimento igual ou múltiplo do tamanho do período utilizado na análise principal, e então a análise espectral é repetida para cada um dos trechos (88). Na figura 16 podemos ver a aplicação desta técnica para explorar o efeito de diferentes sistemas de iluminação no ritmo de atividade e repouso ao longo do desenvolvimento de roedores. Cada coluna da matrix gráfica representa um componente espectral que é avaliado ao longo dos dias. Observamos que os animais expostos à iluminação com variação no espectro de cor que busca

mimetizar variações naturais na luz (RGB-v), apresentam, antes em seu desenvolvimento, o primeiro harmônico de 24h como o principal para determinação de seu ritmo de atividade/repouso.

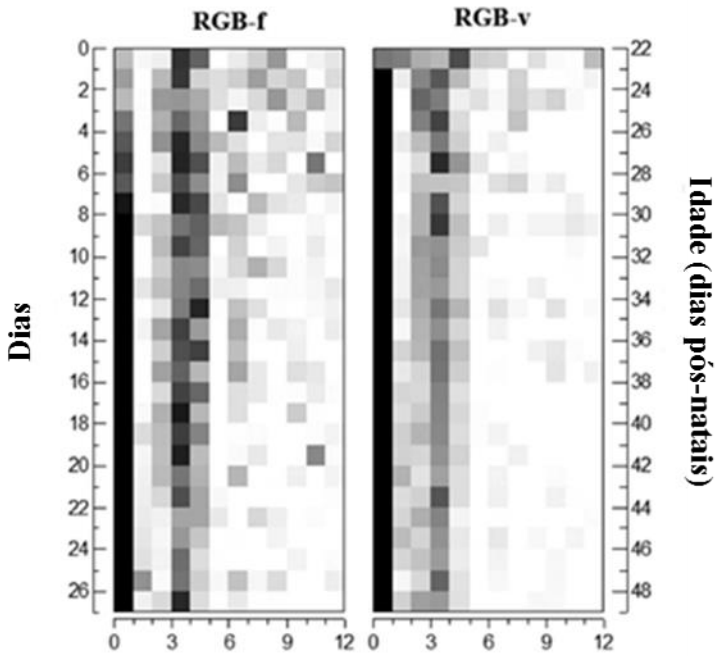


Figura 16. Análise espectral seriada do ritmo de atividade/repouso de roedores. Imagem adaptada de Oliveira et al, 2019.

ESTATÍSTICA CIRCULAR

A estatística circular surge como um conjunto de ferramentas que possibilitam a análise de dados que apresentam característica circular, sejam eles de carácter temporal ou direcional (Figura 17) (98). Dentre as observações tipicamente medidas pelo relógio, estão horários de manifestação de eventos patológicos (e.g. crise epiléptica, infarto), início do período de sono, hora correspondente à oscilação do hormônio cortisol, hora de entrada de paciente em uma emergência hospital e assim por diante. O momento do ano de ocorrência de certas doenças ou quando comportamentos são observados pode estar ligado à sazonalidade e provém de um tipo similar de dados que é coletado, por exemplo, ao longo dos dias do mês e dos meses do ano.

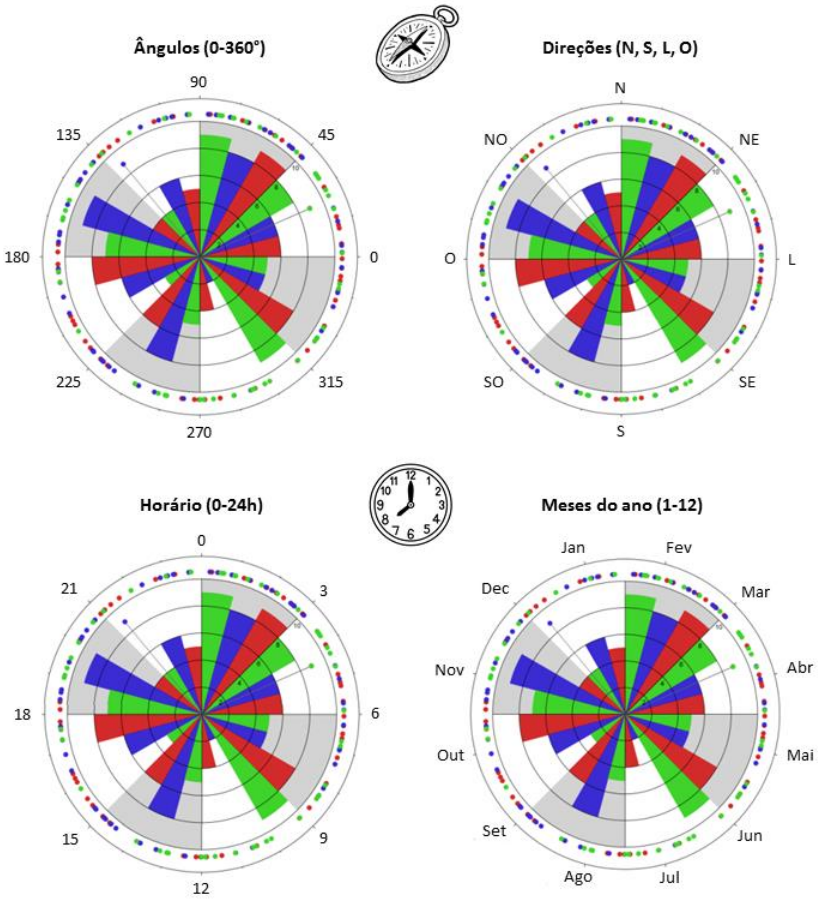


Figura 17. Exemplos de dados circulares. Tipos de dados são representados por ângulos, pontos cardeais, horas e meses do ano e podem representar desde direção de correntes de ar e migração de pássaros até horário de início do período de sono ou mês de ocorrência de depressão sazonal.

Uma observação circular pode ser identificada como um ponto em um círculo que corresponde a um momento do dia ou, no caso de medidas de direção, um vetor em um plano. Após definição do ponto de partida e da orientação do círculo, cada observação pode ser especificada através do ângulo formado entre a posição inicial no círculo e o ponto correspondente à observação (Figura 18). Estes ângulos são medidos em graus os radianos, contudo frequentemente trabalhando com dados circulares de séries temporais, faz-se necessária a transformação entre as unidades de graus, radianos, horas decimais e hh:mm para melhor análise e interpretação dos resultados.

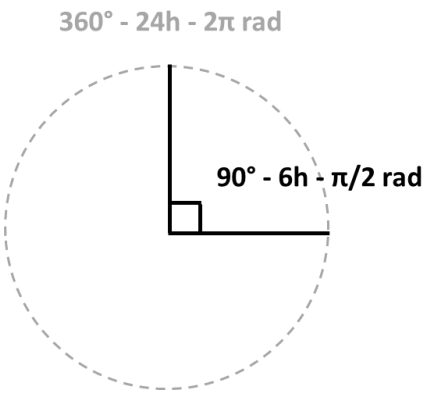


Figura 18. Unidades em observações circulares.

Para dados “d” com valores em graus, radianos, horas decimais ou hh:mm, abaixo os cálculos para a respectivas transformações.

1. *Graus para radianos*

   $360° - 2\pi rad$

   $d° - x$

   $x = d * 2\frac{\pi rad}{360}$

2. *Graus para horas decimais*

   $360° - 24,00\ h$

   $d° - x$

   $x = d * \frac{24,00\ h}{360}$

3. *Radianos para grau*

   $2\pi rad - 360°$

   $d\ rad - x$

   $x = d * \frac{360°}{2\pi}$

4. *Radianos para horas decimais*

   $2\pi rad - 24,00\ h$

   $d\ rad - x$

   $x = d * \frac{24,00\ h}{2\pi}$

5. *Horas decimais para graus*

   $24,00\ h - 360°$

   $d\ h - x$

   $x = d * \frac{360°}{24,00}$

6. *Horas decimais para radianos*

   $24,00\ h - 2\pi rad$

   $d\ h - x$

   $x = d * \frac{2\pi rad}{24,00}$

7. *hh:mm para graus*

   $24\ h - 360°$

   $d\ h - x$

   $x = d * \frac{360°}{24}$

   $+$

   $1440\ min - 360°$

   $d\ min - x$

   $x = d * \frac{360°}{1440}$

8. *hh:mm para radianos*

   $24\ h - 2\pi rad$

   $d\ h - x$

   $x = d * \frac{2\pi rad}{24}$

   $+$

   $1440\ min - 2\pi rad$

   $d\ min - x$

   $x = d * \frac{2\pi rad}{1440}$

Abaixo, a tabela 3 resume e simplifica as orientações para as conversões necessárias:

Tabela 3. Unidades frequentes em dados circulares e orientações para conversões.

	RADIANOS	**HORAS DECIMAIS**	**hh:mm**
GRAUS	Multiplicar por πrad/180 *Cálculo 1*	Multiplicar por 1h/15 *Cálculo 2*	Multiplicar por 1h/15 para obter os dados em horas decimais; depois, o valor inteiro corresponde a "hh"; para obter "mm", multiplicar o valor decimal por 60min. *Por exemplo: 10,75 => 10 horas e 0,75 x 60 = 45 minutos => logo, 10:45.*
	GRAUS	**HORAS DECIMAIS**	**hh:mm**
RADIANOS	Multiplicar por 180°/π *Cálculo 3*	Multiplicar por 12h/π *Cálculo 4*	Multiplicar por 12h/π para obter os dados em horas decimais; depois, o valor inteiro corresponde a "hh"; para obter "mm", multiplicar o valor decimal por 60min.
	GRAUS	**RADIANOS**	**hh:mm**
HORAS DECIMAIS	Multiplicar por 15° *Cálculo 5*	Multiplicar por πrad/12 *Cálculo 6*	O valor inteiro corresponde a "hh"; para obter "mm", multiplicar o valor decimal por 60min.
	GRAUS	**RADIANOS**	**HORAS DECIMAIS**
hh:mm	Multiplicar o valor das horas por 15° e somar este valor ao valor dos minutos multiplicado por 0,25°. *Cálculo 7*	Multiplicar o valor das horas por πrad/12 e somar ao valor dos minutos multiplicado por πrad/720. *Cálculo 8*	O valor de "hh" corresponde ao valor inteiro; o valor de "mm" deve ser multiplicado por 1h/60 para obtenção do valor decimal.

Obs.: As orientações para conversões estão descritas partindo da unidade em cinza escuro para a unidade em cinza claro.

A estatística circular é necessária para preservar as características dos dados circulares. Isso se dá, pois ainda que os dados sejam aferidos para cada indivíduo, por exemplo, nem sempre pode ser comparado entre eles utilizando métodos tradicionais de estatística linear. O exemplo da figura 19 ilustra as médias para os dados em horas se calculada utilizando estatística linear e circular. Neste caso, fica claro que a estatística linear pode ser inadequada e levar a resultados errôneos quando aplicada sem devida compreensão das carartеrísticas dos dados.

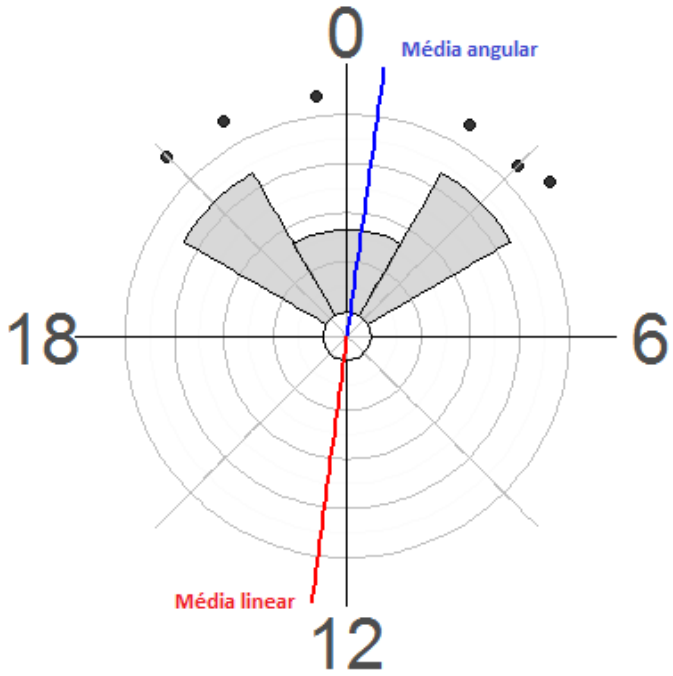


Figura 19. Comparação entre média linear e média circular. Seis dados em horas (23:30, 21:00, 22:00, 3:30, 3:00, 2:00) estão plotados juntamente com as médias linear (12:30) em vermelho e circular (00:32) em azul. Observa-se que a média circular representa adequadamente o ponto médio entre os dados plotados.

A fim de melhor compreender as bases dos cálculos com variáveis circulares, faz-se necessária a retomada de certas funções trigonométricas. Abaixo, o gráfico polar da esquerda (Figura 20), mostra 4 medidas angulares, sendo cada uma representada por um vetor colorido, que podem ser, por exemplo, quantidade água ingerida em 4 momentos diferentes do dia. O vetor resultante que nos levará ao cálculo da média circular está representado em cor preta no gráfico da esquerda, cujas coordenadas são detalhadas juntamente com suas fórmulas no gráfico da direita. O valor do eixo vertical é definido pela média dos senos dos ângulos de cada vetor colorido ($\bar{y}$), enquanto o valor do eixo horizontal é definido pela média dos cossenos destes

mesmos ângulos ($\bar{x}$). O ângulo do vetor resultante será a média circular ($\bar{\phi}$), que é calculada como o arco tangente da média dos senos divido pela média dos cossenos. O comprimento do vetor resultante ($\bar{r}$) representa a medida de dispersão dos dados (e.g. quando mais agrupados os dados, maior o comprimento do vetor).

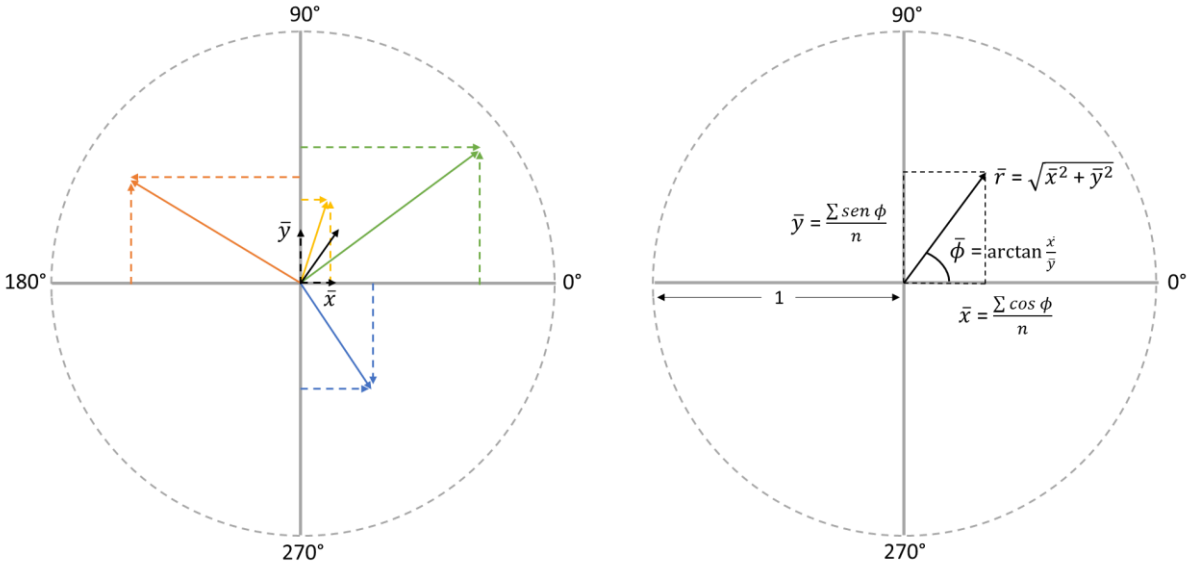


Figura 20. Relembrando funções trigonométricas para cálculos em estatística circular.

Distribuição de Von Mises

Em relação às distribuições circulares, a distribuição de von Mises assume um papel na estatística circular que é análogo ao da distribuição normal nas estatísticas lineares padrão. Na verdade, esta distribuição é simétrica, unimodal e tem a forma da distribuição normal, exceto pelo fato de que suas extremidades são truncadas (Figura 21) (99).

A função de probabilidade de densidade é dada por

$$f(\theta) = [2\pi I_0(K)]^{-1} \exp[K \cos(\theta - \mu)]$$

onde I_0 é a função de Bessel modificada de ordem zero

$$I_0(K) = (2\pi)^{-1} \int_0^{2\pi} \exp[K \cos(\varphi - \mu)]\, d\varphi$$

e K é fator de concentração. Quando maior o K, maior a concentração dos dados. Assim como existe para distribuição normal, Batschelet e Mardia criaram tabelas com valores de distribuição para uma gama de valores (99,100).

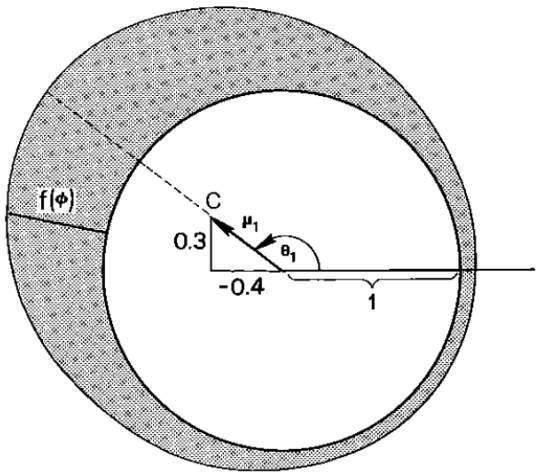


Figura 21. Plot circular da distribuição de Von Mises. O vetor e o ângulo médio estão plotados. O comprimento do vetor médio é de 0.5, calculado com base na relação entre catetos e hipotenusa de um triângulo retângulo. A partir do valor do vetor médio, a tabela B de Bastchelet (99) nos dá o valor convertido para K que é de 1.16. Imagem retirada de Batschelet, 1981.

Teste para avaliar uniformidade na distribuição dos dados circulares

Teste de Rayleigh avalia se existe evidência estatística para direcionalidade nos dados de dada amostra. Desta forma, o comprimento do vetor médio, r, fornece indícios sobre a direcionalidade, sendo que se *r* é grande o bastante, a hipótese de aleatoriedade na distribuição circular (uniforme) dos dados pode ser rejeitada em favor da existência direcionalidade. Abaixo, a figura 22 ilustra a distribuição circular de dois grupos de dados medidos em graus, bem como as respectivas estatísticas do teste de Rayleigh para comparação.

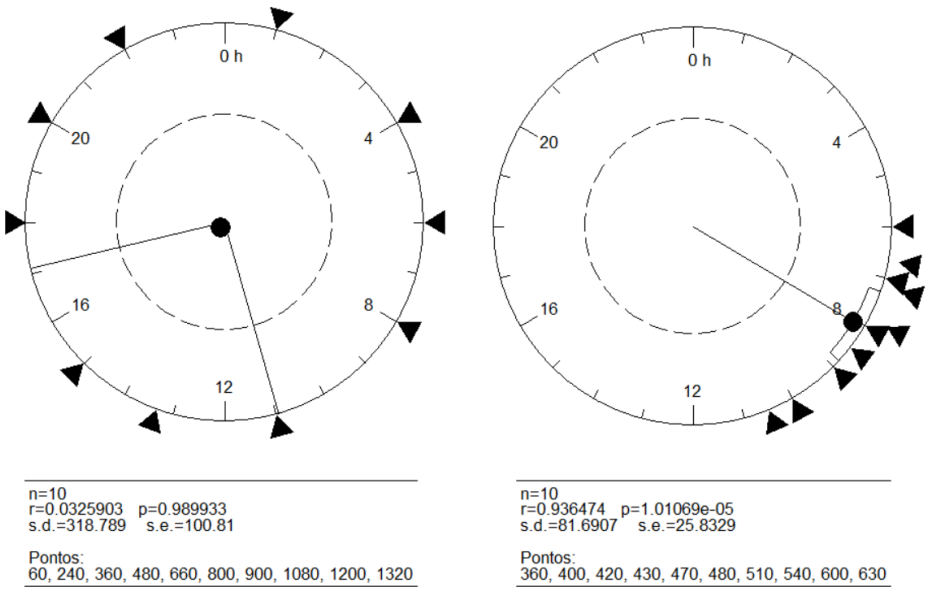
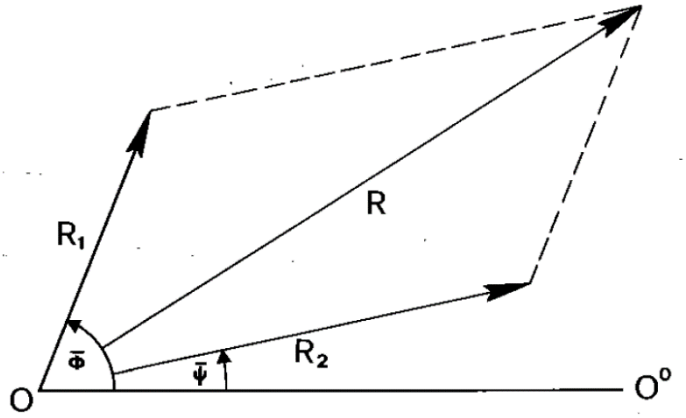


Figura 22. Teste de Rayleigh para dois grupos de dados simulados. O plot circular a esquerda apresenta dados uniformemente distribuídos ao redor do relógio, e com um valor de *r* baixo, como esperado, a hipótese nula não é rejeitada. O relógio da direita, no entanto, mostra dados que estão bastante próximos entre si, agrupando-se em uma região do círculo, ou seja, apresentam evidente direcionalidade, confirmada pela estatística que rejeita a hipótese nula.

Comparação entre 2 ou mais grupos de dados circulares

Watson-Williams

O teste de Watson-Williams é um teste paramétrico utilizado para comparar 2 amostras independentes. Ele é o equivalente circular do teste t de Student e o objetivo é testar se os ângulos médios de duas amostras são distintos ou não. A estatística do teste leva em consideração o comprimento dos vetores resultantes de cada amostra (R_1 e R_2), bem como o vetor resultante das amostras combinadas (R) (Figura 23), o tamanho amostral total (n) e uma constante calculada a partir do fator de concentração K (g). O teste apresenta como suposições que as duas amostras apresentem dados que sigam uma distribuição de von Mises e que o parâmetro de concentração seja igual para ambas amostras, apresentando valor elevado ($K > 2$) (99).



$$F = g(n-2)\frac{R_1 + R_2 - R}{n - (R_1 + R_2)}$$

$$g = 1 + \frac{3}{8\ \mathrm{K}}$$

$$F = g\frac{(n-k)(\sum R_i - R)}{(k-1)(n - \sum R_i)}$$

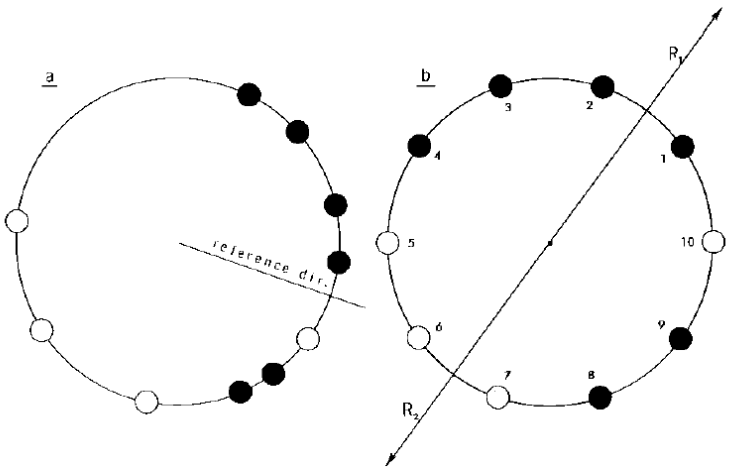
Figura 23. Vetores resultantes de duas amostras (comprimentos R_1 e R_2), juntamente com o vetor da amostra combinada (comprimento R). Quanto mais R se diferencia de $R_1 + R_2$, maior a distância entre as direções médias de cada amostra. Imagem retirada de Batschelet, 1981.

Mardia-Watson-Wheeler

O teste de Mardia-Watson-Wheeler é um teste não paramétrico utilizado para comparar 2 ou mais amostras independentes. Ele é o equivalente circular do teste Kruskal-Wallis e o objetivo é testar se duas amostras são diferentes entre si a partir dos seus ângulos médios, das variâncias angulares ou de ambas as medidas (99).

A ideia do teste é juntar todos os dados de diferentes amostras, observar a ordem dos ângulos de cada dado e distribuí-los de com as mesmas distâncias entre cada ponto. Cada ponto é ranqueado a partir de uma direção definida como referência de forma arbitrária não importanto o sentido (horário ou anti-horário) (Figura 24). Escolhe-se uma amostra e se calcula o comprimento do vetor resultante (R_1). Para comparação entre 2 amostras, o teste estatístico

utiliza o valor de B, que a partir do nível de significância escolhido, pode ser obtido através da tabela Q. Para amostra com n > 17, utilizar o valor de χ^2 disponível na Tabela G do apêndice do livro de Batschelet (99). Para B > B (α), rejeita-se a hipótese nula e se conclui que as amostras são diferentes entre si. Quando a comparação for entre mais de duas amostras, a estatística é obtida através de W. Os valores críticos de W podem ser obtidos da Tabela R no caso de até 3 amostras com pouco dados, o a partir da Tabela G com valores de χ^2.



$$R_1 = (C^2 + S^2)^{1/2}$$

$$B = R_1^2$$

$$\chi^2 = 2(n-1)R_1^2/n_1 n_2$$

$$W = 2\sum_{i=1}^{k} R_i^2 / n_i$$

Figura 24. Princípio do teste proposto por Mardia, Watson e Wheeler. Imagem retirada de Batschelet, 1981.

Correlação Circular

Existem muitas ocasiões onde uma variável circular pode estar relacionada a outra variável circular, mas também é bastante corriqueiro no estudo dos ritmos biológicos correlacionar dados circulares a dados lineares. Por exemplo, numero de nascimentos (linear) e hora do dia ou mês do ano (circular) que ocorreram. Para tanto, assim como para cálculo de média circular não usamos a mesma estatística linear, o mesmo ocorre para correlação que envolva dados circulares (100).

Os cálculos matemáticos por trás das estatísticas de correlação são um pouco extensos, mas podem ser facilmente seguidos com a orientação didática de Batschelet em seu livro *Circular Statistics in Biology* (99), cuja leitura é fortemente recomendada. Abaixo, duas figuras ilustram estes dois tipos de correlações envolvendo dados circulares (Figura 25-26).

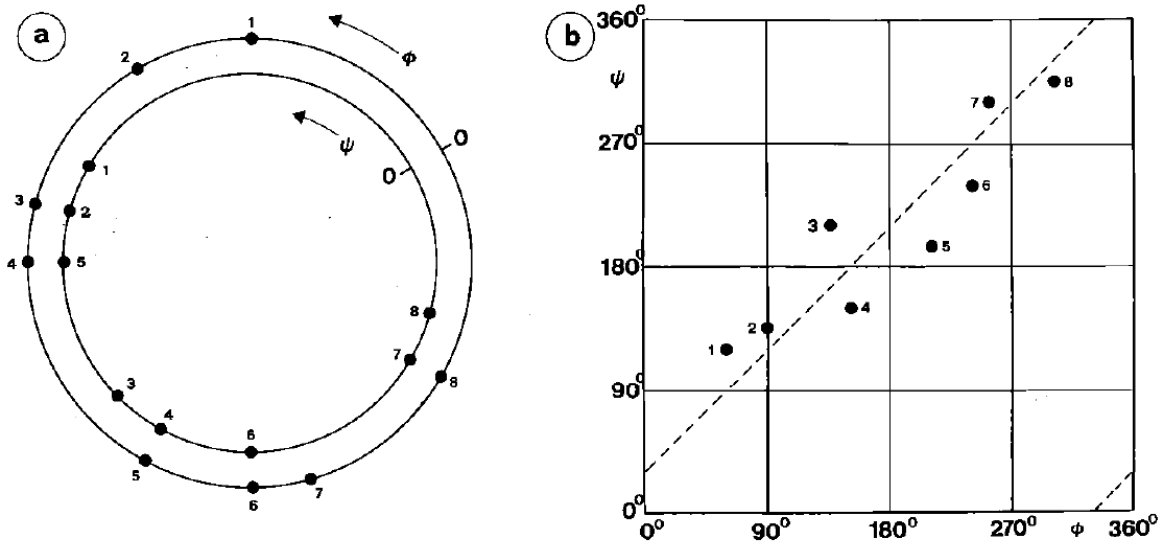


Figura 25. Correlação circular-circular. Em (a) um plot circular das variáveis em questão e em (b) um plot linear dos mesmos dados. A linha de regressão pontilhada sugere uma correlação positiva entre as amostras. Imagem retirada de Batschelet, 1981.

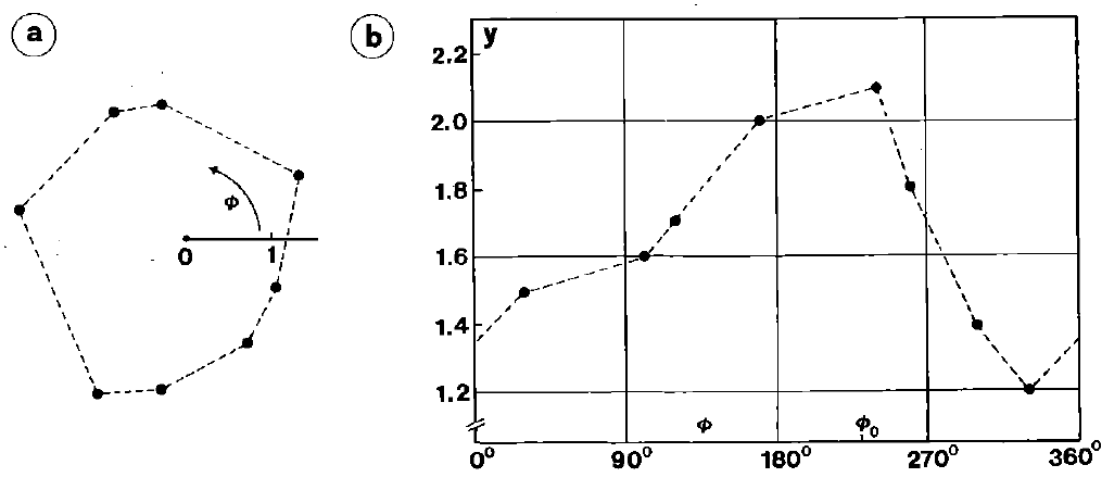


Figura 26. Correlação circular-linear. Em (a) um plot circular das variáveis em questão e em (b) um plot linear dos mesmos dados. A linha de regressão pontilhada é aproximadamente sinusoidal. Imagem retirada de Batschelet, 1981.

IDENTIFICAÇÃO DE DADOS FALTANTES

Um importante passo durante a análise de séries temporais é o cuidadoso pré-processamento dos dados. A depender das ferramentas utilizadas para a coleta de dados, bem como do intervalo de coleta, nem sempre é uma possível obter uma série uniformemente amostrada. Isso significa que é comum identificarmos dados faltantes. No entanto, além de tipos de análise que conseguem lidar com tal fenômeno, também podemos contar com técnicas para minimizar os efeitos de gaps nos dados como, por exemplo, aplicando suavizações ou preenchimentos dos intervalos vazios com médias de outros intervalos equivalentes. Tonon e colaboradores após simulações sugeriram inclusive substituir zeros por NA para evitar invariância gerada nos dados (101). Contudo, antes de trabalhar com os dados faltantes é necessário bem identifica-los, o que nem sempre é uma tarefa fácil, em especial quando se trata de actimetria.

A actimetria, ou actigrafia, é um método objetivo para estudo do ritmo de atividade/repouso e que possibilita inferir sono/vigília com base nos movimentos do punho, onde o aparelho, semelhante a um relógio, é mais usualmente utilizado. Alguns modelos também registram dados de temperatura corporal e exposição à luz. O actímetro é um equipamento cômodo e que possibilita coleta de dados por até meses ininterruptos, dependendo do intervalo de coleta pré-definido, até que haja necessidade de recarregar a bateria. Portanto, a série de dados temporais pode ser bastante longa. Uma das limitações da actimetria é a acurácia reduzida para diferenciar períodos de repouso de período de sono. E também, especialmente em períodos curtos, o desafio passa a ser de diferenciar o não uso do equipamento (os *missings* ou *off-wrist)* e o repouso.

A inspeção visual mencionada anteriormente pode auxiliar na identificação destes períodos de não uso do equipamento, mas acaba tornando o pré-processamento trabalhoso, dependente da disponibilidade de pessoas com conhecimento técnico e demorado considerando a possibilidade de séries muito longas de registro. Diante disso, com o intuito de deixar o processamento dos dados mais automatizado, decidiu-se verificar a viabilidade de algoritmos para detecção de períodos *off-wrist* em dados de actimetria.

Diferentes de outras ferramentas computacionais com similar propósito, no artigo abaixo, o desenvolvimento de algoritmos com abordagem heurística e de machine learning partiu da análise de dados coletado conforme um protocolo para simulação dos períodos de não uso do equipamento. Tentando mimetizar as situações mais frequentes de acordo com a prática

do grupo de pesquisa que, há anos, trabalha com actimetria, o protocolo apresentava situações em que o aparelho deveria estar fora do punho e em certos contextos que poderiam influenciar dados de luz e temperatura. Um exemplo de situação contida no protocolo é “fora do pulso ao sol das 14:00 às 18:00”. Os dados obtidos foram analisados tendo os diários de seguimento do protocolo como padrão ouro, e os algoritmos foram comparados com a inspeção visual e com outro algoritmo já disponível. Todos os métodos utilizados para a detecção dos *off-wrist* tiveram desempenhos bons. Intervalos de menos de 2h de não uso do equipamento ou não foram identificados, ou identificados incorretamente pelos algoritmos. No entanto, diferente da inspeção visual que levou horas para ser concluída, em segundos ou poucos minutos os métodos automatizados terminaram a tarefa. Desta forma, os algoritmos desenvolvidos para detecção do não uso do equipamento são igualmente eficazes à inspeção visual, com a importante vantagem de serem mais rápidos, menos dispendiosos e independentes da atenção/experiência dos avaliadores.

OXFORD Sleep Research Society®

SLEEP, 2022, 1–14

https://doi.org/10.1093/sleep/zsac118
Advance Access Publication Date: 22 May 2022
Original Article

ORIGINAL ARTICLE

Development and testing of methods for detecting off-wrist in actimetry recordings

Luísa K. Pilz[1,2,*], Melissa A. B. de Oliveira[1,2], Eduardo G. Steibel[1], Lucas M. Policarpo[3], Alicia Carissimi[1], Felipe G. Carvalho[1], Débora B. Constantino[1,2], André Comiran Tonon[1,2], Nicóli B. Xavier[1,2], Rodrigo da Rosa Righi[3] and Maria Paz Hidalgo[1,2]

[1]Laboratório de Cronobiologia e Sono—Hospital de Clínicas de Porto Alegre (HCPA)/Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, Brazil, [2]Graduate Program in Psychiatry and Behavioral Sciences—UFRGS, Porto Alegre, Brazil and [3]Applied Computing Graduate Program (PPGCA)—Universidade do Vale do Rio dos Sinos (UNISINOS), São Leopoldo, Brazil

*Corresponding author. Luísa K. Pilz, Laboratório de Cronobiologia e Sono (HCPA/UFRGS), Ramiro Barcelos, 2350, Porto Alegre – RS. 90035-903, Brazil. Email: luisa.pilz@ufrgs.br.

Todas as ferramentas acima descritas vem sendo cada vez mais incorporadas na compressão de fenomenos que detalhem o processo saude-doença. Assim forma-se um arcabouço importante para o entendimento dos transtornos mentais como processos dinâmicos que representem momentos de cronodisrrupção.

8. CRONOMEDICINA

A cronomedicina pode ser compreendida como uma disciplina cujo foco principal é o tempo e o papel que ele exerce sobre fisiologia, metabolismo e comportamento, o que pode afetar a prevenção, a identificação e o tratamento de doenças nos seres humanos. Ainda que haja muito conhecimento sendo obtido através de estudos em cronobiologia e que podem ser transferidos para estratégias em Cronomedicina, ainda se faz necessário investir em estudos clínicos a fim de construir metodologias personalizadas de ação, com foco no indivíduo, e em ações de prevenção que possam ser aplicadas em larga escala com foco na população (102).

Cada vez mais se valoriza o caráter translacional dos estudos científicos, buscando encurtar e facilitar a aplicação dos achados do laboratório na prática clínica. Diante da quantidade crescente de evidências mostrando a relação do relógio biológico com a saúde, a cronobiologia vem buscando estabelecer um caminho entre ciência básica e a medicina (1). Klerman e colaboradores inclusive criaram um esboço para a comunidade científica de base cronobiológica sobre as etapas, processos e prazos necessários para traduzir as descobertas científicas da área básica em cuidados clínicos baseados em evidências e/ou recomendações de saúde e segurança públicas (103).

Kramer e colaboradores também propuseram um modelo sobre o futuro da medicina circadiana (Figura 27) que se baseia no desenvolvimento de novas ferramentas diagnósticas, na melhoria ou reverção da ruptura de ritmos e na utilização de protocolos de tratamento que levem em consideração a hora do dia que seja mais adequada (104).

Atualmente, algumas destas abordagens já estão disponíveis, como a utilização do pico de produção de melatonina como marcador de fase circadiana, do inglês dim light melatonin onset (DLMO) (105); a terapia de luz para transtornos de ritmo e também psiquiátricos (45); e recomendações sobre hora do dia para administração de medicamentos do tipo estatina ou para tratamento de asma brônquica (106,107). No entanto, ainda podemos identificar desafios, por exemplo, para determinação do “tempo interno” em humanos (108), na identificação de marcadores biológicos que possam ser aplicados em estudo clínicos e epidemiológicos e na disponibilidade de instrumentos de avaliação em saúde mental que levem em consideração variações de comportamentos relacionados ao humor.

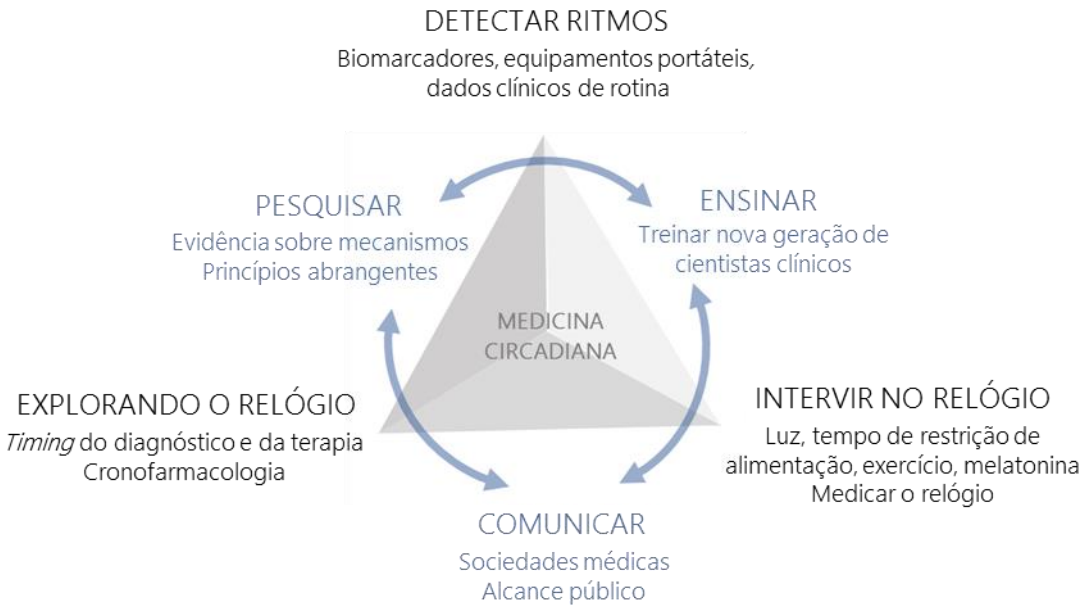


Figura 27. **Modelo de Achim Kramer e colaboradores sobre o futuro da medicina circadiana**. De acordo com os autores, a medicina circadiana compreende 3 abordagens: usar o conhecimento acerca de ritmos fisiológicos para tratamentos adaptados ao momento do dia ideal (explorar o relógio); melhora ou ressincronização de ritmos alterados através de intervenções no relógio (intervir no relógio); e desenvolvimento da medicina circadiana como parte da medicina de precisão através de novas ferramentas diagnósticas que possibilitem intervenções personalizadas de acordo com cronotipo (detectar o relógio). Adaptada de Kramer et al, 2022.

9. OBJETIVOS

Geral

Desenvolver e aprimorar ferramentas de avaliação e intervenção em ritmos biológicos para aplicabilidade na área da Cronomedicina.

Específicos

1. Elaborar orientações para auxiliar pesquisadores que não são da área de Cronobiologia durante desenho do estudo, coleta, análise, interpretação e divulgação dos resultados levando em consideração ritmos biológicos.
2. Investigar os efeitos da exposição a diferentes sistemas de iluminação nas oscilações cerebrais de roedores
3. Avaliar o impacto do uso de equipamentos individuais de proteção contra luz durante a noite no desenvolvimento de neonatos prematuros.
4. Validar o Instrumento de Ritmo de Humor (MRhI) para espanhol e inglês e realizar análise transcultural dos itens do MRhI entre amostras brasileira, espanhola e canadense.

CAPÍTULO 2 ï CONSIDERANDO OS RITMOS BIOLÓGICOS NA PRÁTICA DA PESQUISA

A Cronobiologia possui carácter multidisciplinar e, portanto, suas bases de estudo podem ser aplicadas nas mais diversas áreas do conhecimento. No entanto, é comum observar, pelo menos nas áreas das ciências biológicas e da saúde, uma deficiência metodológica em relação aos ritmos biológicos e como eles podem afetar a obtenção, interpretação e reprodução dos resultados.

A necessidade de compartilhar conhecimento básico para comunidade científica que não está necessariamente familiarizada com a Cronobiologia fomentou a produção de um artigo de revisão que buscasse esclarecer a importância e orientar sobre a incorporação de conhecimento básico sobre ritmos biológicos nos distintos estágios da pesquisa humana e animal, desde o desenho experimental, análise dos dados, até descrição dos resultados.

Artigo publicado na revista *Physiology & Behavior* (IF: 2.9; Qualis A2 área Medicina II).

DOI: 10.1016/j.physbeh.2023.114387

Title: Taking biological rhythms into account: from study design to results reporting

Melissa Alves Braga de Oliveira[1,2], Ana Carolina Odebrecht Vergne de Abreu[1], Débora Barroggi Constantino[3], André C. Tonon[1,2], Antoni Díez-Noguera[4], Fernanda Gaspar do Amaral[5], Maria Paz Hidalgo[1,2,]*

[1] Laboratório de Cronobiologia e Sono do Hospital de Clínicas de Porto Alegre, Universidade Federal do Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil;

[2] Graduate Program in Psychiatry and Behavioral Sciences, Faculty of Medicine, Universidade Federal do Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil;

[3] Chronobiology, Faculty of Health and Medical Sciences, University of Surrey, Guildford, United Kingdom;

[4] Department de Bioquimica i Fisiologia, Facultat de Farmàcia i Ciències de l'Alimentació, Universitat de Barcelona, Barcelona, Spain;

[5] Department of Physiology, Federal University of São Paulo, São Paulo, Brazil;

*Corresponding author: Mailing address - Ramiro Barcelos, 2350, CPC-sala 21514 - Santa Cecilia, Porto Alegre - RS, 90035-903, Brazil; Phone: +5551 3359-6339; email: mhidalgo@hcpa.edu.br.

Abstract

Numerous physiological and behavioral processes in living organisms exhibit strong rhythmicity and are regulated within a 24-hour cycle. These include locomotor activity and sleep patterns, feeding-fasting cycles, hormone synthesis, body temperature, and even mood and cognitive abilities, all of which are segregated into different phases throughout the day. These processes are governed by the internal timing system, a hierarchical multi-oscillator structure conserved across all organisms, from bacteria to humans. Circadian rhythms have been seen across multiple taxonomic kingdoms. In mammals, a hierarchical internal timing system is comprised of so called central and periphereal clocks. Although these rhythms are intrinsic, they are under environmental influences, such as seasonal temperature changes, photoperiod variations, and day-night cycles. Recognizing the existence of biological rhythms and their primary external influences is crucial when designing and reporting experiments. Neglecting these physiological variations may result in inconsistent findings and misinterpretations. Thus, here we propose to incorporate biological rhythms into all stages of human and animal research, including experiment design, analysis, and reporting of findings. We also provide a flowchart to support decision-making during the design process, considering biological rhythmicity, along with a checklist outlining key factors that should be considered and documented throughout the study. This comprehensive approach not only benefits the field of chronobiology but also holds value for various other research disciplines. The insights gained from this study have the potential to enhance the validity, reproducibility, and overall quality of scientific investigations, providing valuable guidance for planning, developing, and communicating scientific studies.

Keywords: human experimentation, animal experimentation, chronobiology, research methods

1. Introduction

Imagine yourself performing a clinical study to investigate the effect of a drug on pineal melatonin levels. Participants are instructed to take the drug in the morning, but blood samples for melatonin dosage were collected at random times of the day, according to their availability. This study has a few methodological problems that may be obvious to chronobiology specialists, but not to researchers across other diverse fields. Indeed, the issues can be observed mainly because pineal melatonin release occurs only during the dark phase, with its peak during the sleep/rest phase. Numerous factors invariably influence research performed in the field of Natural Sciences as the one aforementioned. From basic physiological regulation to complex behaviors, living beings undergo constant biological changes throughout the day. This means that blood pressure, body temperature, expression of dopamine receptors in the striatum, cognitive performance, sleep and appetite are all functions that can exhibit varying patterns depending on the time of data collection (1). To ensure transparency and reproducibility, it is crucial to consider these factors when designing and reporting experiments. When data is collected, the moment of the day can drastically impact results obtained in numerous fields, from cognitive and behavioral sciences to genetic analysis. While general guidelines of experimental and clinical design apply universally, studies involving biological rhythms, particularly *circadian rhythms*, often overlook certain intricacies that non-chronobiology researchers may not be aware of.

Life on Earth has evolved to adapt to the natural alternation of light and darkness caused by the planet's rotation on its axis. The ability to anticipate cyclic environmental changes has conferred a significant survival advantage to species, leading to the ubiquitous presence of biological rhythms across all living organisms. In humans and practically all other organisms, biological rhythms are regulated by a *circadian system* that coordinates *peripheral clocks.* This intricate system is responsible for aligning internal rhythms with external cues, facilitating the adaptation of organisms to their environment (2). Every cell possesses its own molecular machinery that sustains its biological rhythm. Distinguishable clock and clock-controlled genes form a macromolecular transcription-based oscillation within all cells, having further implications on the physiology of peripheral and central tissues and body systems (3). The timing regulatory system utilizes neural and humoral signaling to orchestrate oscillations in a series of physiological events throughout the body, ensuring optimal adaptation and synchronization to the environment (4,5).

Some examples of rhythmic variables are ATP release, serotonin levels, immune cell reactivity, cortisol or corticosterone release, pineal melatonin synthesis and release, gene transcription, feeding-fasting cycle, body temperature and sleep-wake cycle (6–8). Many of these variables encompass hormones and peptides that are intricately linked to emotions, cognition, and behavior.(9,10). It is important to note that not all biological rhythms are circadian in nature. Some present a cycle longer than 24 hours spanning weekly, monthly (e.g., menstrual cycle), or even yearly (e.g., hibernation) rhythms. These are called ***infradian rhythms***. There are also ***ultradian rhythms***, such as blood circulation, growth hormone secretion, and appetite (a glossary of relevant terms is provided in Table 1) (11,12). Thus, the physiological rhythmic organization directly and indirectly influences the variables of interest of clinical studies and experimental models using non-human species.

Table 1 | Glossary

Term	Definition
Aliasing	Effect observed when sampling occurs in intervals longer than half of the real period of the rhythm, inducing the identification of a nonexistent rhythm.
Biological Clock	A self-sustained oscillator that receives environmental information and produces periodicity to physiological functions.
Chronopharmacology	An area of chronobiology that investigates the effect of pharmacologic agents on the biological timing of biological rhythms and the effect of administering drugs in different timings.
Circadian phase	The circadian phase indicates the current position of the organism within its circadian cycle. The circadian phase is primarily influenced by environmental signals, especially light-dark cycles.
Circadian Rhythm	A biological rhythm with a period of about 24hr (e.g., the sleep-wake cycle). Circadian rhythms exist in organisms ranging from bacteria to humans and continue to oscillate in a constant environment with their own period (tau).
Endogenous rhythm	A rhythm generated by the organism itself, not by an environmental cycle to which the organism is exposed, which can be quantified in free-running conditions presenting a period tau.

Entrainment	Process that occurs when an internal oscillator is synchronized by an environmental cue.
Free-running	When the intrinsic circadian rhythm is not adjusted (entrained) to the 24-hour cycle. It reflects the intrinsic oscillation of the endogenous rhythm when there are no external time cues.
Infradian Rhythm	Rhythms that are repeated in a cycle longer than 24 hours (e.g., once a month). The sexual and reproductive behavior of most animals follows an infradian rhythm.
Peripheral Clocks	Circadian oscillators located in tissues and cells that receive information from the central pacemaker and present localized rhythmic outputs.
Phase	A defined point on an oscillation that reflects a time point on the rhythm.
Photoperiod	It is the interval/length of light phase in a day. For example, the 12:12 photoperiod, which means 12 hours of light and 12 hours of dark.
Time Series	Set of successive observational values of the same variable (discrete or continuous) made over time.
Circadian System	In mammals, the circadian timing system is composed of inputs, outputs (neural and humoral), and the SCN, which determine the circadian and seasonal biological rhythmicity in order to time physiological functions and synchronize them with the external environmental fluctuations. Gene and biochemical loops are part of the cellular rhythmic fluctuation machinery.
Ultradian Rhythm	Rhythms that are repeated in a cycle shorter than 24 hours (e.g., four times a day), like the heart rate.
Zeitgeber	From the German for “time-giver”, refers to an environmental cue that provides stimulus to reset the biological clock. The most powerful *zeitgeber* is the light-dark cycle.
Zeitgeber time (ZT)	As opposed to the chronological time, the *zeitgeber* time represents the time (hours) passed since a *zeitgeber* is given (usually the lights on). This measurement is used in experimental studies with controlled photoperiod.

Several studies take into account rhythmicity when investigating behavioral and cognitive aspects (9,13), genetics (14), pharmacotherapy (15–19), neuroimaging (20) and other research fields. However, it is not uncommon for articles to overlook the reporting of crucial methodological details. Nelson and colleagues evaluated the top 50 cited papers across 10 domains of the biological sciences, such as immunology, neurosciences and pharmacology in 2015 and then repeated the analysis in 2019. They found that most publications failed to include sufficient temporal details when describing methods and that there were no differences in time-of-day reporting between 2015 and 2019, even after the 2017 Nobel Prize in Medicine and Physiology for discoveries of molecular mechanisms controlling the circadian rhythms, which they hypothesized would improve appreciation and reporting of temporal information (21). This oversight often occurs due to researchers' limited understanding of chronobiology or the absence of a requirement to include such vital information. Since aspects regarding biological rhythms can drastically impact the quality of results, the accuracy of conclusions, and the reproducibility of experiments, it is imperative not to neglect them. The acknowledgment of inner biological rhythmicity and its main external influences considerably improves the validity and reliability of the collected data. On the other hand, ignoring this essential part of normal physiology may lead to inconsistent results, erroneous conclusions (Figure 1), and irreproducible experiments.

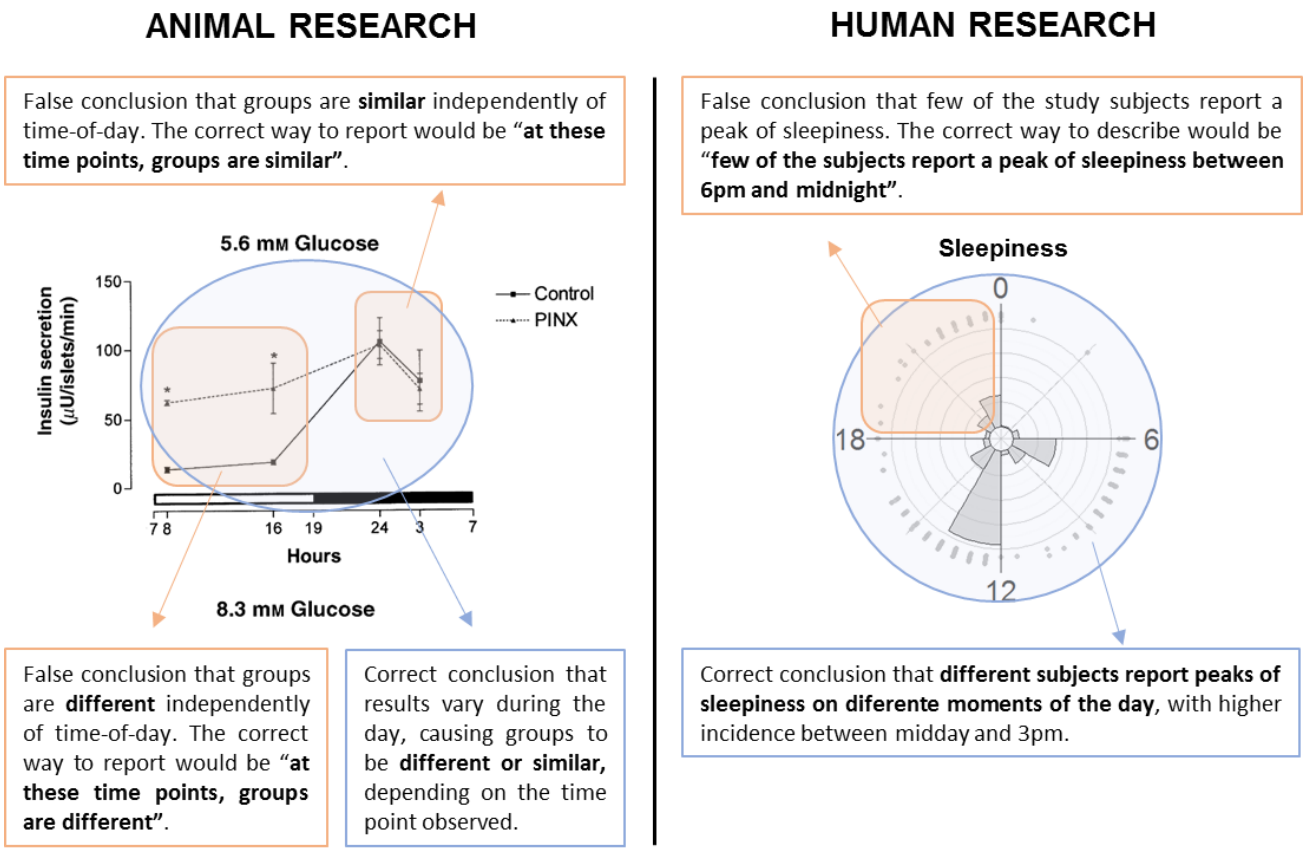


Figure 1. Examples of misleading conclusions when working with rhythmic variables. Even when we do not intend to analyze rhythmicity, we cannot ignore the possibility of its existence and, thus, we should present coherent conclusions and report real data. When it is possible to observe only a part of the rhythm cycle (orange), the conclusion must not extrapolate this sampling feature (orange). The best way to report the findings properly is also shown (blue). Results taken from Picinato et al, 2002 and Pilz et al, 2018 (22,23).

Given that rhythms are inherent to life, and are observed in our study subjects, including humans, mammals, rodents, plants, bacteria, and cells, we present a set of guidelines for non-chronobiology experts. These guidelines aim to equip researchers and professionals involved in the development, approval, and publication of high-quality scientific research with essential knowledge of methodological aspects that account for biological rhythms. The guidelines will focus on human and rodent research; however, some aspects may also be extended to other species and study areas. We address appropriate experimental design, sampling methodology and statistical methods for analysis of rhythmic patterns, besides advising how to describe the methodology and report study results.

A flowchart to support researchers while designing and reporting studies can be found in Figure 2, where we summarized decision-making and reporting processes considering

biological rhythmicity. The only situation left out of the flowchart was the case when searching in literature for data regarding rhythmicity of the study variable turns out unfruitful, which lead to further studies to fill out this gap.

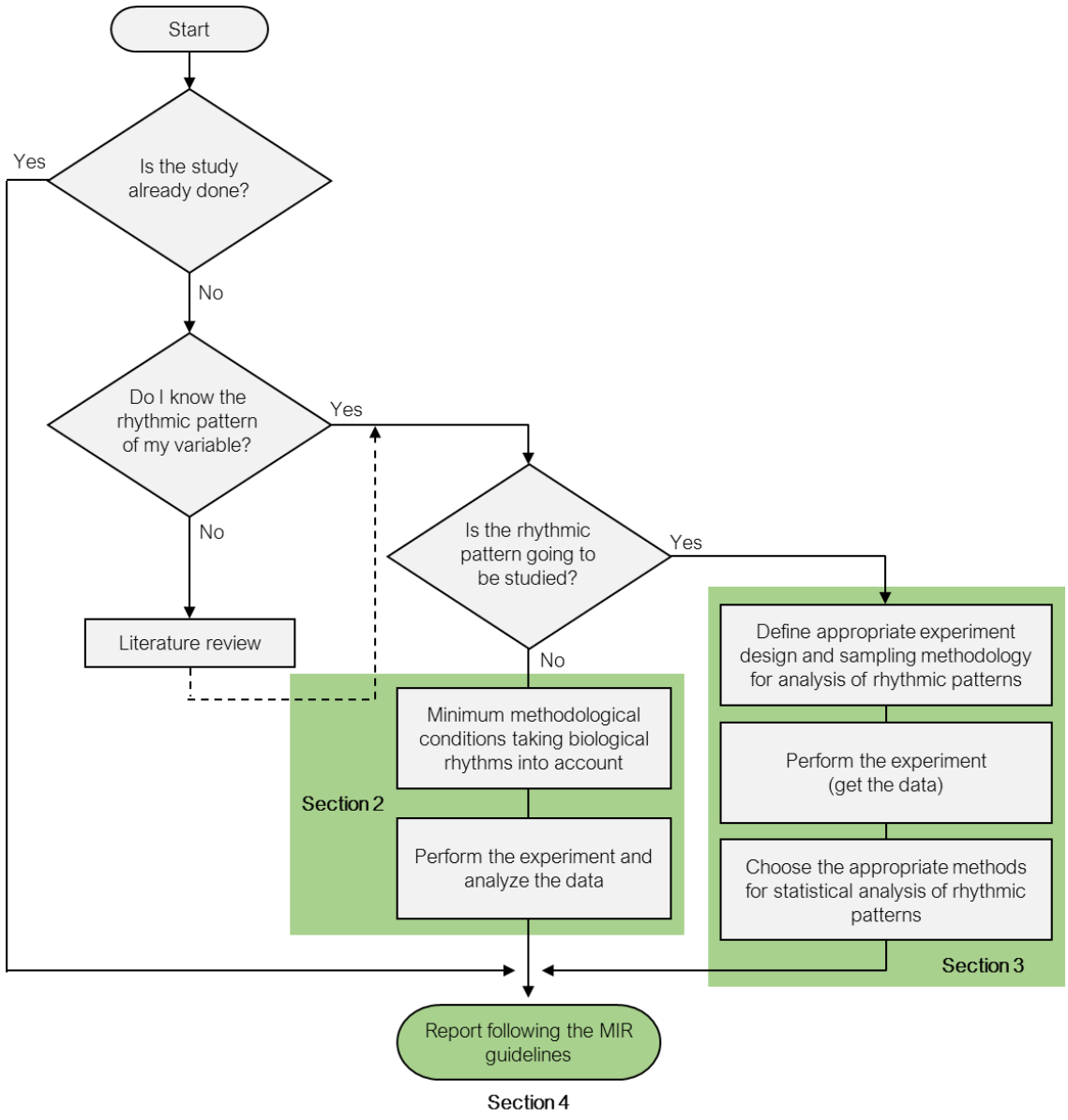


Figure 2. Flowchart to guide decision making and the reporting of information, taking into account biological rhythmicity. When there is still the possibility to think about the study design, methodological decisions should consider whether biological rhythms are going to be studied or not. If they are not, *minimum methodological conditions* should still be followed, which can be found in section 2. On the other hand, if studying rhythmic patterns is one of the goals, it is important to *define appropriate experiment design and sampling methodology* and to *choose the appropriate methods for statistical analysis of rhythmic patterns*, both topics explored in section 3. In the case of studies that have already been performed, even if they were

not designed with the goal of measuring rhythms, some information should be provided to support conclusions and improve reproducibility. Finally, the methodology should be reported following the MIR guidelines described in section 4.

2. Minimum methodological conditions taking biological rhythms into account

Ideally, in all clinical, experimental, and observational research projects, the entrained endogenous rhythms should be taken into account, irrespective of whether biological rhythms are the primary focus of the study. While it may not always be feasible to incorporate this consideration fully, applying a minimum level of rigor in study design can help minimize sampling and measurement biases.

2.1. Avoiding bias due to sampling and time-sensitive interventions

When assessing participants or subjects, it is essential to consider the appropriate timing for sampling or performing interventions. If researchers do not intend to analyze rhythmic patterns, we are unaware of the minimum number of sampling times, however for those planning multiple-timepoint studies, defining optimal number to sample will depend on literature regarding the rhythm in question, available funds and study logistics. Even though there is no specific number, for circadian rhythms, more than three samples and ideally six during 24h are suggested (24). In order to define the best time to collect data, researchers should be aware of the periodicity of the variable and the pattern of variability (e.g., the timing of the highest and lowest values during a cycle) (25). This precaution allows the researcher, for example, to hypothesize when differences may be expected when study groups are compared. For instance, resuming the example given at the beginning of introduction, if one desires to see the effect of a drug upon pineal melatonin levels, the best time to dose melatonin would be during its production and release in the bloodstream (e.g. during dark phase at night). There is no impediment to making collections during morning or afternoon, when perhaps is more feasible in terms of research logistics, however, unless one hypothesizes the drug is capable of modifying the phase of pineal activity, night time would be the most suitable time to access melatonin levels.

It is also essential to collect the data always at the same time points in order to avoid misleading conclusions due to time-of-day differences in normal oscillatory patterns. Defining time slots such as “morning” or “afternoon” is relevant, but does not provide enough information, particularly in animal research. When studying human participants with varying

chronotypes and sleep/wake patterns, assessments should be adjusted to account for each participant's habitual sleep/wake timing. This adjustment helps minimize inter-individual variance in circadian phases of measurements and reduces the variance associated with participants altering their behaviors due to the study protocol (26). The currently available instruments to determine chronotype are the Morningness-Eveningness Questionnaire (MEQ), that provides a self-evaluation of chronotype, which is a unidimensional construct and offers a classification that encompasses categories from morning to evening types. The questionnaire presents questions relative to the time when each individual feels to be more prone to perform daily activities and to sleep (27). Another well-established tool is the Munich Chronotype Questionnaire (MCTQ), which was introduced by Roenneberg et al. (2003), that defines chronotype from the calculation of the midpoint of sleep, which is defined as the half-way point between sleep onset and sleep end (28).

Time patterns of variables such as weekend/weekdays behavioral variations, ultradian and infradian hormonal fluctuations, monthly variations, and seasonality should be considered. This means that it is also important to consider the duration and frequency of the interventions in relation to the rhythmic patterns under investigation. As described, some rhythms may exhibit short-term fluctuations, while others may span longer periods. Adjusting the timing and duration of interventions accordingly allows for capturing the dynamics and characteristics of the study variable accurately.

The study conducted by Mure et al. (29) gives us the real notion of rhythmic control exerted across a wide range of tissues in order to afford the best timing fitness. Collecting samples every 2 hours over a day from 64 distinct tissues and brain areas of baboons, researchers conclude that a daily pattern is observed in the expression of more than 80% of protein-coding genes encompassing many biochemical and cellular functions. Interestingly, most of these genes coded proteins that are drug targets, which leads us to *chronopharmacology* and the importance of considering the time of day to pharmacological treatments. The well-established knowledge that both drug pharmacokinetics and pharmacodynamics are modulated by the circadian clock highlights that temporal organization is a fundamental factor modulating both the efficacy and safety of pharmacotherapy protocols (15,30). Thus, if investigating the effects of a drug or treatment on circadian rhythms, it is necessary to administer it at specific times that coincide with the relevant biological processes. This ensures that the intervention targets the intended rhythms and maximizes the potential impact. There is evidence that patients

respond differently to pharmacological treatment depending on the time of drug administration (15). After choosing the appropriate time, repeat the protocol always in the same manner unless comparing time-of-day effects.

When it comes to cognitive and behavioral interventions, it is important to be aware of the differences between chronotype in humans (31) and the time-of-day differences in animal protocols. The same goes for physically demanding tasks (e.g., running wheel interventions), where chronotype and light-dark cycles can also impact results. Lastly, if post-mortem analyses are going to be performed, the time of death or euthanasia should be considered.

2.2. Avoiding bias due to environmental cues

It is crucial to acknowledge that all living organisms are influenced by environmental cues. However, not all daily patterns are clock driven, meaning that environmental factors can directly impact physiology and behavior. When these cues have the ability to entrain rhythms they are commonly referred to as ***zeitgebers***. Therefore, it is essential to address and control these environmental cues during research studies. Aschoff, in his classical study with participants that volunteered to spend a few weeks isolated in bunkers, precisely showed the impact of living without temporal cues and raised some important questions about their impact on behavior and metabolism (2).

When thinking about the study's environmental conditions, light is of extreme importance (32,33). The timing properties of the light stimulus (e.g. clock time, duration and pattern) as well as its intensity, spectral composition are relevant factors that can actually affect entrainment, behavior and hormones (34–36), and thus should be addressed and reported (37). We also encourage researchers to provide the *zeitgeber* time (ZT), which is a way to report when lights were on, but can be used to represent the time in hours passed since a *zeitgeber* is given.

An individual's response to light also depends on many inter-individual differences, such as age and chronotype. The same occurs with spectral sensitivity to light that varies considerably between experimental species (37–39). In experiments where the light/dark cycle is managed, defining a ***photoperiod*** for experimental protocols (e.g., 12 hours of light and 12h of dark) and rigorously following it should be standard practice, unless manipulation of light

exposure is a factor to be investigated. In spite of variances in behavioral patterns between mammals that are active during the night (nocturnal) and those active during the day (diurnal), there are striking similarities in the physiology and function of the suprachiasmatic nucleus (SCN), the central clock of our circadian timing system. Experiments conducted during the usual working hours of humans take place during a rodent's inactive phase. These differences regarding the relationships between circadian timing and behavioral rhythms among diurnal and nocturnal species can introduce significant confounding factors.

Regardless of your specific interventions and research outcomes, if possible, it is important to prioritize the use of a reversed light/dark cycle when studying nocturnal animals. This ensures that their active phase aligns with your own, facilitating animal handling and sampling without disrupting their rest period. Depending on the animal strain, when performing tests, interventions, or manipulating samples during dark hours, red light should be preferable due to its smaller impact on the circadian timing system, which is the case for nocturnal rodents (40,41).

Avoid interrupting natural behavior whenever possible. For example, it is not advisable to wake individuals to collect blood samples or conduct behavioral tests on nocturnal animals during their light phase. Instead, choose the appropriate activity and rest periods to perform the necessary tasks. For instance, performing the light-dark box test in nocturnal rats to verify spontaneous exploration behavioral of new environments should prioritize the animal's period of highest activity (dark phase) in order to enable observing differences caused by the intervention (42).

If the study subjects are human, gather information about shift work or irregular routines and transmeridian flights, that are considered possible factors of chronodisruption that may interfere with the results and could be exclusion factors depending on the study research question (43,44).

Additionally, be aware that non-photic zeitgebers (e.g. physical exercise, food intake, temperature and social rhythms) can also contribute to the synchronization of rhythms (45). When dealing with non-human animals, use randomization to mitigate the zeitgeber effect that some necessary habits may assume (e.g., handling, cage cleaning). The well-established non-photic zeitgebers affect peripheral oscillators in a highly tissue-specific manner. For instance, consider that food timing can impact the rhythmic features of the gut, liver, and pancreas (46).

Literature has provided evidence that food intake restricted to the normal activity phase displays great synchronization of the circadian system (47,48). Escobar and colleagues showed that depending on the type of food, a timed piece of chocolate, for example, might be sufficient stimulus for circadian synchrony (49).

While this guide does not provide specific guidance on controlling external factors such as noise, vibration, and feed intake, it is important to recognize their potential influence and consider appropriate measures to minimize their impact on studies.

3. Recommendations for well-designed experiments

This section was designed to assist researchers in the conduction of experiments where rhythms are going to be explored. Nevertheless, the previous section should also be considered as basic knowledge regarding sampling, time of data collection and impact of environmental factors are addressed.

Define appropriate experiment design and sampling methodology for analysis of rhythmic patterns

When it comes to studies on biological rhythms, it is imperative that researchers think over the study design carefully before data collection. Knowing the existing methods for sampling *time series*, such as longitudinal, cross-sectional, and hybrid, as well as their strengths and weaknesses, assists in choosing the most appropriate and feasible strategy (25). Besides, establishing adequate data collection intervals and considering environmental factors affecting sample acquisition is also necessary to enable reliable conclusions (24).

There are basically three methods of sampling for time series (25). Longitudinal sampling is characterized by a collection of data that is continuously made over time, encompassing many cycles of the studied phenomenon, which enables the assessment of temporal features of that one subject. For more accurate conclusions regarding the components responsible for the manifested behavior under analysis, the longer the data collection, the better. In a cross-sectional sampling, each subject or individual is the source of one data point in the

time series. Thus, a large number of subjects is necessary to assess rhythmic behavior. Nevertheless, this is a useful approach when longitudinal sampling is not possible (e.g., circadian variation in ischemic stroke occurrence) (50). Lastly, the hybrid sampling combines longitudinal and cross-sectional designs. If possible, this should be the chosen method in chronobiological studies, for it enables the assessment of variability within the rhythm. For example, there are substantial natural inter-individual differences in circulating melatonin levels, making more time points from different individuals necessary for reliable interpretations (51).

Perhaps the most essential step while planning the study design is defining the intervals of data collection and the number of time points that are going to be sampled. An insufficient sampling rate can result in misleading conclusions about the studied rhythm (e.g., *aliasing*: If Δt is between T and T/2, the estimated T will be longer than real T) (52). Thus, repeated measures should be collected at regular intervals to cover at least one complete cycle of the rhythm, be it *ultradian* (<24h), *circadian* (≅ 24h), or *infradian* (>24h) (25). The shorter period of data collection that one can analyze in a time series is determined by the Nyquist frequency (f_N) and can be calculated as follows:

$$f_N = \frac{1}{2\,\Delta t} \qquad T = \frac{1}{f_N} = 2\Delta t$$

Where Δt is the sampling interval and T the period of data collection (52).

To ensure characterization of circadian rhythms in multiple-timepoint studies, four samples per cycle taken at regular intervals can be considered the minimum for data analysis, but it will provide little information about accurate rhythmic characteristics. It is important to acknowledge that several factors, including the volume of existing literature and financial resources, play a significant role in determining the optimal number of time points for such studies (25).

By intervals, we mean the amount of time between samples collected from the same subject and the interval between the sampling of different subjects. It is already known that seasonality exerts an important impact on many functions, from mood to gene expression (53–55). Given this, unless intending to explore variations across seasons, data collection should be driven at the same periods of the year. In the same line, samples should also be collected at the

same time points within a day for each subject from a longitudinal or hybrid study. Taking interleukin-6 (IL-6) as an example, a recent meta-analysis confirmed the diurnal variation exhibited by IL-6 and suggested that this temporal information is important to avoid confounding by the time of day in studies of IL-6 in plasma or serum (56). The same also occurs with circulating glucocorticoids (57) and many other physiological variables under circadian organization.

As a matter of fact, many pathological processes also display a rhythmic pattern. This occurs when the pathology is related to a process under circadian modulation, and thus, it is expected that symptoms also follow the same pattern of rhythmicity (58). For example, manifestations of rheumatoid arthritis, allergic rhinitis or bronchial asthma are more frequent at night and around awakening when proinflammatory cytokines present higher levels in the circulation (59,60). This rhythmicity reinforces the importance of knowing the variation behavior of the parameter as well as being attentive to sampling timing.

Choose the appropriate methods for statistical analyses of rhythmic patterns

Once the data has been collected, it is recommended to plot it as a function of time (chronogram) and perform a visual inspection. This step should come first because it reveals more evident rhythms and assists in the process of selecting appropriate analytic methods (25,52,61). Next, the characterization and quantification of the oscillations of a rhythmic variable can be determined through various statistical and mathematical procedures, such as Fourier analysis, Cosinor procedure, Rayleigh test, and periodograms, which will be briefly explained below. Table 2 lists some of the softwares and analysis tools currently available for the assessment of rhythmic characteristics. It is worth mentioning that there is much open source-code available (e.g., in R, Python) that can be used to visualize and analyze data with the advantage of being accessible in an open source environment.

The **analysis of variance (ANOVA)** can be used in Chronobiology research to determine uniformity in a time series: if all class means are not different, it is possible to infer that there is no rhythmicity in the series. However, it does not provide specific information about rhythmic patterns or periods.

Periodograms are methods used to estimate the period of a time series (e.g., a circadian rhythm usually shows a period (T) of 1440 minutes, or 24 hours, but it could be different under *free-running* conditions). The **Whitaker/Enright periodogram** proposed in the 1960s (62) is

employable in the case of time series with equidistant measurements (data collected in regular intervals), which are more common under laboratory conditions. The Chi-square periodogram (also known as Sokolove-Bushell periodogram) is its most popular implementation and uses the $\chi 2$ distribution to detect circadian rhythmicity in a series (63). It is recommendable to have more than eight complete cycles to perform these periodograms. The **Lomb-Scargle periodogram**, on the other hand, can be applied to data that has been sampled at irregular intervals or contains missing values (64). The **classical periodogram** is based on the Fourier analysis (65,66) and consists of fitting the data to sinusoids of increasing periods and detecting the period that has a higher correlation with the data.

The **Fourier analysis**, also known as "spectral analysis", is used to uncover distinct frequency components of a rhythm through a decomposition process called Fourier transformation. Its output maps the time series into different frequencies called harmonics. If a major powerful spectral component is identified, the investigator can deduce that the variable under study displays rhythmicity. For example, if the harmonic of greater power is compatible with 1440 min, the rhythm found follows a circadian pattern (52). The power spectrum is a characteristic of the "shape" or the profile of the rhythm.

The **Cosinor analysis** is a procedure for investigating the periodic aspects of a time series by fitting a cosine function to the data through regression techniques. It can be applied to equidistant and non-equidistant time series and to serially independent data as well. From this procedure, it is possible to determine parameters such as the MESOR, the amplitude, and the acrophase (61,67).

The **MESOR** (M, Midline Estimating Statistic of Rhythm) is a rhythm-adjusted mean. It is different from arithmetic mean in the case of non-equidistant data and/or data that does not cover a complete number of cycles. The **amplitude** (A) is defined as half of the distance between the highest and the lowest point of the oscillation (or the difference between the mesor and the crest or trough of the wave). The **acrophase** (ϕ) is a parameter that indicates the time when a variable reaches the peak of the curve.

The **Rayleigh test** assesses data uniformity by distributing data points around a circle. This test is specific for circular data, which, unlike linear data, has a periodic nature. On the circle, an angle of 345º is nearer to an angle of 15º than to an angle of 300º, just like 11 p.m. is closer to 1 a.m. than to 8 p.m. on the clock. On a linear scale, the distance between these

measurements would be different, so one can understand the need for specific circular analysis procedures (68,69). Since a day can be represented as a 24-hour clock, the Rayleigh test is often used in Chronobiology to investigate if the periodicity with which events occur is uniform or occurs at a specific time (showing a unimodal distribution). An example can be found in Figure 1, where the peaks of sleepiness reported by subjects are plotted around a 24h clock.

Biological rhythms, including circadian rhythms, are known to exhibit patterns beyond the traditional sinusoidal shape. **Waveform analysis** is useful for characterizing the shape of a wave. For example, if a period of 24 hours is chosen, similar times of activity levels among days will be averaged successively. Therefore, the mean waveform should be considered in inferential statistical chronobiology (25). Another approach to study rhythmic patterns was proposed by Van Someren and colleagues (70) with the use of "**nonparametric variables**" (71) in the study of biological rhythms, as these are sensitive indicators of disturbances to rhythms. These strategies are most used when the rhythm is not adjusted to a sinusoidal function, i.e., a curve that follows a smooth periodic oscillation. The **interdaily stability (IS)** is a measure that indicates how constant or stable the pattern of activity is across days. IS is reduced when the day-to-day variation of activity-rest rhythms is higher. This parameter is often used as an indication of how synchronized to the environment an individual is. The **intradaily variability (IV)** indicates fragmentation of the 24-hour rhythm based on successive time intervals and reflecting transitions between rest and activity. IV is increased when there is daytime napping and/or nighttime arousals. Even though IS and IV are frequently applied in the analysis of 24h rhythms, the formula for calculation does not depend on this assumption. The **M10** corresponds to the mean values for the 10 continuous hours with the highest values of a variable (e.g., activity, temperature, or light). The **L5** is the parameter related to the 5 continuous hours with the lowest values (71). The **relative amplitude (RA)** is a nonparametric alternative to estimate the sinusoidal amplitude, and it is calculated as the difference between M10 and L5 in the average 24h pattern with the formula (M10-L5)/(M10+L5) (72). It is worth mentioning that actimetry data, which can provide information on activity-rest and body temperature rhythms, together with light exposure behavior during long term recordings, is commonly targeted for calculating many of the parameters cited above. Nonparametric variables are especially explored in actimetry for the study of sleep and psychiatry disorders. For example, lower RA in wrist actimetry has been associated with depressive symptoms and increased risk of lifetime major depressive disorder (73,74).

Table 2 | Examples of available tools for the assessment of rhythmic characteristics	
CATkit	https://564394709114639785.weebly.com/cosinor.html by Cathy Lee Gierke, Ruth Helget and Germaine Cornelissen-Guillaume Chronomics Analysis Toolkit extract period and phase and other key characteristic of rhythms (actogram, periodogram, smoothing, auto-correlation, cosinor and more)
ChronoSapiens	https://www.chronsulting.org/services-products by Till Roenneberg Tool for all levels of circadian and sleep research, with special emphasis on in-depth analysis and visualization of long-term actimetry and light recordings
Chronos-fit	https://chronos-fit.software.informer.com/ by Björn Lemmer Detection of rhythmic organization in arbitrary data and modules for group analysis, power spectrum analysis and actograms are implemented.
CircaCompare	https://github.com/RWParsons/circacompare/ by Rex Parsons Performs analyses regarding mesor, phase, and amplitude, reporting on estimates and statistical differences, for each, between groups of circadian rhythms
Circadian Software	http://www.circadian. org/main.html by Roberto Refinetti Perform cartesian plots, actograms, acrophase, cosinor method, circadian period and more
COMPARERHYTHMS	https://github.com/bharathananth/compareRhythms by Bharath Ananthasubramaniam Find features with altered circadian rhythm parameters (amplitude and phase) at molecular level (e.g. profiling the transcriptome at multiple time points)
El temps	http://www.el-temps.com/principal.html by Antoni Díez-Noguera Integrates many chronobiological procedures for data analysis (actograms, periodograms, cosinor, waveforms, serial analysis and more)
Pyactigraphy	https://github.com/ghammad/pyActigraphy by Grégory Hammad Comprehensive python toolbox for in-depth and large scale actigraphy data analyses

4. Minimum Information to Report taking biological rhythms into account: MIR guidelines

Below is a checklist with items that should be included in a study report to allow reproducibility and a critical analysis of the research (Table 3).

Conclusions

This article provides comprehensive guidelines for conducting and reporting research, specifically emphasizing the importance of considering biological rhythmicity. Even though we acknowledge that the information brought here would benefit from discussions in a broader international context, we aimed to offer valuable insights not only to researchers but also to editors, reviewers, ethic committee members, and other professionals involved in scientific development, approval, and publication processes. The accompanying flowchart is designed to be accessible and beneficial at any stage of the study, whether it's experimental design, data collection, result reporting, or even for individuals who wish to avoid misleading results but do not specifically focus on studying rhythms. In addition to the general aspects of Chronobiology presented here, these principles can also be incorporated into other research areas to enhance the quality and reproducibility of publications. Specific guidelines and general implications of accounting for rhythms can also be found in the literature, focusing on clinical studies (26,37,75,76), experimental studies (77), genomic analysis (78), tissue culture (79,80), ethics (24), and statistical analysis (25,52).

It's worth noting that while the recognition of the importance of biological rhythms is still relatively recent, awareness on the subject is steadily increasing. Consequently, there is a growing expectation that biological rhythmicity will be widely acknowledged and incorporated into biomedical research practices.

Table 3 | Minimum information to report

General	Sampling	a. Specify time-of-day, interval and duration of data collection. b. Explain how biological material was processed and stored, including information on exposure to light.
	Environmental factors	a. If subjects are in non-research environments/natural habitats, provide details about geographical localization, the season of the year, and dawn and dusk times. b. In controlled environments, describe the light/dark cycle providing information on length of photoperiod, ZT0, as well as basic characteristics of the light source (e.g. lux and spectral composition) during the study and during procedures and data collection (e.g. surgeries, behavioral tests, blood sampling); in the case of studies where light stimulus is an intervention, see Spitschan et al, 2019 for minimum reporting guidelines. c. Provide details on possible interruption of natural behavior (e.g. biological sampling or behavioral tests during sleep). d. Detail treatment/intervention protocols (what, when, where and why).
	Statistical methods	a. Describe method used for pre-processing data and handling missing. b. Describe the methodology for rhythm analysis.
Animal Research	Experimental animals	a. Describe species-appropriate details (strain, sex, age, health status, genetic manipulations status, previous procedures such as maternal isolation).
	Environmental factors	a. Describe procedures related to animal care detailing time-of-day and frequency (food and water availability, cage changes, handling and environmental enrichment). b. Describe the details on the diet and whether animals were deprived of any type of food before assessments. c. Report any other possible temporal cues (e.g. exercise promotion, environment temperature variations, noise, stress protocols)
Human Research	Human subjects	a. Describe subjects (age, sex, health condition and, if possible, assess chronotype, usual sleep times and light exposure behavior). b. Report current and/or recent pharmacological treatments
	Environmental factors	a. Provide information regarding transmeridian flights and night/rotating shift work, and whether they were considered inclusion/exclusion criteria. b. Report daylight saving time changes in case they occur during the study c. Describe the details on tobacco use, caffeine intake, and alcoholic beverage consumption before and during the data collection. d. Report whether participants avoided consumption of any time of food before assessments (e.g. banana, pineapple and chocolate should be avoided for melatonin measurement).

Conflict of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

Author Contributions

M. A. B. Oliveira, D. B. Constantino, A. C. Tonon, F. G. Amaral and M.P. Hidalgo designed the study. M. A. B. Oliveira, D. B. Constantino, A. C. Tonon and A. C. O. V. Abreu wrote the manuscript. A.D. Noguera, F. G. Amaral, M.P. Hidalgo revised the manuscript. All authors approved the final manuscript.

Acknowledgements

Financial support was provided by Coordenaç˜ao de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001 CAPES (DBC, ACT, FGA), CNPq (MABO, ACOVA), PQ-CNPq and PrINT/CAPES (MPH). The funding bodies had no role in the study design, data collection, analysis, or interpretation, or in the writing of the manuscript.

REFERENCES

1. Mendoza J, Challet E. Circadian insights into dopamine mechanisms. Neuroscience. 2014 Dec 12;282:230–42.

2. Aschoff J. CIRCADIAN RHYTHMS IN MAN. Science. 1965 Jun 11;148(3676):1427–32.

3. Reppert SM, Weaver DR. Coordination of circadian timing in mammals. Nature. 2002 Aug;418(6901):935–41.

4. Aschoff J. CIRCADIAN SYSTEM PROPERTIES. In: Obál F, Benedek G, editors. Environmental Physiology [Internet]. Pergamon; 1981 [cited 2023 Jan 18]. p. 1–17. Available from: https://www.sciencedirect.com/science/article/pii/B9780080273396500063

5. Moore RY. Chapter 8 Entrainment pathways and the functional organization of the circadian system. In: Buijs RM, Kalsbeek A, Romijn HJ, Pennartz CMA, Mirmiran M,

editors. Progress in Brain Research [Internet]. Elsevier; 1996 [cited 2023 Jan 18]. p. 103–19. Available from: https://www.sciencedirect.com/science/article/pii/S0079612308604033

6. Takahashi JS, Hong HK, Ko CH, McDearmon EL. The genetics of mammalian circadian order and disorder: implications for physiology and disease. Nature Reviews Genetics. 2008 Oct;9(10):764–75.

7. Suárez-Trujillo A, Casey TM. Serotoninergic and Circadian Systems: Driving Mammary Gland Development and Function. Frontiers in Physiology [Internet]. 2016 [cited 2022 Feb 21];7. Available from: https://www.frontiersin.org/article/10.3389/fphys.2016.00301

8. Marpegan L, Swanstrom AE, Chung K, Simon T, Haydon PG, Khan SK, et al. Circadian Regulation of ATP Release in Astrocytes. J Neurosci. 2011 Jun 8;31(23):8342–50.

9. Gritton HJ, Stasiak AM, Sarter M, Lee TM. Cognitive Performance as a Zeitgeber: Cognitive Oscillators and Cholinergic Modulation of the SCN Entrain Circadian Rhythms. PLoS One [Internet]. 2013 Feb 18 [cited 2020 Oct 7];8(2). Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3575350/

10. Logan RW, McClung CA. Rhythms of life: circadian disruption and brain disorders across the lifespan. Nat Rev Neurosci. 2019 Jan;20(1):49–65.

11. Laje R, Agostino PV, Golombek DA. The Times of Our Lives: Interaction Among Different Biological Periodicities. Frontiers in Integrative Neuroscience [Internet]. 2018 [cited 2022 Feb 21];12. Available from: https://www.frontiersin.org/article/10.3389/fnint.2018.00010

12. Gamble KL, Berry R, Frank SJ, Young ME. Circadian Clock Control of Endocrine Factors. Nat Rev Endocrinol. 2014 Aug;10(8):466–75.

13. Todd WD, Fenselau H, Wang JL, Zhang R, Machado NL, Venner A, et al. A hypothalamic circuit for the circadian control of aggression. Nat Neurosci. 2018;21(5):717–24.

14. Rakshit K, Qian J, Ernst J, Matveyenko AV. Circadian variation of the pancreatic islet transcriptome. Physiol Genomics. 2016 01;48(9):677–87.

15. Dallmann R, Okyar A, Lévi F. Dosing-Time Makes the Poison: Circadian Regulation and Pharmacotherapy. Trends Mol Med. 2016;22(5):430–45.

16. Bowles NP, Thosar SS, Herzig MX, Shea SA. Chronotherapy for Hypertension. Curr Hypertens Rep. 2018 28;20(11):97.

17. Hermida RC, Ayala DE, Fernández JR, Mojón A, Crespo JJ, Ríos MT, et al. Bedtime Blood Pressure Chronotherapy Significantly Improves Hypertension Management. Heart Fail Clin. 2017 Oct;13(4):759–73.

18. Sanghani HR, Jagannath A, Humberstone T, Ebrahimjee F, Thomas JM, Churchill GC, et al. Patient fibroblast circadian rhythms predict lithium sensitivity in bipolar disorder. Mol Psychiatry. 2020 May 13;

19. Hermida RC, Ayala DE, Mojón A, Fontao MJ, Fernández JR. Chronotherapy with valsartan/hydrochlorothiazide combination in essential hypertension: improved sleep-time blood pressure control with bedtime dosing. Chronobiol Int. 2011 Aug;28(7):601–10.

20. Byrne JEM, Tremain H, Leitan ND, Keating C, Johnson SL, Murray G. Circadian modulation of human reward function: Is there an evidentiary signal in existing neuroimaging studies? Neurosci Biobehav Rev. 2019; 99:251–74.

21. Nelson RJ, Bumgarner JR, Liu JA, Love JA, Meléndez-Fernández OH, Becker-Krail DD, et al. Time of day as a critical variable in biology. BMC Biology. 2022 Jun 15;20(1):142.

22. Pilz LK, Carissimi A, Oliveira MAB, Francisco AP, Fabris RC, Medeiros MS, et al. Rhythmicity of Mood Symptoms in Individuals at Risk for Psychiatric Disorders. Scientific Reports. 2018 Jul 30;8(1):11402.

23. Picinato MC, Haber EP, Carpinelli AR, Cipolla-Neto J. Daily rhythm of glucose-induced insulin secretion by isolated islets from intact and pinealectomized rat. Journal of Pineal Research. 2002;33(3):172–7.

24. Portaluppi F, Smolensky MH, Touitou Y. Ethics and methods for biological rhythm research on animals and human beings. Chronobiol Int. 2010 Oct;27(9–10):1911–29.

25. REFINETTI R, LISSEN GC, HALBERG F. Procedures for numerical analysis of circadian rhythms. Biol Rhythm Res. 2007;38(4):275–325.

26. Klerman EB, Brager A, Carskadon MA, Depner CM, Foster R, Goel N, et al. Keeping an eye on circadian time in clinical research and medicine. Clin Transl Med. 2022 Dec;12(12):e1131.

27. Horne JA, Ostberg O. A self-assessment questionnaire to determine morningness-eveningness in human circadian rhythms. Int J Chronobiol. 1976;4(2):97–110.

28. Roenneberg T, Wirz-Justice A, Merrow M. Life between clocks: daily temporal patterns of human chronotypes. J Biol Rhythms. 2003 Feb;18(1):80–90.

29. Mure LS, Le HD, Benegiamo G, Chang MW, Rios L, Jillani N, et al. Diurnal transcriptome atlas of a primate across major neural and peripheral tissues. Science [Internet]. 2018 Mar 16 [cited 2020 Oct 8];359(6381). Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5924732/

30. Dallmann R, Brown SA, Gachon F. Chronopharmacology: New Insights and Therapeutic Implications. Annu Rev Pharmacol Toxicol [Internet]. 2014 Jan 6 [cited 2020 Oct 15];54. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3885389/

31. Adan A, Archer SN, Hidalgo MP, Di Milia L, Natale V, Randler C. Circadian typology: a comprehensive review. Chronobiol Int. 2012 Nov;29(9):1153–75.

32. Daan S, Aschoff J. The Entrainment of Circadian Systems. In: Takahashi JS, Turek FW, Moore RY, editors. Circadian Clocks [Internet]. Boston, MA: Springer US; 2001 [cited

2023 Jan 18]. p. 7–43. (Handbook of Behavioral Neurobiology). Available from: https://doi.org/10.1007/978-1-4615-1201-1_2

33. LeGates TA, Fernandez DC, Hattar S. Light as a central modulator of circadian rhythms, sleep and affect. Nat Rev Neurosci. 2014 Jul;15(7):443–54.

34. de Oliveira MAB, Scop M, Abreu ACO, Sanches PRS, Rossi AC, Díez-Noguera A, et al. Entraining effects of variations in light spectral composition on the rest-activity rhythm of a nocturnal rodent. Chronobiol Int. 2019;36(7):934–44.

35. Harb F, Hidalgo MP, Martau B. Lack of exposure to natural light in the workspace is associated with physiological, sleep and depressive symptoms. Chronobiology International. 2015 Mar 16;32(3):368–75.

36. Juda M, Liu-Ambrose T, Feldman F, Suvagau C, Mistlberger RE. Light in the Senior Home: Effects of Dynamic and Individual Light Exposure on Sleep, Cognition, and Well-Being. Clocks Sleep. 2020 Dec 14;2(4):557–76.

37. Spitschan M, Stefani O, Blattner P, Gronfier C, Lockley SW, Lucas RJ. How to Report Light Exposure in Human Chronobiology and Sleep Research Experiments. Clocks Sleep. 2019 Jul 1;1(3):280–9.

38. Gooley JJ, Rajaratnam SMW, Brainard GC, Kronauer RE, Czeisler CA, Lockley SW. Spectral responses of the human circadian system depend on the irradiance and duration of exposure to light. Sci Transl Med. 2010 May 12;2(31):31ra33.

39. Murphy MJ, Westerman EL. Evolutionary history limits species' ability to match colour sensitivity to available habitat light. Proceedings of the Royal Society B: Biological Sciences. 2022 May 18;289(1975):20220612.

40. Emmer KM, Russart KLG, Walker WH, Nelson RJ, DeVries AC. Effects of Light at Night on Laboratory Animals and Research Outcomes. Behav Neurosci. 2018 Aug;132(4):302–14.

41. Hattar S, Liao HW, Takao M, Berson DM, Yau KW. Melanopsin-containing retinal ganglion cells: architecture, projections, and intrinsic photosensitivity. Science. 2002 Feb 8;295(5557):1065–70.

42. Sasso EM, Vieira JL, Dantas G, de Souza CM, Levandovski RM, Hidalgo MPL. Impact of the time in an animal model of mood disorder. Journal of Neuroscience Methods. 2012 Oct 15;211(1):84–7.

43. Haus E, Smolensky M. Biological clocks and shift work: circadian dysregulation and potential long-term effects. Cancer Causes Control. 2006 May;17(4):489–500.

44. Wittmann M, Dinich J, Merrow M, Roenneberg T. Social jetlag: misalignment of biological and social time. Chronobiol Int. 2006;23(1–2):497–509.

45. Roenneberg T, Merrow M. The Circadian Clock and Human Health. Curr Biol. 2016 23;26(10):R432-443.

46. Lewis P, Oster H, Korf HW, Foster RG, Erren TC. Food as a circadian time cue — evidence from human studies. Nat Rev Endocrinol. 2020 Apr;16(4):213–23.

47. Zarrinpar A, Chaix A, Panda S. Daily Eating Patterns and Their Impact on Health and Disease. Trends in Endocrinology & Metabolism. 2016 Feb 1;27(2):69–83.

48. Ekmekcioglu C, Touitou Y. Chronobiological aspects of food intake and metabolism and their relevance on energy balance and weight regulation. Obesity Reviews: An Official Journal of the International Association for the Study of Obesity. 2011 Jan;12(1):14–25.

49. Escobar C, Espitia-Bautista E, Guzmán-Ruiz MA, Guerrero- Vargas NN, Hernández-Navarrete MÁ, Ángeles-Castellanos M, et al. Chocolate for breakfast prevents circadian desynchrony in experimental models of jet-lag and shift-work. Scientific Reports. 2020 Apr 10;10(1):6243.

50. Argentino C, Toni D, Rasura M, Violi F, Sacchetti M L, Allegretta A, et al. Circadian variation in the frequency of ischemic stroke. Stroke. 1990 Mar 1;21(3):387–9.

51. Lockley SW. Journal of Pineal Research guideline for authors: Measuring melatonin in humans. Journal of Pineal Research. 2020;69(2):e12664.

52. Díez-Noguera A. Methods for serial analysis of long time series in the study of biological rhythms. J Circadian Rhythms. 2013 Jul 18;11:7.

53. Wirz-Justice A. Seasonality in affective disorders. Gen Comp Endocrinol. 2018 01;258:244–9.

54. Stewart S, Keates AK, Redfern A, McMurray JJV. Seasonal variations in cardiovascular disease. Nature Reviews Cardiology. 2017 Nov;14(11):654–64.

55. Dopico XC, Evangelou M, Ferreira RC, Guo H, Pekalski ML, Smyth DJ, et al. Widespread seasonal gene expression reveals annual differences in human immunity and physiology. Nature Communications. 2015 May 12;6(1):7000.

56. Nilsonne G, Lekander M, Åkerstedt T, Axelsson J, Ingre M. Diurnal Variation of Circulating Interleukin-6 in Humans: A Meta-Analysis. PLOS ONE. 2016 Nov 10;11(11):e0165799.

57. Oster H, Challet E, Ott V, Arvat E, de Kloet ER, Dijk DJ, et al. The Functional and Clinical Significance of the 24-Hour Rhythm of Circulating Glucocorticoids. Endocrine Reviews. 2017 01;38(1):3–45.

58. Smolensky MH, Peppas NA. Chronobiology, drug delivery, and chronotherapeutics. Adv Drug Deliv Rev. 2007 Aug 31;59(9–10):828–51.

59. Buttgereit F, Smolen JS, Coogan AN, Cajochen C. Clocking in: chronobiology in rheumatoid arthritis. Nature Reviews Rheumatology. 2015 Jun;11(6):349–56.

60. Smolensky MH, Lemmer B, Reinberg AE. Chronobiology and chronotherapy of allergic rhinitis and bronchial asthma. Adv Drug Deliv Rev. 2007 Aug 31;59(9–10):852–82.

61. Cornelissen G. Cosinor-based rhythmometry. Theoretical Biology and Medical Modelling. 2014 Apr 11;11(1):16.

62. Enright JT. The search for rhythmicity in biological time-series. Journal of Theoretical Biology. 1965 May 1;8(3):426–68.

63. Sokolove PG, Bushell WN. The chi square periodogram: Its utility for analysis of circadian rhythms. Journal of Theoretical Biology. 1978 May 8;72(1):131–60.

64. Ruf T. The Lomb-Scargle Periodogram in Biological Rhythm Research: Analysis of Incomplete and Unequally Spaced Time-Series. Biological Rhythm Research. 1999 Apr 1;30(2):178–201.

65. Scargle JD. Studies in astronomical time series analysis. II - Statistical aspects of spectral analysis of unevenly spaced data. The Astrophysical Journal. 1982 Dec 1;263:835–53.

66. Frescura F a. M, Engelbrecht CA, Frank BS. Significance of periodogram peaks and a pulsation mode analysis of the Beta Cephei star V403 Car. Mon Not R Astron Soc. 2008 Aug 21;388(4):1693–707.

67. Halberg F, Tong YL, Johnson EA. Circadian System Phase — An Aspect of Temporal Morphology; Procedures and Illustrative Examples. In: von Mayersbach H, editor. The Cellular Aspects of Biorhythms: Symposium on Rhythmic Research Sponsored by the VIIIth International Congress of Anatomy Wiesbaden 8–14 August 1965 [Internet]. Berlin, Heidelberg: Springer; 1967 [cited 2020 Nov 30]. p. 20–48. Available from: https://doi.org/10.1007/978-3-642-88394-1_2

68. Cremers J, Klugkist I. One Direction? A Tutorial for Circular Data Analysis Using R With Examples in Cognitive Psychology. Front Psychol [Internet]. 2018 [cited 2020 Nov 30];9. Available from: https://www.frontiersin.org/articles/10.3389/fpsyg.2018.02040/full

69. Landler L, Ruxton GD, Malkemper EP. Circular data in biology: advice for effectively implementing statistical procedures. Behav Ecol Sociobiol. 2018 Jul 11;72(8):128.

70. Van Someren EJ, Swaab DF, Colenda CC, Cohen W, McCall WV, Rosenquist PB. Bright light therapy: improved sensitivity to its effects on rest-activity rhythms in Alzheimer patients by application of nonparametric methods. Chronobiol Int. 1999 Jul;16(4):505–18.

71. Witting W, Kwa IH, Eikelenboom P, Mirmiran M, Swaab DF. Alterations in the circadian rest-activity rhythm in aging and Alzheimer's disease. Biol Psychiatry. 1990 Mar 15;27(6):563–72.

72. van Someren EJ, Hagebeuk EE, Lijzenga C, Scheltens P, de Rooij SE, Jonker C, et al. Circadian rest-activity rhythm disturbances in Alzheimer's disease. Biol Psychiatry. 1996 Aug 15;40(4):259–70.

73. Lyall LM, Wyse CA, Graham N, Ferguson A, Lyall DM, Cullen B, et al. Association of disrupted circadian rhythmicity with mood disorders, subjective wellbeing, and cognitive function: a cross-sectional study of 91 105 participants from the UK Biobank. Lancet Psychiatry. 2018 Jun;5(6):507–14

74. Tonon AC, Constantino DB, Amando GR, Abreu AC, Francisco AP, de Oliveira MAB, et al. Sleep disturbances, circadian activity, and nocturnal light exposure characterize high risk for and current depression in adolescence. Sleep. 2022 Jul 11;45(7):zsac104.

75. Halberg F, Cornélissen G, Bingham C, Fujii S, Halberg E. From experimental units to unique experiments: chronobiologic pilots complement large trials. In Vivo. 1992 Aug;6(4):403–27.

76. Blatter K, Cajochen C. Circadian rhythms in cognitive performance: methodological constraints, protocols, theoretical underpinnings. Physiol Behav. 2007 Feb 28;90(2–3):196–208.

77. Halberg E, Cornélissen G, Halberg F. Optimization of the chronotherapeutic index in the experimental animal laboratory. In Vivo. 1992 Aug;6(4):371–85.

78. Hughes ME, Abruzzi KC, Allada R, Anafi R, Arpat AB, Asher G, et al. Guidelines for Genome-Scale Analysis of Biological Rhythms. J Biol Rhythms. 2017 Oct;32(5):380–93.

79. Kaeffer B, Pardini L. Clock genes of mammalian cells: Practical implications in tissue culture. In Vitro CellDevBiol-Animal. 2005 Nov 1;41(10):311–20.

80. Paulose JK, Rucker EB, Cassone VM. Analysis of Circadian Rhythms in Embryonic Stem Cells. In: Rich IN, editor. Stem Cell Protocols [Internet]. New York, NY: Springer; 2015 [cited 2023 Jun 13]. p. 73–9. (Methods in Molecular Biology). Available from: https://doi.org/10.1007/978-1-4939-1785-3_7.

CAPÍTULO 4 – ESTUDO DOS RITMOS EM HUMANOS

1. APLICAÇÃO DOS CONHECIMENTOS DE CRONOBIOLOGIA NA UTI NEONATAL DO HOSPITAL POMPÉIA DE CAXIAS DO SUL

Artigo publicado na revista *Journal of Biological Rhythms* (IF: 3.5; Qualis A2 área Medicina II).

DOI: 10.1177/07487304231201752

Title: Use of Light Protection Equipment at Night Reduces Time Until Discharge from the Neonatal Intensive Care Unit: A Randomized Interventional Study

Ana Carolina Odebrecht Vergne de Abreu[1], Melissa Alves Braga de Oliveira[1,2], Tamila Alquati[3], André Comiran Tonon[1], Mariana de Novaes Reis[1], Augusto Camargo Rossi[1], Fernanda Sbaraini Bonatto[1,2], Maria Paz L. Hidalgo[1,2,4]

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

[2] Graduate Program in Psychiatry and Behavioral Sciences, School of Medicine, Universidade Federal do Rio Grande do Sul (UFRGS). Porto Alegre, RS, Brazil.

[3] Neonatology Department, Hospital Nossa Senhora de Pompéia, Caxias do Sul, RS, Brazil.

[4] School of Medicine, Universidade Federal do Rio Grande do Sul (UFRGS). Porto Alegre, RS, Brazil.

Corresponding author:

Maria Paz L. Hidalgo
mhidalgo@hcpa.edu.br
R. Ramiro Barcelos, 2350 - Rio Branco, Porto Alegre - RS, 90035-007, Brazil
+55 51 3359-8000

ABSTRACT

Newborn infants' circadian systems are not completely developed and rely on external temporal cues for synchronizing their biological rhythms to the environment. In Neonatal Intensive Care Units (NICU), lighting is usually continuous or irregular and infants are exposed to artificial light at night, which can have negative health consequences. Therefore, the aim of this study was to evaluate the impact of the use of individual light protection equipment at night on the development and growth of preterm neonates. Infants born at less than 37 gestational weeks who no longer needed constant intensive care were admitted into a newborn nursery and randomized to either use eye masks at night (intervention, n = 21) or not (control, n = 20). Infants who used eye protection at night were discharged earlier than those in the control group (8 [5] days vs 12 [3.75] days; $p < 0.05$). A greater variation within the day in heart rate was observed in the intervention group, with lower values of beats per minute (bpm) at 1400 h and 2000 h. There was no significant difference in weight gain between groups. In view of our results and of previous findings present in the literature, we suggest that combining a darkened environment at night with individual light protection devices creates better conditions for the development of preterm infants in the NICU. In addition, eye masks are an affordable and simple to use tool that can reduce hospitalization costs by decreasing the number of days spent in the NICU.

Keywords: preterm infants, light pollution, light protection equipment, neonatology, chronobiology

INTRODUCTION

Preterm birth is defined by the World Health Organisation (WHO) as delivery before the completion of 37 gestational weeks (Vogel et al., 2018). Worldwide, approximately 15 million infants are born prematurely every year, with varying rates among countries that range from 5% to 18% of total births (World Health Organization et al., 2012). Premature infants are at higher risk of neonatal respiratory and neurological conditions, sepsis, visual and hearing problems and poorer neurodevelopmental outcomes (Vogel et al., 2018). Given that specialized care is critical during the first weeks or months of a preterm infant's life, when proper health infrastructure is available, the newborns are admitted to a Neonatal Intensive Care Unit (NICU). In this environment, infants are often exposed to constant lighting conditions and artificial light at night (ALAN), in contrast to the uterine environment in which their development took place (Hazelhoff et al., 2021).

During the gestational period, maternal circadian signals, such as temperature variations, activity-rest cycle, feeding schedule and melatonin secretion are responsible for entraining fetal rhythms (Serón-Ferré et al., 2012). After delivery, in the absence of these maternal signals and with an immature circadian system, the newborn uses social interaction patterns, feeding schedule and, especially, environmental light variations as timing cues (Thomas et al., 2014). The light-dark cycle is the main external cue responsible for the entrainment of circadian rhythms (Aschoff, 1965). In adults, lighting variations are perceived by intrinsically photosensitive ganglion cells in the retina, which send this information to the suprachiasmatic nucleus (SCN) of the hypothalamus through the retinohypothalamic tract (RHT). The SCN acts as a master clock, synchronizing peripheral circadian oscillators present in the whole organism (Welsh et al., 2010). Although it is currently unknown in which developmental stage the RHT becomes functional in humans, studies in non-human primates indicate that the SCN is responsive to light since the equivalent to 24 weeks of gestation in humans, suggesting that lighting can also influence the rhythms of premature infants (Hao and Rivkees, 1999).

When timed correctly, the exposure to bright light can synchronize the circadian clock to the external environment, regulating behavior and physiology (LeGates et al., 2014). However, studies in adults indicate that exposure to even very low levels of artificial light at night (<10 lx) can interfere with melatonin biosynthesis, alter sleep structure and aggravate poor sleep quality (Cho et al., 2016; Phillips et al., 2019; Stebelova et al., 2020; Tähkämö et

al., 2019). Exposure to ALAN has been associated in adults with several negative health outcomes, such as psychiatric and metabolic disorders and higher cancer risk (Bechtold et al., 2010; Dauchy et al., 2015; Haus and Smolensky, 2013). Unfortunately, creating a completely dark environment during the night is not always possible, as in the case of intensive care facilities where some light is necessary at all times for visibility reasons. According to Brazilian regulations, light levels for nighttime observation should be around 20 lux (Associação Brasileira de Normas Técnicas, 2013).

Several studies documented improvement in clinical outcomes of preterm infants exposed to light-dark cycles in NICUs, when compared to the typical constant light or constant near-darkness conditions (Hazelhoff et al., 2021; Morag and Ohlsson, 2016). The cycled light condition, characterized by light-dark cycles with decreased light exposure at night, has been shown to accelerate weight gain and the beginning of oral feeding (Miller et al., 1995; Vásquez-Ruiz et al., 2014), shorten the stay in the NICU and improve peripheral oxygen saturation and heart rate stability throughout the days (Vásquez-Ruiz et al., 2014), decrease activity, respiratory rate and heart rate during the night (Blackburn and Patteson, 1991; Shiroiwa et al., 1986) and accelerate the emergence of circadian activity-rest rhythms (Rivkees et al., 2004). Cycled light has also been shown to improve weight gain when compared with continuous near darkness (5-10 lux) (Brandon et al., 2002) and to reduce fussing and crying in very preterm infants when compared with dim lighting (97.6 ± 45.3 lux during the day and 20.8 ± 20.7 lux at night) (Guyer et al., 2012). Other studies, however, have found no difference between cycled and continuous dim light conditions (Boo et al., 2002; Mirmiran et al., 2003).

In these studies, the light-dark pattern is typically achieved through changes in the unit's lighting system and eventually with the use of individual helmets (Vásquez-Ruiz et al., 2014), curtains (Guyer et al., 2012) or incubator coverings (Brandon et al., 2002; Guyer et al., 2012). Although designed to resemble day-night alterations, the cycled condition usually consists of dim light exposure (~25 lux) during the "lights-off" phase (Guyer et al., 2012; Vásquez-Ruiz et al., 2014). The use of individual light protection equipment, such as eye masks, offers a potential solution to this problem (Hu et al., 2010).

In this context, the aim of the present randomized interventional study was to investigate the impact of the use of individual light protection equipment at night on the development and growth of preterm infants.

MATERIALS AND METHODS

Participants

The sample consisted of forty-one infants born under 37 gestational weeks (average gestational age of 31.99 ± 2.09 weeks) that no longer required intensive care and were admitted at a nursery adjacent to the NICU dedicated to clinical stabilization before hospital discharge. The eligibility criteria of the study were weight greater than 1500 g and stability of vital signs for at least 48 hours. Infants with congenital malformation relevant to the measurement of the desired outcomes (e.g., pre-chiasmatic blindness; brain, cardiac and digestive tract malformations; bronchopulmonary dysplasia) were not included. Moreover, patients were not receiving phototherapy during the study, but some of them did receive while in the NICU. This information was included in the analysis.

The randomization was performed as follows: prior to the start of the study, the research team determined that infants in the control group would be identified by numbers 1-20 and those in the intervention group, by numbers 21-41. The numbers were then randomized by a computer to generate the order in which participants would be included (e.g. the first infant included in the study would receive the number 37 and therefore be part of the intervention group). Envelopes containing information of which group the infant should be assigned to were labeled in the randomized order and sealed. Every time a participant was included in the study, the next envelope in line was opened by the NICU staff and the infant was allocated accordingly. Infants in the control group were exposed to the typical lighting conditions of the nursery during day and night, while those in the intervention group wore eye masks during the night (see Figure 1). Twins were included and each infant was randomly assigned to the intervention (n = 6) or control (n = 4) group, meaning that siblings could either be assigned to the same group or not.

The study was conducted following the Declaration of Helsinki (World Medical Association, 2013) and was approved by the Research Ethics Committees of Hospital de Clínicas de Porto Alegre and Hospital Nossa Senhora de Pompéia (CAAE 65311417.9.1001.5327). Verbal informed consent was provided by a legal guardian.

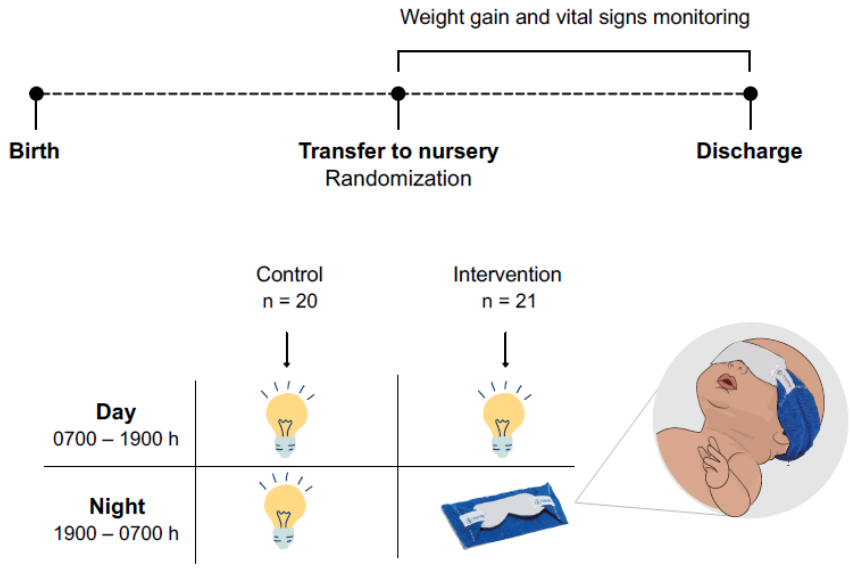


Figure 1. Visual representation of the study design. Time elapsed between birth and transfer to nursery, and from transfer to nursery until discharge varied among participants.

Eye masks

Infants in the intervention group were blindfolded from 1900 h to 0700 h with an eye protector typically used for phototherapy (Baby Block, Impacto Medical, Brazil). In the case a mother came to breastfeed after 1900 h, the eye mask was removed during breastfeeding to allow eye contact between mother and child.

Room Characterization

The study took place in a five-bed newborn nursery adjacent to the NICU. The room has three windows facing other indoor facilities (one facing a corridor and two facing the nursing station) and no external windows (see Fig. 2). Light was provided by two ceiling luminaires with dimmable halogens light bulbs (2700K) that were mostly on and four fluorescent lamp fixtures (4000K) that were only switched on if necessary, such as in the case of a medical emergency. Decisions regarding when lights were switched on, dimmed, or switched off were made by the nurse or nurse technician in charge of the room, according to the need for clinical assistance.

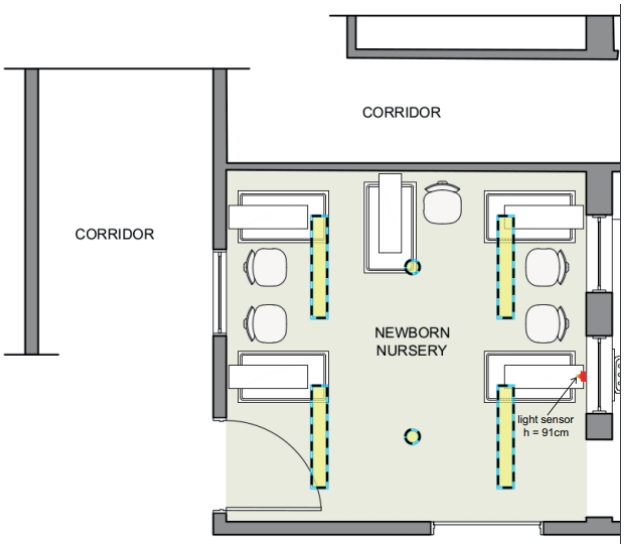


Figure 2. Architectural plan of the five-bed newborn nursery where the study took place. The location of the luminaires is shown in dashed lines.

lluminance, temperature, and humidity were measured every 10 minutes for 15 consecutive days by a sensor developed by the Biomedical Engineering Department of Hospital de Clínicas de Porto Alegre. The sensor was attached to a wall near the height of the head of an infant's bed, at 92 cm from the ground, facing the opposite wall (see Fig. 2). The average temperature in the room was 24.9 ± 0.78 ºC and the average humidity was 62.73 ± 6.28%. The illuminance detected at the point where the sensor was located ranged from 0 to 47 lx (average illuminance ranged between 0.6-12.5 lx during the day and 0.4-4.5 lx during the night) (see Fig. 3).

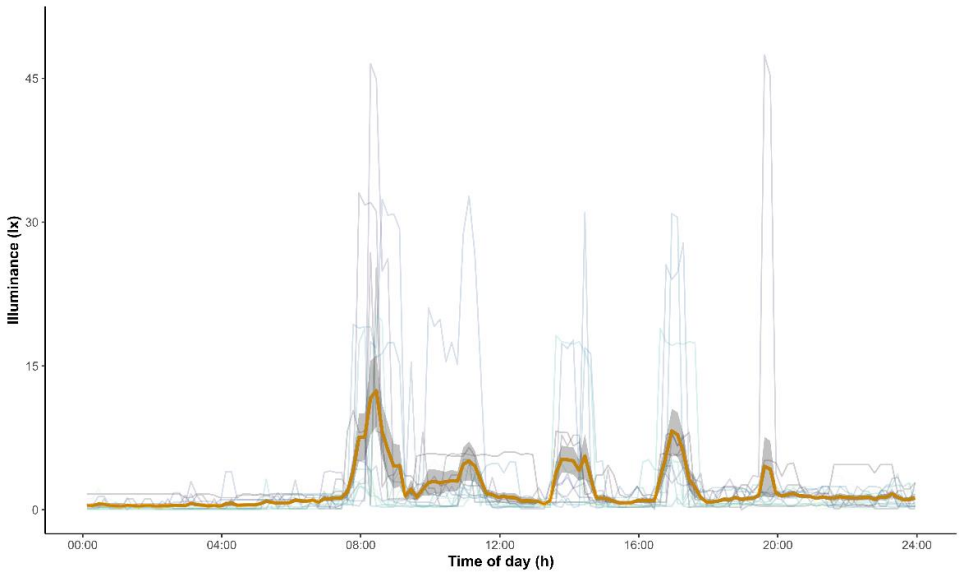


Figure 3. Daily profile of illuminance. Each line represents illuminance measurements from one day. Means of illuminance (in lux) are presented according to clock time in a thicker orange line, standard error is presented in gray.

Measures

Days until discharge

The primary outcome was the number of days spent in the room until discharge. Discharge criteria included stable weight gain (15-30 g per day), weight greater than 1900g, the removal of the orogastric tube and complete oral feeding established for at least 48 hours, and the absence of other health conditions that required constant supervision.

Weight gain

Infants were weighed daily by a nurse or nurse technician. Body weight gain per day was calculated dividing the total weight gain by the number of days until discharge. Because weight can vary according to postmenstrual age (PMA) and sex of the infant, weight Z-scores were also calculated using PediTools (Chou et al., 2020).

Vital signs

Heart and respiratory rate, blood-oxygen saturation and body temperature were measured every 6 hours (0200 h, 0800 h, 1400 h and 2000 h) by the nurses using patient monitors (DX 2023, Dixtal Biomédica, Brazil; and Efficia CM120, Philips, Brazil).

Statistical analyses

Measures of age, sex, weight, head circumference and body length at birth, days until oral feeding and days until discharge were compared between groups using chi-square, Student's t or Mann-Whitney U tests when appropriate. The variations of heart rate over time were analyzed by generalized estimating equations (GEE), which is one of the methods to analyze repeated measurements using a clustering variable to account for the covariance between data points (Vagenas and Totsika, 2018). Differences between groups at each time point were assessed by Bonferroni's test for pairwise comparisons and statistical significance for multiple-factor interactions was set at $p < 0.1$ as we prioritized the detailing of the interactions. Data were presented as mean ± SD, median [IQR] or percentage and statistical significance was set at $p < 0.05$ for the remaining tests. Analyses were performed on SPSS Statistics Subscription (IBM Corp., Armonk, NY, USA) and RStudio version 1.3.1056 (RStudio, PBC, Boston, MA, USA). Graphs were made on SPSS, RStudio and Microsoft Excel version 16.47 (Microsoft Corp., Redmond, WA, USA).

RESULTS

A total of 41 infants were included in the study, 21 of which were randomized into the intervention group. No loss or exclusion occurred after randomization. Groups were similar in age and weight at birth, at the time of enrollment and at discharge. A summary of sample characteristics can be found in Table 1.

Table 1 Characteristics of the study population

	Intervention (n = 21)	**Control (n = 20)**	**Test contrast, p**
GA (wk)	32.86 [1.65]	31 [3.65]	U = 255, p = 0.239
Sex (% female)	57.14	35.00	$\chi 2 = 2.02$, p = 0.155
Birth weight (g)	1686.67 ± 339.42	1631.5 ± 340.75	*t* = -0.519, p = 0.607
Birth weight (Z-score)	-0.3 ± 0.87	-0.18 ± 0.97	*t* = 0.425, p = 0.673
Birth length (cm)	40.76 ± 2.88	40.29 ± 2.57	*t* = -0.545, p = 0.589
Head circumference at birth (cm)	30 [2]	28 [3.5]	U = 225, p = 0.201
PMA at check-in (wk)	34.57 [2]	34.07 [2.11]	U = 229, p = 0.620
Weight at check-in (g)	1873.33 ± 203.23	1883.75 ± 188.29	*t* = 0.170, p = 0.866
Weight at check-in (Z-score)	-1.03 [1]	-0.98 [1.37]	U = 204, p = 0.876
Previous phototherapy (% of infants)	61.9	78.9	$\chi 2 = 1.38$, p = 0.240
Duration of phototherapy (days)	4 ± 1.41 (n=13)	3.93 ± 1.16 (n=15)	*t* = 0.137, p = 0.892

Chi-square (χ^2), Mann-Whitney (U) or Student's *t*-test was used as appropriate. Data are presented as median [IQR], mean ± SD or percentage. GA: gestational age. PMA: postmenstrual age.

Table 2 Study outcomes

	Intervention (n = 21)	**Control (n = 20)**	**Test contrast, p**
PMA at discharge (wk)	35.57 [1.72]	35.79 [1.64]	U = 192, p = 0.638
Weight at discharge (g)	2090 [362.5]	2215 [336.25]	U = 151, p = 0.124
Weight at discharge (Z-score)	-1.01 [1.04]	-1 [1.34]	U = 199.5, p = 0.784
Days until oral feeding (d)	6 [4.5]	8 [7.5]	U = 138.5, p = 0.061
Days until discharge (d)	8 [5]	12 [3.75]	U = 124.5, p = 0.025

Chi-square (χ^2), Mann-Whitney (U) or Student's *t*-test was used as appropriate. Data are presented as median [IQR], mean ± SD or percentage. PMA: postmenstrual age.

Days until discharge

Infants stayed a median of 11 [7.5 - 13] days in the nursery. Preterm infants randomized to the intervention group were discharged earlier than infants in the control group (control, 12 [10.25 - 14]; intervention, 8 [7 - 12]; Mann-Whitney U test = 124.5, p = 0.025) (Fig. 4). The calculated effect size was intermediate to large (η^2= 0.121). Two infants stayed in the room much longer than the others, one from the control group (29 days) and one from the intervention group (35 days). If removed from the sample, the significant difference between groups was maintained, therefore we decided to include them in the remaining analyses.

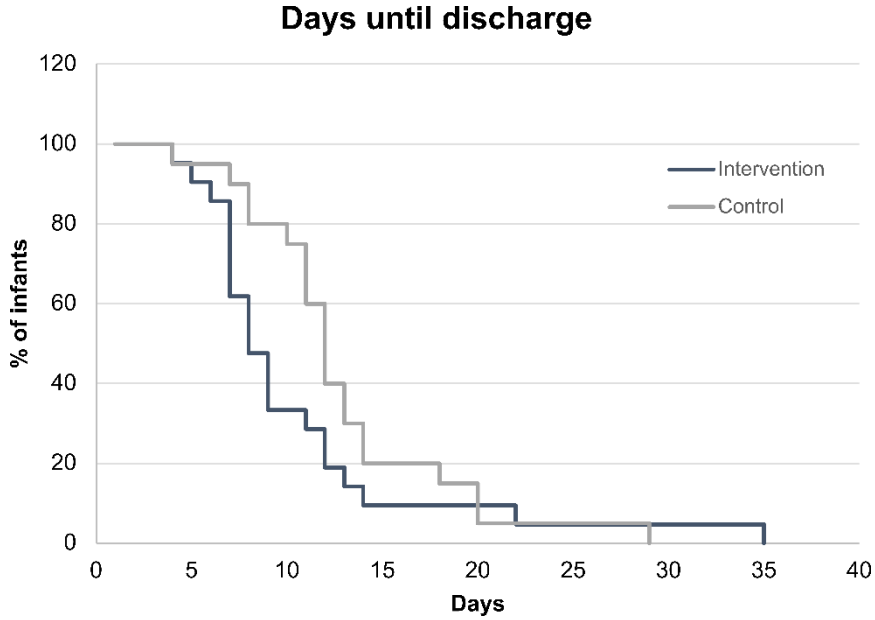


Figure 4. Percentage of infants in the nursery room at a given time point. Infants in the intervention group (black line) were discharged earlier than those in the control group (gray line).

Weight gain

No significant difference was found between groups regarding average weight gain per day (control, 31.35 ± 8.27; intervention, 27.26 ± 7.7; t = 1.641, p = 0.11) (Fig. 5).

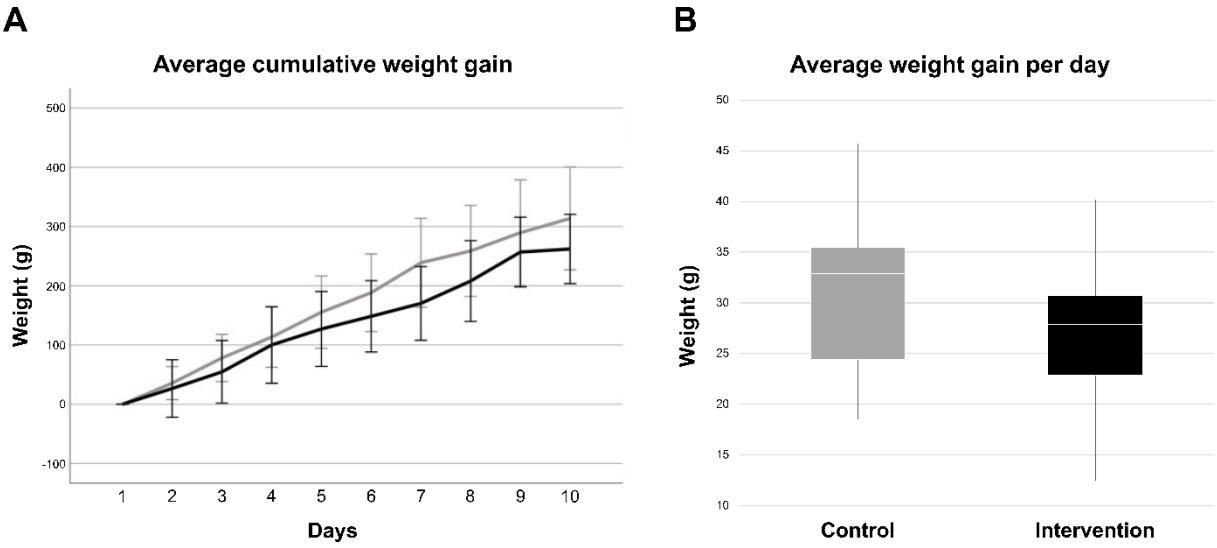


Figure 5. A) Cumulative weight gain of the intervention group (black line) and the control group (gray line) in the first ten days (error bars represent one standard deviation). B) Boxplot of the average weight gain per day during the whole stay in the nursery room. ns, non-significant.

Of the 41 infants, 34 entered the study before transitioning to oral feeding (control, n = 18; intervention, n = 16). No significant difference was found between the groups regarding number of days until oral feeding (control, 8 [7.5]; intervention, 6 [4.5]; Mann-Whitney U test = 138.5, $p = 0.06$).

Vital signs

Means of heart rate, respiratory rate, SpO2 and body temperature for each group are presented in Figure 6. Statistical differences were found between groups for average heart rate at 1400 h and 2000 h ($t = 2.4$, $p = 0.021$ and $t = 2.4$, $p = 0.02$, respectively). Mean and standard deviation values can be found in Supplementary Table S1 and the distribution of data and their means through the days in Figures S1 and S2.

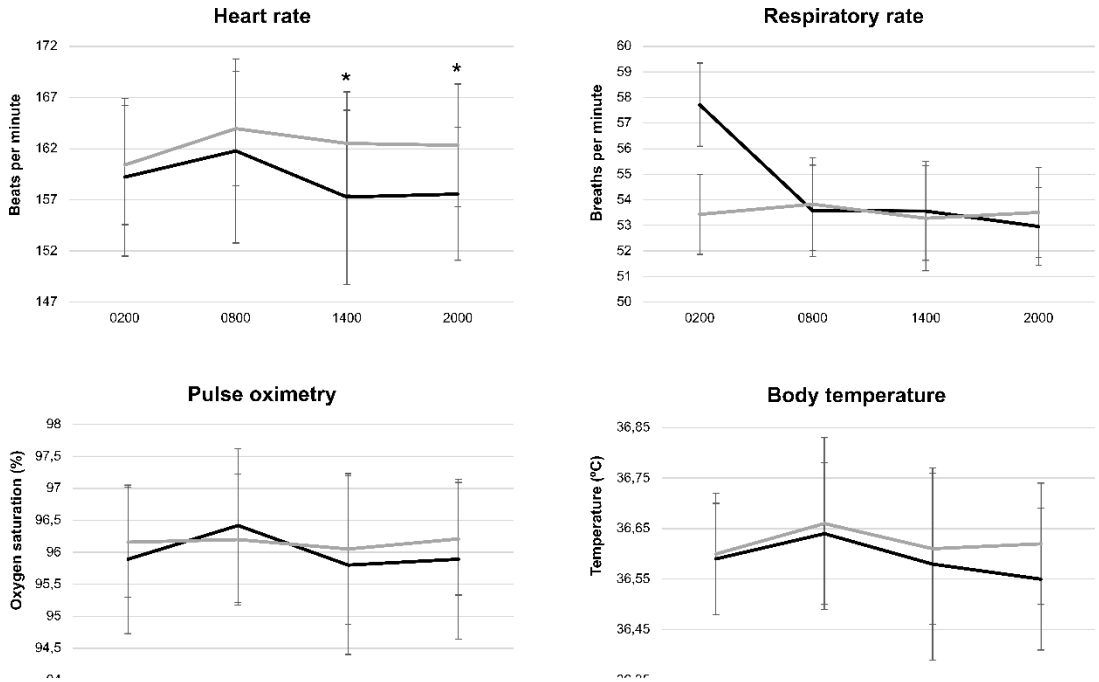


Figure 6. Group means and standard deviations of heart rate, respiratory rate, pulse oximetry and body temperature at four time points. *$p < 0.05$.

The GEE performed for heart rate showed that the interactions time*day and group*time*day were significant ($p = 0.09$ and 0.08, respectively) and residuals were well distributed (Fig. S3). This indicates that the heart rate difference between groups varies across

days and time points. We then compared the estimated marginal means of heart rates at each time point for the first ten days, when the majority of infants were still in the nursery and found that the heart rate was significantly higher in the control group at 1400 h from day 4 onwards and at 2000 h from day 1 until day 8 (Fig. 7). When we performed the same analysis intra-group, we found significant differences in heart rate from day 7 until day 10 in both groups (Supplementary Table S2).

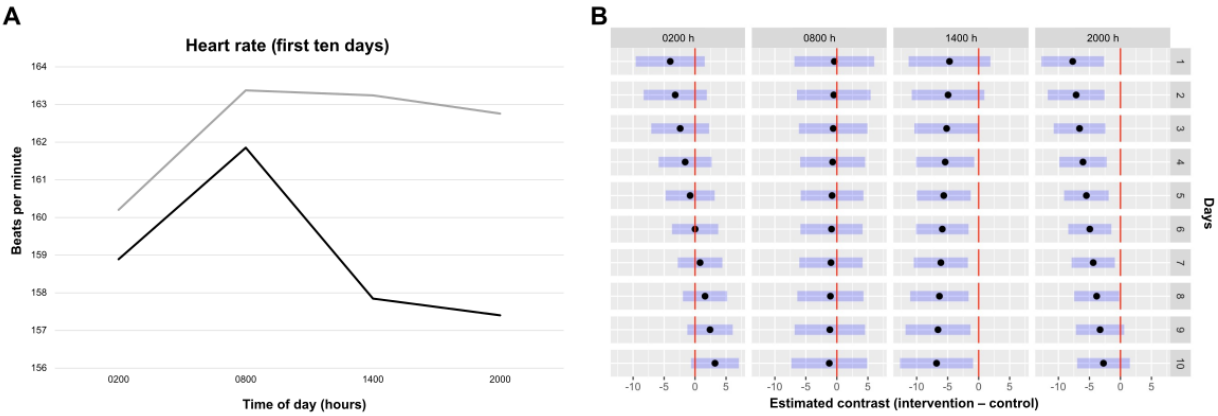


Figure 7. A) Average heart rate of the intervention group (black line) and the control group (gray line) at different time points during the first ten days. B) Multiple comparison of the estimated marginal means at different time points across days. The black dots represent the difference between the estimated marginal means of the two groups, the blue bars represent 95% confidence intervals for the estimated marginal means, and the red lines represent equal values between groups, allowing for comparison. When a blue bar is not crossing or touching the red line, there is a significant difference between groups. Negative values indicate that the intervention group had lower estimated marginal means than the control group and positive values indicate that the intervention group had higher estimated marginal means.

DISCUSSION

In the present study, preterm infants wearing individual light protection equipment at night were discharged earlier from the hospital than infants exposed to very low levels of light. The intervention group also showed greater heart rate variation throughout the day during several days. Furthermore, we observed no significant differences in weight gain and median of days until the beginning of oral feeding between groups

Days until discharge

Infants wearing eye masks at night were discharged earlier from the hospital than infants in the control group, who were exposed to very low levels of light at night. Light is the main environmental clue responsible for entraining human circadian rhythms (Duffy and Wright, 2005). The timing and duration of the exposure, as well as the intensity and wavelength of light that individuals are exposed to, have physiological and behavioral impacts and can be related to either positive or negative health outcomes (Souman et al., 2018; Vethe et al., 2021). Over time, these parameters of light and dark exposure create a light history that influences human circadian responses to artificial and natural light (Foster et al., 2020). In our study, both groups seemed to be exposed to near darkness, with little variation in light assessment. Here we were able to collect lighting information in the nursery by using a sensor attached to the wall next to one of the infant's beds. It is important to note that the sensor provides information about lighting levels in that point of the room, not an average of the environment or a precise measurement of light reaching the infants' eyes. Although the difference in light exposure between the groups was small, the implementation of eye masks that blocked the very low levels of ALAN in the room led to a significant difference in recovery.

Earlier findings pointed to light intensities of >100 lux at night having deleterious health impacts (Cho et al., 2015), but recent research suggests that exposure to less than 10 lux can reduce melatonin biosynthesis, and even 1 lux can aggravate sleep quality in adults (Cho et al., 2016; Phillips et al., 2019; Stebelova et al., 2020; Tähkämö et al., 2019). Given that melanopsin is present in the eye tissue since early weeks of gestation and that the RHT and the SCN are formed during the second trimester, it is likely that even extreme preterm infants can sense and respond to light variations (Hazelhoff et al., 2021), leading to the results observed in this study.

Heart rate variation

Infants in the control group showed a significantly higher heart rate at 1400 h from day 4 onwards and at 2000 h from day 1 until day 8 when compared to the intervention group. Inside each group, significant differences in heart rate according to time were observed starting at day 7.

Studies show that, during the last gestational trimester, a day-night variation of fetal heart rate synchronized to maternal rhythms can be observed (Mirmiran et al., 2003). However, research on the postnatal development of circadian heart rate rhythms in preterm newborns is

still lacking. Begum et. al measured physiological variables in preterm and term neonates exposed to a light-dark cycle and reported that the circadian amplitude of pulse rate increased with the gestational age (Begum et al., 2006). In 1991, Blackburn and Patteson observed that preterm infants exposed to cycled lighting had lower heart rates in the period when the light was decreased compared to when lights were on (Blackburn and Patteson, 1991). A year later, D'Souza et al. published a study with nine extremely preterm infants, in which four of them developed circadian rhythms after being reallocated to a nursery where they were exposed to light-dark cycles (D'Souza et al., 1992). Vásquez-Ruiz et al. reported that neonates exposed to light-dark cycles presented stable heart rate means across days, while those exposed to constant light exhibited an irregular pattern (Vásquez-Ruiz et al., 2014).

Prenatal factors such as intrauterine growth, gestational age, fetal and maternal illnesses can influence the development of an infant's circadian rhythms (Mirmiran and Ariagno, 2000); and postnatal environmental conditions, such as light exposure, feeding schedules and nursing care can entrain the rhythms (Bueno and Menna-Barreto, 2016). These factors may also interfere with heart rate rhythmicity development, making it difficult to establish what a "normal" development curve would look like, or to determine what to expect at each postnatal week.

In our study, because there was no need for 24-hour monitoring of the infants' vital signs, these were only recorded at four time points. It would be interesting to increase the number of time points in order to achieve a more precise characterization of rhythmic patterns.

Weight gain and days until oral feeding

In the present study, although not implementing a light-dark cycle, we also observed a decrease in time until discharge in infants wearing light protection devices at night. However, no significant difference was found between groups regarding weight gain and days until the beginning of oral feeding, possibly due to the small variation between day and night light conditions. Daytime bright light exposure has been previously shown to increase nocturnal melatonin levels and maintain circadian phase in young adults when compared to dim light (Takasu et al., 2006). In our study, since infants were exposed to a near dark condition during the day, the use of eye masks at night might have had a smaller effect than it would if participants were exposed to daytime bright light.

Factors that can influence the postnatal growth of preterm infants include degree of prematurity, birth weight (appropriate or small for gestational age), nutrition type (breast milk or formula), food intolerance and morbidities (Silveira and Procianoy, 2019). We found no statistically significant difference between the two groups regarding degree of prematurity or birth weight, and infants presenting several morbidities such as brain, cardiac and digestive tract malformations were not included.

It is also important to note that, given that the study took place in a nursery where infants with stable vital signs are admitted in order to gain weight, transition to oral feeding and get ready for discharge, the average number of days in the study was lower than if they had been included since birth. The setting, on the other hand, is one of the study's strengths. Since the nursery is separated by walls and a corridor from the intensive ward, it provides a more controlled environment for investigating the effect of light variations, with less noise and circulation.

Limitations

A methodological limitation of this study is that, since the intervention consists of wearing a mask, the nurses, nurse technicians and neonatologists that also collect daily data are aware of the intervention allocation, and therefore a double-blind or single-blind study design was not possible. Nonetheless, statistical analyses were blinded.

For the sample size estimation, we used as reference a similar study carried out by Vásquez-Ruiz et al. (2014). This study has a slightly different methodology, in which infants were included since birth and therefore had a longer stay in the NICU. Considering the p value of 0.014 found in the reference study for an average difference of 16.74 days of hospitalization time between groups in a total sample of 38 individuals, we calculated the necessary sample size for a significance of 5% and a power of 90%. The calculation resulted in a required sample of 67 individuals. At the end of data collection, our sample consisted of 41 infants. Even though the sample size was smaller than originally estimated, we calculated the effect size of our main outcome (days until discharge), which was intermediate to large (Lenhard and Lenhard, 2016).

Cost-benefit

According to the nursing staff, the masks were easy to wear and did not seem to increase restlessness or bother the infants. They were already available in the hospital, since typically

used for phototherapy, and were extremely affordable, costing R$11,68 per participant at the time of this study (R$ = Brazilian real). The costs of a daily stay in the NICU through Brazil's Unified Public Health System (SUS), on the other hand, ranged between R$139 and R$700 in June of 2022 (SIGTAP DATASUS, 2022), and it is not uncommon for premature infants, especially very premature ones, to stay over a month in the NICU (AlJohani et al., 2020; Manktelow et al., 2010; Seaton et al., 2019). The results presented here, together with findings of studies in adults (Hu et al., 2010), suggest that this low-cost intervention has the potential of bringing health benefits for patients in an intensive care unit.

Conclusion and perspectives

Lighting needs in the NICU and other intensive care facilities are different among occupants. A cycled pattern consisting of bright light during the day and dim light at night is potentially beneficial for both infants and staff (Rea and Figueiro, 2016), and based on our results we suggest that coupling a darkened environment at night with individual light protection devices can create the best conditions for the development of preterm infants. Our next step will be the implementation of a dynamic lighting system in the nursery, with color temperature variations that aims to mimic the natural daily ones, in order to investigate the effect of the use of light protection equipment at night coupled with bright light exposure during the day.

REFERENCES

AlJohani E, Qaraqei M, and Al-Matary A (2020). Estimating the Neonatal Length of Stay for Preterm Babies in a Saudi Tertiary Hospital. J Clin Neonatol 9:13-17.

Aschoff J (1965). Circadian Rhythms in Man. Science 148:1427–1432.

Associação Brasileira de Normas Técnicas (2013). ABNT NBR ISO/CIE 8995-1:2013: Iluminação de ambientes de trabalho Parte 1: Interior. p22.

Bechtold DA, Gibbs JE, and Loudon ASI (2010). Circadian dysfunction in disease. Trends Pharmacol Sci 31:191–198.

Begum E, Bonno M, Obata M, Yamamoto H, Kawai M, and Komada Y (2006). Emergence of Physiological Rhythmicity in Term and Preterm Neonates in a Neonatal Intensive Care Unit. J Circadian Rhythms 4: Art. 11.

Blackburn S and Patteson D (1991). Effects of cycled light on activity state and cardiorespiratory function in preterm infants. J Perinat Neonatal Nurs 4:47–54.

Boo NY, Chee SC, and Rohana J (2002). Randomized controlled study of the effects of different durations of light exposure on weight gain by preterm infants in a neonatal intensive care unit. Acta Paediatr Oslo Nor 1992 91:674–679.

Brandon DH, Holditch-Davis D, and Belyea M (2002). Preterm infants born at less than 31 weeks' gestation have improved growth in cycled light compared with continuous near darkness. J Pediatr 140:192–199.

Brasil, Ministério da Saúde (2022). DATASUS. Sistema de Gerenciamento da Tabela de Procedimentos, Medicamentos, Órteses, Próteses e Materiais Especiais (SIGTAP, OPM) do SUS. Retrieved from http://sigtap.datasus.gov.br/tabela-unificada/app/sec/inicio.jsp

Bueno C and Menna-Barreto L (2016). Environmental Factors Influencing Biological Rhythms in Newborns: From Neonatal Intensive Care Units to Home. Sleep Sci 9:295–300.

Cho C-H, Lee H-J, Yoon H-K, Kang S-G, Bok K-N, Jung K-Y, Kim L, and Lee E-I (2016). Exposure to dim artificial light at night increases REM sleep and awakenings in humans. Chronobiol Int 33:117–123.

Cho Y, Ryu S-H, Lee BR, Kim KH, Lee E, and Choi J (2015). Effects of artificial light at night on human health: A literature review of observational and experimental studies applied to exposure assessment. Chronobiol Int 32:1294–1310.

Chou JH, Roumiantsev S, and Singh R (2020). PediTools Electronic Growth Chart Calculators: Applications in Clinical Care, Research, and Quality Improvement. J Med Internet Res 22: e16204.

Dauchy RT, Wren MA, Dauchy EM, Hoffman AE, Hanifin JP, Warfield B, Jablonski MR, Brainard GC, Hill SM, Mao L, Dobek GL, Dupepe LM, and Blask DE (2015). The influence of red light exposure at night on circadian metabolism and physiology in Sprague-Dawley rats. J Am Assoc Lab Anim Sci JAALAS 54:40–50.

D'Souza SW, Tenreiro S, Minors D, Chiswick ML, Sims DG, and Waterhouse J (1992). Skin Temperature and Heart Rate Rhythms in Infants of Extreme Prematurity. Arch Dis Child 67:784–788.

Duffy JF and Wright KP (2005). Entrainment of the human circadian system by light. J Biol Rhythms 20:326–338.

Fleury G, Masís-Vargas A, and Kalsbeek A (2020). Metabolic Implications of Exposure to Light at Night: Lessons from Animal and Human Studies. Obesity 28:S18–S28.

Foster RG, Hughes S, and Peirson SN (2020). Circadian Photoentrainment in Mice and Humans. Biology 9:180.

Guyer C, Huber R, Fontijn J, Bucher HU, Nicolai H, Werner H, Molinari L, Latal B, and Jenni OG (2012). Cycled light exposure reduces fussing and crying in very preterm infants. Pediatrics 130:e145-151.

Hao H and Rivkees SA (1999). The biological clock of very premature primate infants is responsive to light. Proc Natl Acad Sci U S A 96:2426–2429.

Harrison MS and Goldenberg RL (2016). Global burden of prematurity. Semin Fetal Neonatal Med 21:74–79.

Haus EL and Smolensky MH (2013). Shift work and cancer risk: potential mechanistic roles of circadian disruption, light at night, and sleep deprivation. Sleep Med Rev 17:273–284.

Hazelhoff EM, Dudink J, Meijer JH, and Kervezee L (2021). Beginning to See the Light: Lessons Learned From the Development of the Circadian System for Optimizing Light Conditions in the Neonatal Intensive Care Unit. Front Neurosci 15.

Hu R, Jiang X, Zeng Y, Chen X, and Zhang Y (2010). Effects of earplugs and eye masks on nocturnal sleep, melatonin and cortisol in a simulated intensive care unit environment. Crit Care 14:R66.

Koritala BSC, Porter KI, Arshad OA, Gajula RP, Mitchell HD, Arman T, Manjanatha MG, Teeguarden J, Van Dongen HPA, McDermott JE, and Gaddameedhi S (2021). Night shift schedule causes circadian dysregulation of DNA repair genes and elevated DNA damage in humans. J Pineal Res e12726.

LeGates TA, Fernandez DC, and Hattar S (2014). Light as a central modulator of circadian rhythms, sleep and affect. Nat Rev Neurosci 15:443–454.

Lenhard W and Lenhard A. (2016). Computation of effect sizes. Retrieved from: https://www.psychometrica.de/effect_size.html. Psychometrica.

Manktelow B, Draper ES, Field C, and Field D (2010). Estimates of Length of Neonatal Stay for Very Premature Babies in the UK. Arch Dis Child Fetal Neonatal Ed 95:F288-292.

Miller CL, White R, Whitman TL, O'Callaghan MF, and Maxwell SE (1995). The effects of cycled versus noncycled lighting on growth and development in preterm infants. Infant Behav Dev 18:87–95.

Mirmiran M and Ariagno RL (2000). Influence of Light in the NICU on the Development of Circadian Rhythms in Preterm Infants. Semin Perinatol 24:247–257.

Mirmiran M, Baldwin RB, and Ariagno RL (2003). Circadian and sleep development in preterm infants occurs independently from the influences of environmental lighting. Pediatr Res 53:933–938.

Mirmiran M, Maas YGH, and Ariagno RL (2003). Development of Fetal and Neonatal Sleep and Circadian Rhythms. Sleep Med Rev 7:321–334.

Morag I and Ohlsson A (2016). Cycled light in the intensive care unit for preterm and low birth weight infants. Cochrane Database Syst Rev 2016(8):CD006982.

Nelson RJ and Chbeir S (2018). Dark matters: effects of light at night on metabolism. Proc Nutr Soc 77:223–229.

Phillips AJK, Vidafar P, Burns AC, McGlashan EM, Anderson C, Rajaratnam SMW, Lockley SW, and Cain SW (2019). High sensitivity and interindividual variability in the response of the human circadian system to evening light. Proc Natl Acad Sci U S A 116:12019–12024

Rea MS and Figueiro MG (2016). The NICU Lighted Environment. Newborn Infant Nurs Rev NAINR 16:195–202.

Rivkees SA, Mayes L, Jacobs H, and Gross I (2004). Rest-Activity Patterns of Premature Infants Are Regulated by Cycled Lighting. Pediatrics 113:833–839.

Seaton SE, Barker L, Draper ES, Abrams KR, Modi N, Manktelow BN, and UK Neonatal Collaborative (2019). Estimating Neonatal Length of Stay for Babies Born Very Preterm. Arch Dis Child Fetal Neonatal Ed 104:F182–F186.

Serón-Ferré M, Mendez N, Abarzua-Catalan L, Vilches N, Valenzuela FJ, Reynolds HE, Llanos AJ, Rojas A, Valenzuela GJ, and Torres-Farfan C (2012). Circadian rhythms in the fetus. Mol Cell Endocrinol 349:68–75.

Shiroiwa Y, Kamiya Y, Uchibori S, Inukai K, Kito H, Shibata T, and Ogawa J (1986).

Activity, cardiac and respiratory responses of blindfold preterm infants in a neonatal intensive care unit. Early Hum Dev 14:259–265.

Silveira RC and Procianoy RS (2019). Preterm newborn's postnatal growth patterns: how to evaluate them. J Pediatr (Rio J) 95:42–48.

Souman JL, Borra T, de Goijer I, Schlangen LJM, Vlaskamp BNS, and Lucassen MP (2018). Spectral Tuning of White Light Allows for Strong Reduction in Melatonin Suppression without Changing Illumination Level or Color Temperature. J Biol Rhythms 33:420–431.

Stebelova K, Roska J, and Zeman M (2020). Impact of Dim Light at Night on Urinary 6-Sulphatoxymelatonin Concentrations and Sleep in Healthy Humans. Int J Mol Sci 21.

Tähkämö L, Partonen T, and Pesonen A-K (2019). Systematic review of light exposure impact on human circadian rhythm. Chronobiol Int 36:151–170.

Takasu NN, Hashimoto S, Yamanaka Y, Tanahashi Y, Yamazaki A, Honma S, and Honma K (2006). Repeated Exposures to Daytime Bright Light Increase Nocturnal Melatonin Rise and Maintain Circadian Phase in Young Subjects under Fixed Sleep Schedule. Am J Physiol-Regul Integr Comp Physiol 291:R1799–R1807.

Thomas KA, Burr RL, Spieker S, Lee J, and Chen J (2014). Mother–infant circadian rhythm: Development of individual patterns and dyadic synchrony. Early Hum Dev 90:885–890.

Touitou Y, Reinberg A, and Touitou D (2017). Association between light at night, melatonin secretion, sleep deprivation, and the internal clock: Health impacts and mechanisms of circadian disruption. Life Sci 173:94–106.

Vagenas D and Totsika V (2018). Modelling correlated data: Multilevel models and generalized estimating equations and their use with data from research in developmental disabilities. Res Dev Disabil. 81:1-11.

Vásquez-Ruiz S, Maya-Barrios JA, Torres-Narváez P, Vega-Martínez BR, Rojas-Granados A, Escobar C, and Ángeles-Castellanos M (2014). A light/dark cycle in the NICU accelerates body weight gain and shortens time to discharge in preterm infants. Early Hum Dev 90:535–540.

Vethe D, Scott J, Engstrøm M, Salvesen Ø, Sand T, Olsen A, Morken G, Heglum HS, Kjørstad K, Faaland PM, Vestergaard CL, Langsrud K, and Kallestad H (2021). The evening light environment in hospitals can be designed to produce less disruptive effects on the circadian system and improve sleep. Sleep 44.

Vogel JP, Chawanpaiboon S, Moller A-B, Watananirun K, Bonet M, and Lumbiganon P (2018). The global epidemiology of preterm birth. Best Pract Res Clin Obstet Gynaecol 52:3–12.

Welsh DK, Takahashi JS, and Kay SA (2010). Suprachiasmatic Nucleus: Cell Autonomy and Network Properties. Annu Rev Physiol 72:551–577.

World Health Organization, March of Dimes, The Partnership for Maternal, Newborn & Child Health, and Save the Children (2012). Born too soon: the global action report on preterm birth.

World Medical Association (2013). World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. JAMA 310:2191–2194.

SUPPLEMENTARY MATERIAL

Table S1 Mean of vital signs at different time points

		Heart rate	**Respiratory rate**	**SpO2**	**Temperature**
0200 h	Intervention	159.21 ± 7.7	52.72 ± 1.62	95.89 ± 1.16	36.59 ± 0.11
	Control	160.39 ± 5.82	53.43 ± 1.56	96.16 ± 0.86	36.6 ± 0.12
	p	0.585	0.163	0.410	0.843
0800 h	Intervention	161.78 ± 9	53.57 ± 1.78	96.42 ± 1.2	36.64 ± 0.14
	Control	163.96 ± 5.59	53.83 ± 1.81	96.2 ± 1.02	36.66 ± 0.17
	p	0.360	0.652	0.529	0.660
1400 h	Intervention	157.26 ± 8.51	53.56 ± 1.93	95.8 ± 1.4	36.58 ± 0.19
	Control	162.53 ± 5.01	53.28 ± 2.06	96.05 ± 1.18	36.61 ± 0.15
	p	0.021	0.658	0.536	0.683
2000 h	Intervention	157.57 ± 6.49	52.96 ± 1.52	95.89 ± 1.25	36.55 ± 0.14
	Control	162.33 ± 5.99	53.51 ± 1.76	96.21 ± 0.88	36.62 ± 0.12
	p	0.020	0.287	0.358	0.102

ata are presented as mean ± SD and compared with Student’s t-test.
SpO2: oxygen saturation determined by pulse oximetry.

Table S2 Comparison of the estimated marginal means of heart rate (bpm) at different time points across days in each group

	Intervention				Control			
Day	**0200 h**	**0800 h**	**1400 h**	**2000 h**	**0200 h**	**0800 h**	**1400 h**	**2000 h**
1	157	161	159	157	161	161	164	164
2	157	161	159	157	161	161	164	164
3	158	161	158	157	160	162	164	164
4	159	162	158	158	160	162	164	164
5	159	162	158	158	160	163	164	163
6	160	162	158	158	160	163	164	163
7	161^{b}	162^{ab}	157^{ab}	158^{a}	160^{a}	163^{b}	164^{ab}	162^{ab}
8	162^{b}	163^{ab}	157^{ab}	158^{a}	160^{a}	164^{b}	163^{ab}	162^{ab}
9	162^{b}	163^{ab}	157^{a}	158^{a}	160^{a}	164^{b}	163^{ab}	162^{ab}
10	163^{b}	163^{ab}	157^{a}	159^{a}	160^{a}	165^{b}	163^{ab}	161^{ab}

Confidence level used: 0.95
Conf-level adjustment: Bonferroni method for 4 estimates
P value adjustment: Bonferroni method for 6 tests
Significance level used: alpha = 0.05
Superscript labels indicate differences. Values labeled with different letters are statistically different.

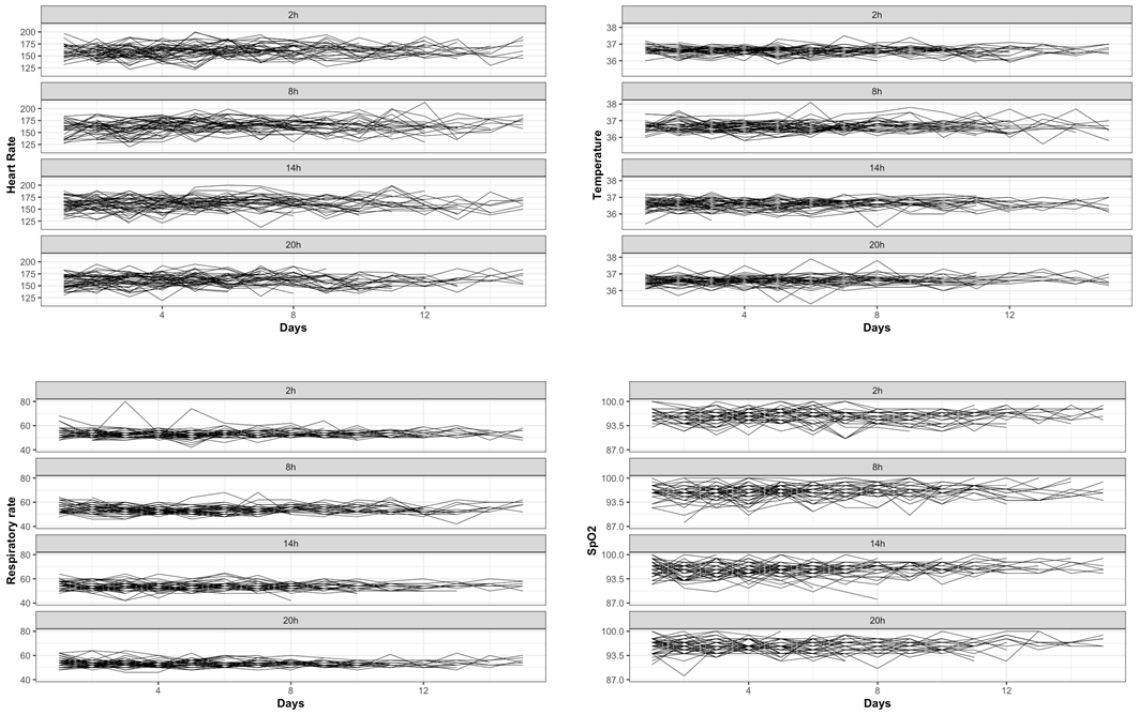


Figure S1. Distribution of data collected continuously from infants until discharge. Data are plotted according to the time of the day and each black line represents a subject.

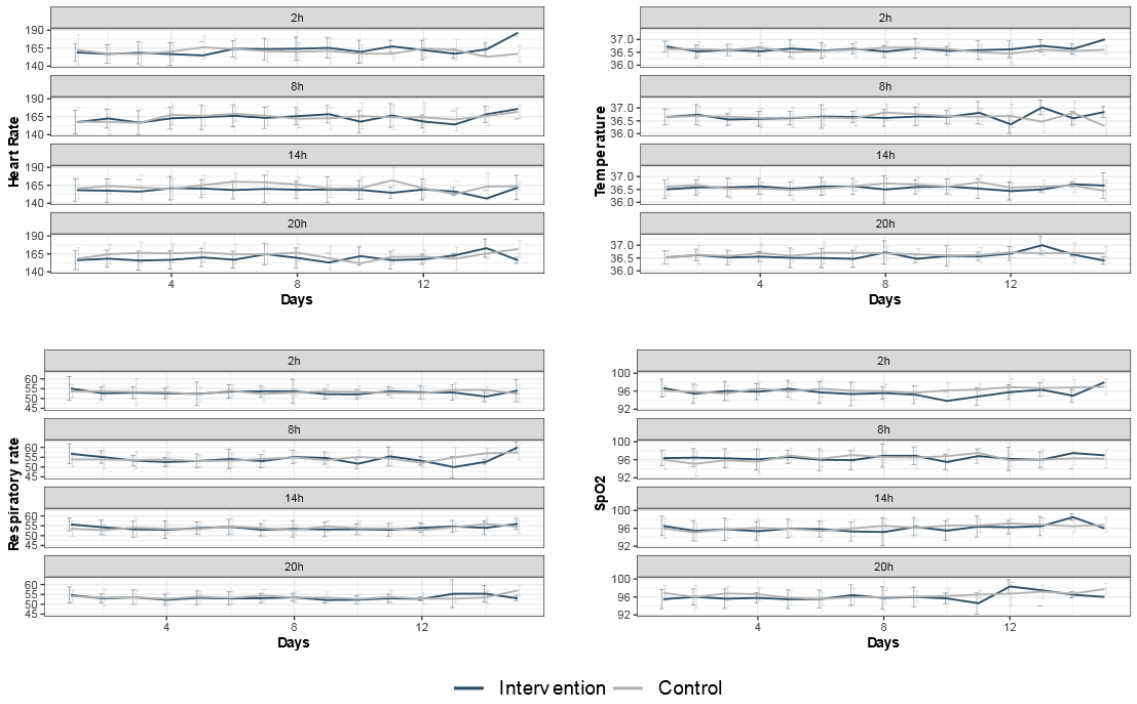


Figure S2. Mean data collected continuously from infants until discharge. Data are plotted as mean ± SD according to the time of the day. The dark gray line stands for the intervention group, while the light gray for the control group.

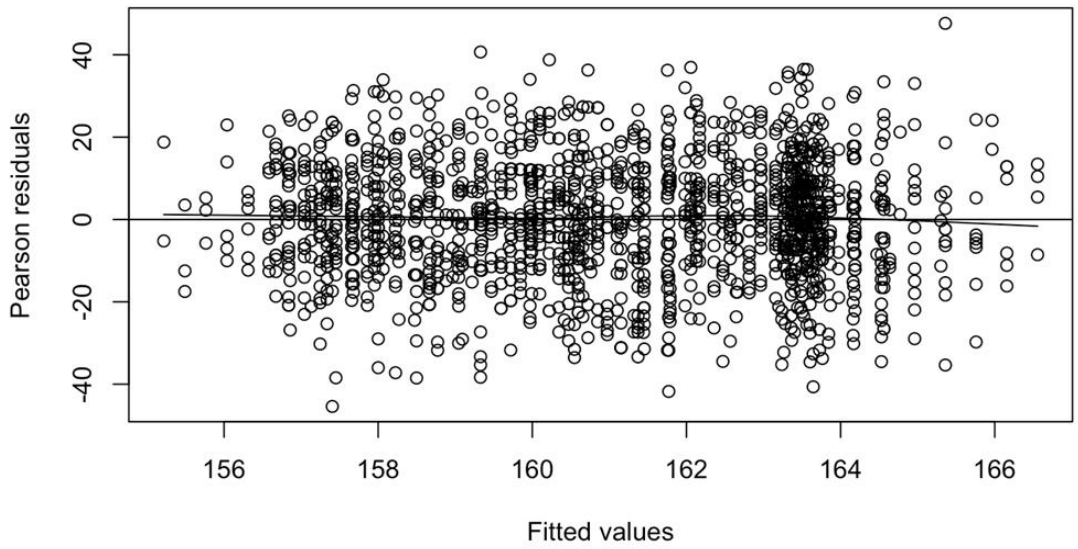


Figure S3. Distribution of residuals obtained from the GEE analysis performed with heart rate data.

2. INSTRUMENTO DE RITMO DE HUMOR

Como visto anteriormente, o sistema de regulação circadiana age em diversas áreas cerebrais cuja modulação pode impactar funções comportamentais e neurobiológicas como humor, memória, secreção hormonal, ingesta de alimentos e sono (58). No entanto, o padrão rítmico destas funções nem sempre é bem caracterizado ao longo das 24 hrs. O MRhI foi desenvolvido para suprir a lacuna existente diante da falta questionários clínicos disponíveis que levem em consideração a possibilidade de a frequência dos sintomas de humor seguirem um padrão rítmico ou mesmo a hora do dia em que os sintomas de humor geralmente atingem o pico. Em seguida, apresento os três trabalhos que foram desenvolvidos para validação do MRhI para diferentes línguas a fim de contribuir para a difusão desta importante ferramenta para avaliação do ritmo de humor.

2.1 TRADUÇÃO E VALIDAÇÃO DO MRhI PARA LÍNGUA ESPANHOLA

Artigo publicado na revista *Biological Rhythms Research* (IF: 1.1; Qualis B2 área Medicina II).

DOI: 10.1080/09291016.2019.1675023

Title: Validation and psychometric properties of the Spanish Mood Rhythm Instrument

Alicia Carissimi[1,2], Melissa Alves Braga de Oliveira[1,2], Benicio N. Frey[3], José Francisco Navarro[4], Maria Paz Hidalgo,[1,2†], Ana Adan[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] 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] Department of Psychobiology, School of Psychology, University of Málaga, Spain;
[5] Department of Clinical Psychology and Psychobiology, School of Psychology, University of Barcelona, Barcelona, Spain;
[6] Institute of Neurosciences, University of Barcelona, Barcelona, Spain;
[†] These authors contributed equally to this work as senior authors.

* Contact: Ana Adan, e-mail: aadan@ub.edu
Department of Clinical Psychology and Psychobiology, University of Barcelona, Passeig de la Vall d'Hebron, 171. 08035 Barcelona. Spain.

ABSTRACT

Mood-related behavioral display a daily rhythmic pattern. However, most clinical instruments do not assess mood rhythm aspects. The Mood Rhythm Instrument (MRI) intends to contribute to filling this gap. We evaluated the psychometric properties and validated the MRI in a Spanish sample (N=457), exploring the relationship with chronotype and social jetlag through the reduced version of the Morningness-Eveningness Questionnaire (rMEQ) and the Munich Chronotype Questionnaire (MCTQ), respectively. The MRI proved to have adequate internal reliability. Somatic and cognitive variables displayed higher self-reported frequency of rhythmic pattern than affective variables. Sex differences were only observed for sexual arousal, with females reporting higher rates of rhythmicity. The frequency of self-reported rhythmicity was similar between chronotypes. Chronotype and social jetlag scores correlated with most MRI variables, though rhythmicity depended primarily on age. This study underlines the importance of assessing mood variability through a reliable and user-friendly instrument that could be useful in cross-cultural studies to further our knowledge on the association among mood symptoms, circadian rhythms and mental illness.

Keywords: chronobiology; sex; chronotype; social jetlag; mood

Introduction

Most of the cognitive and behavioral variables present a daily variation. Some of them are directly associated with chronotype and social jet-lag (SJL) (Arbabi et al. 2015; Beauvalet et al. 2017). Chronotype represents the inter-individual variation in a temporal organization (Adan et al. 2012). Whereas SJL represents the misalignment between biological time (the individual's internal clock) and social demands (e.g., work and school routines) that arises from alternating work and free days (Roenneberg and Merrow 2016). Both of these chronobiological parameters can be associated with chronodisruption and health problems, such as metabolic alterations and mood disorders (Müller and Haag 2018; Smolensky et al. 2016). As an example, evening-type individuals tend to have a higher level of SJL. Subsequently, it was observed that SJL >2 hours is correlated to depressive symptoms, probably due to a poor adaptation to social demands (Levandovski et al. 2011). In individuals diagnosed with major depression, worsening of symptoms is often observed at the beginning of the day (i.e., "melancholic subtype"), whereas in other cases this diurnal variation results in evening or night falling (Kronfeld-Schor and Einat 2012).

Additionally, self-esteem, social relationships, sadness, sexual arousal, pessimism, sleep and alertness, often display a rhythmic and dynamic pattern, observed by a periodicity or frequency of occurrence within 24 hours in healthy individuals (Adan and Guàrdia 1993; Boivin et al. 2016; Díaz-Morales et al. 2015; Dzogang et al. 2018) and characterize a behavioral feature related to mood states. However, controversy in literature still exists regarding the peaks of depressive symptoms in individuals with major mood disorders (Wirz-Justice 2008). In part, the limited knowledge regarding the rhythmicity of mood-related behaviors in clinical and non-clinical populations is due to the fact that most available clinical instruments do not adequately assess daily fluctuations of mood symptoms. The majority of the scales focus in the intensity of depressive mood and none of them assess rhythmicity (Daviss WB et al. 2006, Byrne JEM, Bullock B & Murray G. 2017). The Positive and Negative Affect Schedule (Watson D, Clark LA & Tellegen A. 1988.) has the peculiarity of evaluating the intensity of positive and negative affect and its duration as it asks for "moment", "past few days", "week", "past few weeks" and so on, nevertheless, the options do not resemble daily rhythms or peak time of the symptoms. In the other hand there are the chronotype scales. In the case of scales which authors stat to represents the range of diurnal variation, the lack of time of the day information is an important limitation (Dosseville F, Laborde S & Lericollais R. 2013; Randler C et al. 2016). To contribute

to filling this gap, we have recently developed the Mood Rhythm Instrument (MRI), a self-reported questionnaire that evaluates mood-related symptoms and behaviors (de Souza et al. 2016; Pilz et al. 2018a, b).

Table 1. Demographic characteristics and circadian parameters according age groups and total samples.

	≤21yrs (n=189)	**22-40 yrs (n=202)**	**>40 yrs (n=66)**	**Total (n=457)**
Demographic characteristics				
Sex (% female)	136 (72)	117 (58)	30 (46)	283 (62)
Age, mean ± SD	20.32 ± 0.92	26.46 ± 4.42	50.09 ± 6.10	27.33 ± 10.48
Years of schooling, mean± SD	16.83 ± 3.19	18.74 ± 4.34	16.09 ± 7.00	17.56 ± 4.42
Circadian parameters				
rMEQ score	13.71 ± 3.52	14.15 ± 4.04	17.43 ± 3.24	14.43 ± 3.92
MCTQ variables				
MSW	4:05 ± 1:04	4:19 ± 1:10	3:32 ± 0:98	4:00 ± 1:09
MSF	5:92 ± 1:39	5:80 ± 1:44	4:45 ± 1:15	5:66 ± 1:47
Social jetlag	1:89 ± 1:13	1:66 ± 1:01	1:14 ± 0:96	1:68 ± 1:08

Abbreviations: rMEQ, Reduced Morningness-Eveningness Questionnaire; MCTQ, Munich Chronotype Questionnaire; MSW, Midpoint of sleep on workdays; MSF, Midpoint of sleep on free days.

The aim of this study is to assess the psychometric properties of the Spanish version of the MRI in a non-clinical sample, as well as to explore the relationship between MRI and well-known chronobiological parameters, such as chronotype and social jetlag, as external validation.

Methods

Sample

Our sample was composed of 457 Spanish individuals (62% female, age 18-65 years; Table 1), 232 university students and 225 individuals from the general population collected by

snowball method (Biernacki and Waldorf 1981). Females were younger than males (25.7±9.2; 30.08±11.8 years, respectively; t=4.18; p<0.001), while the number of years of schooling was similar in both sexes (females=17.6±4.5; males=17.2±4.3), as well as in age groups, (<21years old: 16.83±3.19; 22 to 40 years old: 18.74±4.34; >40 years old: 16.09±7.00).

Procedure

Participants answered MRI, rMEQ, and MCTQ between September and October 2016. All participants provided written informed consent before study entry and were not compensated for their participation. The study was approved by the Ethics Committee of Hospital de Clínicas de Porto Alegre (#2015-0539-GPPG/HCPA) and University of Barcelona (IRB00003099) and was conducted in accordance with the Declaration of Helsinki.

Instruments

Mood Rhythm Instrument

The MRI is a 15-item self-reported questionnaire (de Souza et al. 2016) to detect rhythmic patterns of mood-related domains (affective, cognitive, somatic, physical), taking the past fifteen days into consideration. It is a one-time application instrument that asks whether or not the individual symptoms have a peak (acrophase) during the 24 hours of the day and, if so, the exact hour in which the peak in symptoms occurs. This instrument has also revealed not presenting recency or recall biases, being a valid tool to investigate daily patterns of mood symptoms over 24 hours (Pilz et al. 2018a). It displayed a satisfactory internal consistency in a Brazilian population (de Souza et al. 2016). The Spanish translation of MRI, developed by Francisco and colleagues (2017), shows semantic agreement compared to the Portuguese version.

Reduced Morningness-Eveningness Questionnaire

The rMEQ is a five-item questionnaire validated for the Spanish population by Adan and Almirall (1991). Items refer to rising, peak and retiring time, morning freshness and self-

evaluation of chronotype. It assigns subjects into the three chronotypes considering scores from 4 to 11 as evening type, from 12 to 17 as intermediate type and from 18 to 25 as morning type. The psychometric properties of the rMEQ were adequate (Di Milia et al. 2013) and the internal reliability for the present sample was 0.73.

Munich Chronotype Questionnaire

MCTQ is an instrument introduced by Roenneberg et al. (2003) from which it is possible to calculate the midpoint of sleep on workdays (MSW), the midpoint of sleep on free days (MSF) and the social jetlag (SJL). The midpoint of sleep is defined as the half-way point between sleep onset and sleep end, and represents individuals' sleep period timing. SJL was computed by the absolute difference between the midpoints of sleep on workdays and free days and categorized into three groups, according to the cut points previously defined (<2; 2-4;>4 hours) based on a review by Beauvalet et al. (2017) showing that SJL correlated with psychiatric symptoms, BMI, cortisol levels and heart rate. In this study, we used the MCTQ version translated to Spanish and available at https://www.euclock.org/.

Statistical analysis

Psychometric properties of the MRI were tested with exploratory factor analysis and Cronbach's alpha. Considering that our data is dichotomous (yes/no), the factor analysis was carried out on a tetrachoric correlation matrix (R Software version 3.4.1 using "psych" package). Data were subjected to factor analysis using the Max Likelihood extraction method with Varimax rotation. The number of factors to retain was based on Kaiser criteria (Eigenvalues above 1) and the screen plot suggested the findings of retaining five factors. Based on the meaningful theoretical contribution of the items in each of the five factors as well as the relevance of each of them to our context, the model that provided the most desirable rotated

factor structure was the three-factor model. We conducted a confirmatory factor analysis, setting it up on three factors, and the results are described on Table 2. Missing data were excluded listwise which resulted in 440 answers that were considered for all the factor analysis performed.

Table 2. Factor analysis of the 15 items that composed the Mood Rhythm Instrument (MRI).

MRI Items	**Factor 1 Somatic**	**Factor 2 Affective**	**Factor 3 Cognitive**
1. Alertness			0.63
2. Sleepiness	0.93		
3. Problem-Solving	0.48		
4. Self-esteem		0.51	
5. Concentration			0.82
6. Appetite	0.38		
7. Sexual Arousal	0.55		
8. Irritability		0.43	
9. Anxiety		0.54	
10. Sadness		0.85	
11. Motivation to Exercise			0.35
12. Memory			0.45
13. Pessimism		0.91	
14. Talking to Friends			0.46
15. Energy			0.42
Eigenvalues	2.07	2.71	2.18
% of variance	0.14	0.18	0.15

Data were expressed as means ± standard deviation, n (%) or median and interquartile range. Comparisons of MRI questions according to sexes were analyzed by Chi-square test (χ^2). The peaks for the statistically significant MRI items for sex and age groups (<21; 22 to 40; and >40 years old) were described using the Rayleigh test. The groups were classified according to these age categories based on preference towards eveningness (Adan et al. 2012; Roenneberg et al. 2007) and because there are marked changes in daily rhythms –for example, in the sleep-wake cycle – after 40 years of age (Adan and Almirall 1991; Waterhouse et al. 2012). The normality of MRI variables was tested using Kolmogorov-Smirnov, which indicates non-normal distribution of data. The MRI time variables were transformed so we could analyze them as linear data (e.g. 2am as 26.0 hours, 3:30am as 27.5 hours).

Comparisons of MRI items between SJL groups (>2; 2-4; and >4 hours) were tested by Kruskal-Wallis test. The Spearman test was used to analyze the correlations between MRI and circadian parameters (rMEQ score, MSW, MSF, and SJL). R version 3.4.1 (package "psych") and PASW Statistics Version 18 were used for statistical analyses (SPSS Inc., Chicago, IL).

Results

Psychometric properties and sex and age influence

Table 2 depicts the results from the factor analysis for the MRI. We observed three principal components with eigenvalues higher than 1. The somatic items – sleepiness, problem-solving, appetite and sexual arousal were grouped in the first factor. In the second factor there were the affective items – self-esteem, irritability, anxiety, sadness, and pessimism. In the third factor were the cognitive items – alertness, concentration, motivation to exercise, memory, talking to friends and energy. In the whole sample, the MRI had a Cronbach's alpha of 0.70 (0.70 in males and 0.72 in females), which suggests adequate internal consistency.

Table 3 shows comparison between sexes and age groups for frequency of rhythmicity in each of the MRI items. Variables in which more than 75% of participants present rhythmicity in a 24h cycle were related to cognitive and somatic domains such as concentration, sleepiness and appetite. On the other hand, less than 40% of subjects reported rhythmicity in affective domains such as self-esteem, anxiety, sadness and pessimism. Sex differences were significant for sexual arousal rhythmicity (χ^2 =5.24; p=0.022), where females reported higher rates of

rhythmicity than males. Comparing age groups, subjects <21 years old presented higher frequency in the indicated peaks for concentration, irritability, sadness, and pessimism than 22-40 and >40 age groups; and subjects in the22-40 age group presented higher frequency in the indicated peaks for alertness and sexual arousal. The >40 years old age group showed no differences in the indicated peaks for MRI variables.

Table 3. Frequency of self-reported rhythmicity of Mood Rhythm Instrument (MRI) items - Spanish version.

MRI items	**Males**	**Females**	**<21 yr**	**22-40 yr**	**>40 yr**
	N (%)				
1. Alertness	110 (64)	194 (70.0)	116 (62.4)	148 (74.4)*	41 (63.1)
2. Sleepiness	**166 (96.0)**	**277 (98.6)**	**185 (97.9)**	**193 (96.5)**	**66 (100)**
3. Problem-Solving	114 (66.3)	199 (70.8)	126 (66.7)	144 (72.0)	44 (67.7)
4. Self-esteem	60 (34.7)	96 (34.2)	73 (38.6)	59 (29.5)	24 (36.4)
5. Concentration	**145 (83.8)**	**248 (87.6)**	**170 (89.9)***	**175 (87.9)**	**49 (74.2)**
6. Appetite	**156 (90.7)**	**254 (90.4)**	**174 (92.1)**	**174 (87.4)**	**62 (93.9)**
7. Sexual Arousal	72 (41.6)	148 (52.7)*	94 (50)	107 (53.2)*	19 (28.8)
8. Irritability	100 (57.8)	179 (63.7)	129 (68.3)*	119 (59.5)	31 (47.0)
9. Anxiety	47 (27.2)	95 (33.7)	57 (30.3)	66 (32.7)	19 (28.8)
10. Sadness	36 (20.9)	83 (29.4)	66 (34.9)*	43 (21.5)	10 (15.2)
11. Motivation to Exercise	129 (75.4)	216 (76.9)	147 (77.8)	156 (78.4)	43 (66.2)
12. Memory	73 (42.4)	133 (47.3)	90 (47.9)	92 (46.0)	24 (36.4)
13. Pessimism	37 (21.5)	85 (30)	65 (34.4)*	43 (21.5)	14 (21.2)
14. Talking to Friends	92 (53.5)	158 (56.2)	108 (57.1)	109 (54.8)	33 (50.0)
15. Energy	93 (53.8)	165 (58.7)	107 (56.6)	113 (56.8)	38 (57.6)

The values in bold indicate the items with the greatest indicated frequency of rhythmicity by participants in a 24h cycle. Chi-square test; *$p<0.05$*.

Figure 1 depicts the comparisons between sex and age groups on MRI items according to the Rayleigh test. It shows the peak in the 24-hour day, reported by each of the participants.

The peaks in the 24-hour for memory, energy and motivation to exercise were phase advanced, while sleepiness showed a phase delaying the older group.

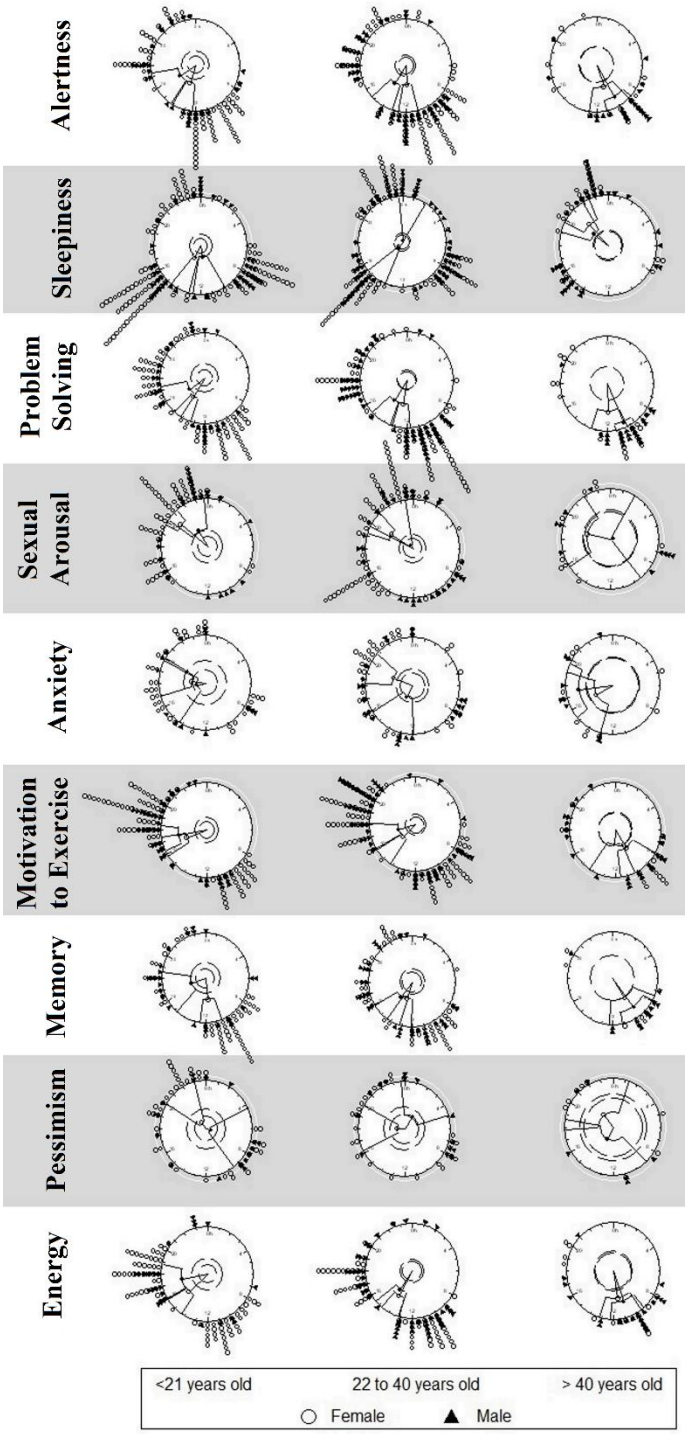


Figure 1. Reported peaks of the MRI items according to sex and age. Circles represent the 24-hour day. During the study period, the shortest dark phase began at 8:00 p.m. and finished at 7:30 a.m., while the longest phase began at 6:30 p.m. and finished at 8:50 a.m. Black triangles and white circles represent males and females, respectively.

External validity: relationship between MRI and chronobiological parameters

The average scores of the circadian parameters (Table 1) in the total sample were: 14.43±3.92 for rMEQ score, 4:00±1:09h for MSW, 5:66±1:47h for MSF and 1:68±1:08h for SJL. Out of the 457 participants, 410 answered the rMEQ and were classified as morning (*n*=85; 20.7%), intermediate (*n*=233; 56.8%) or evening (*n*=82; 22.4%) types. Comparing the sample according to age groups, young adults (≤21yrs and 22-40 yrs) tended to be classified as evening or intermediate types, and older adults (>40 yrs) as morning or intermediate types.

Figure 2 shows how frequently individuals perceived these variables with a rhythm throughout the day according to the individuals' chronotype (morning, intermediate or evening). The frequency of self-reported rhythmicity was similar among chronotypes, although morning and evening chronotypes reported a peak at different periods of the day in all parameters, except for appetite and sadness. The intermediate chronotype reported more broadly distributed frequency of self-reported rhythmicity throughout daily hours.

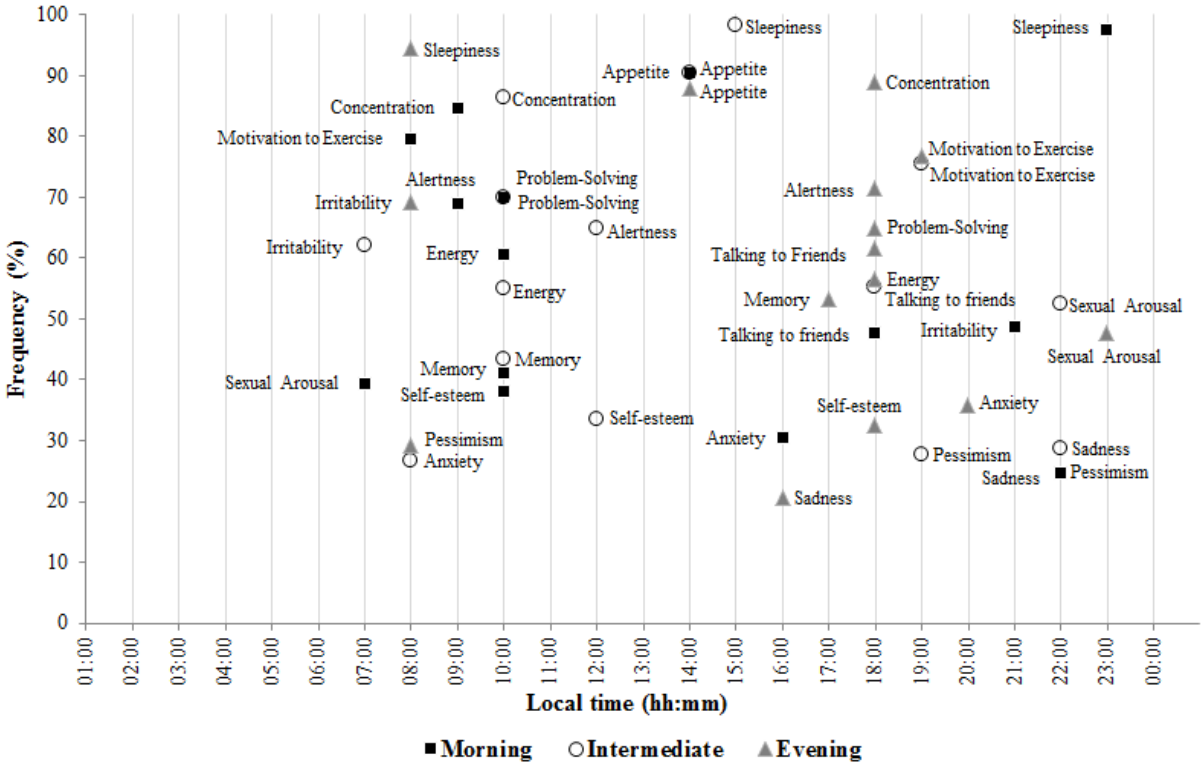


Figure 2. 24-hour peaks of the MRI items (x) and frequency of respondents who perceived rhythmicity during 24-hour periods (y). Data is stratified by chronotype.

Individuals who presented 24-hour peaks of behaviors and physiology needs were classified according to three groups of SJL duration (Table 4). Self-esteem, sexual arousal, irritability, motivation to exercise and energy were different among the SJL groups. Also, self-esteem, sexual arousal, motivation to exercise and energy peaks occurred earlier in those with less than 2h of SJL in comparison with 2-4h of SJL. Irritability peaks were similar when comparing the SJL groups in a pairwise test.

Table 4. 24-hour peaks of Mood Rhythm Instrument (MRI) items among social jetlag groups.

	Social jetlag groups			
MRI items	**<2 h (n=287)**	**2–4 h (n=127)**	**>4 h (n=31)**	**p-value**
1. Alertness	12 [10-18]	14 [11-18]	13 [09-18]	0.098
2. Sleepiness	15.50 [09-22]	15 [08-22]	15 [09.50-21]	0.205
3. Problem-Solving	11.5 [10-17.5]	14.25 [11-18]	15 [11-19]	0.067
4. Self-esteem	12 [10-17.63][a]	18 [10.5-21][b]	14.25 [10.75-18.50][ab]	**0.045**
5. Concentration	11 [10-17]	12 [10.5-18]	12 [09.75-18.75]	0.383
6. Appetite	14 [13-17]	14 [13.5-16.50]	14 [13-19]	0.202
7. Sexual Arousal	19 [15.50-22][a]	22 [18-23][b]	22 [16-23][ab]	**0.001**
8. Irritability	10 [08-20]	08.50 [07.5-18]	08 [07.13-14]	**0.043**
9. Anxiety	17.50 [13-20.50]	15.75 [10-20]	16.50 [12.25-19.25]	0.586
10. Sadness	21 [15-23]	19.50 [09.5-22.50]	19.50 [09-22.38]	0.811
11. Motivation to Exercise	15.50 [10-19][a]	18.50 [17-20][b]	18 [10-20][ab]	**<0.001**
12. Memory	11 [10-18]	12 [10-18]	17 [11-19]	0.160
13. Pessimism	19 [09-22]	11 [08-20.50]	16 [6.75-22.75]	0.237
14. Talking to Friends	18 [17-19.5]	18 [17-20.13]	18.50 [17-19]	0.619
15. Energy	12 [10-17.25][a]	17.25 [12-18][b]	17 [12-18.25][ab]	**<0.001**

When the participants answer "no" for the dichotomous questions, they did not indicate a peak for these variables. Thus, the sample size in each MRI time items were different (described in median [25-75th percentile]). [ab]Pairwise Comparisons were performed by Kruskal-Wallis Test; Significant differences are in bold.

Moderate correlation was found between rMEQ scores and the 24-hour peaks of most MRI items. Morningness-oriented participants (higher scores in the rMEQ) reported a peak of alertness, problem-solving, self-esteem, concentration, appetite, sexual arousal, motivation to

exercise, memory, talking to friends and energy, earlier than those who were eveningness-oriented (lower scores in the rMEQ). Sleepiness, irritability, and pessimism were positively correlated with rMEQ scores. Further, delayed alertness, problem-solving, self-esteem, sexual arousal, motivation to exercise, and energy were correlated with longer SJL, whereas delayed peaks of sleepiness, irritably and pessimism were correlated with shorter SJL. See Table 5.

Table 5. Spearman correlations between peak of MRI items, rMEQ scores, MSW, MSF and SJL (in hours).

MRI items	rMEQ	MSW	MSF	SJL
Alertness	-0.63**	0.35**	0.41**	0.18*
Sleepiness	0.33**	-0.21**	-0.26**	-0.14**
Problem-Solving	-0.59**	0.31**	0.34**	0.13*
Self-esteem	-0.43**	0.33**	0.43**	0.23**
Concentration	-0.50**	0.32**	0.28**	0.06
Appetite	-0.22**	0.23**	0.22*	0.06
Sexual Arousal	-0.28**	0.14*	0.30**	0.27**
Irritability	0.32**	-0.11*	-0.23**	-0.17**
Anxiety	0.04	0.11	0.07	-0.10
Sadness	0.09	0.15	0.12	-0.01
Motivation to Exercise	-0.46**	0.21**	0.36**	0.30**
Memory	-0.55**	0.33**	0.35**	0.07
Pessimism	0.26**	-0.02	-0.07	-0.07
Talking to friends	-0.24**	0.23**	0.28**	0.12
General motivation	-0.65**	0.37**	0.46**	0.25**

Abbreviations: rMEQ, Reduced Morningness-Eveningness Questionnaire; MSW, Midpoint of sleep on workdays; MSF, Midpoint of sleep on free days; SJL, Social Jetlag. *p<0.05; **p<0.001.

Discussio

This study tested the validation steps of the Spanish version of the MRI, an instrument created in Portuguese (de Souza et al. 2016). For psychometric characterization, sex and age were analyzed, and external validity was carried out comparing the MRI items to several chronobiological parameters (chronotype, MSF, MSW, and SJL). The factor analysis showed that the MRI is multidimensional, with three factors related to somatic (factor 1), affective (factor 2), and cognitive symptoms (factor 3). The factor with the highest eigenvalue was the related to affective variables, which includes some of the core symptoms of mood disorders diagnosis (American Psychiatric Association 2013) such as sadness, pessimism, irritability, anxiety, and self-esteem. A Cronbach's alpha of 0.70 indicates that the 15 items are related to each other and are examining the same construct, but are not redundant.

In this sample, males and females reported a similar frequency of perceived rhythms across almost all variables. In both sexes, the distribution of somatic variables (sleepiness and appetite), and cognitive variables (concentration, alertness, and problem-solving) peaked more frequently than affective variables. Geographic location and culture (Bagayogo et al. 2013) are known to influence the variability of mood since emotions can be influenced by genetics, environment, and social/cultural factors (Lim 2016). Moreover, a study using data from Twitter messages found that diurnal and seasonal mood varied depending on factors such as work, sleep, and day length in various cultures (Golder and Macy 2011). In the Spanish sample, the variable most frequently perceived as rhythmic was "sleepiness" for both sexes, while in the Brazilian sample variables were "sleepiness" for females and "appetite" for males (de Souza et al. 2016). Sleepiness and appetite are essential behaviors to the species' survival. Thus, a rhythmicity pattern is conserved over generations and across cultures. We noticed that the peak in sleepiness after lunchtime happen only in intermediate chronotype. Morning and evening types depict their peak in the evening and morning time, respectively. Despite recent studies pointing that this sleep need is a result of the homeostatic process of sleep regulation and, thus, regardless of the chronotype (Fernández-Mendoza et al. 2010), our data suggests the opposite. Therefore, the circadian characteristic plays an important role in the regulation of sleepiness, in spite of the homeostatic characteristics. Also, the intermediate could be more influenced by the cultural behavior of napping, which is common in Spanish communities and might be acting as a social zeitgeber (Vela-Bueno et al. 2008).

Age influenced the MRI variables' peak distribution in the 24-hour day (Figure 1). We found that older adults (> 40) had a phase advance in the memory, energy, and motivation to exercise, as well as a phase delay in sleepiness compared to the <21 and the 22-40 age groups, indicating more activity at an earlier time. These findings are consistent with the age-related shift to morningness observed in studies using the MEQ scores in adults (Paine et al. 2006). Age-related changes in hormonal (Logan and McClung 2019), body temperature (Carrier et al. 2002), and sleep-wakefulness rhythms (Carpenter et al. 2017) all play a role in phase advancing the circadian rhythms in aging (Cornelissen and Otsuka 2017).

Regarding sex differences, females reported a higher frequency of perceived sexual arousal rhythmicity than males. A recent study has shown differences in the rhythmicity of sexual arousal between sexes, with male subjects showing a more robust peak in the morning (Jankowski et al. 2014). Similarly, our findings demonstrated that the 24-hour peaks of sexual arousal occur significantly earlier in men (14.89 ± 7.39) compared to women (18.76 ± 4.95), consistent with the evidence that testosterone levels show circadian rhythmicity and are at their peak value during the morning in healthy males (Gupta et al. 2000).

Appetite and sadness were variables reported to peak at almost the same time over the course of a 24 hour day, independent of chronotype. In this Spanish sample, there might be a social influence behind the distribution of appetite throughout a 24-hour day, since it is frequently to have lunch around 2 p.m. in Spain (Vela-Bueno et al. 2008). Studies demonstrated that the evening type had a higher probability of presenting symptoms related to depression compared to morning and intermediate types in healthy young adults (Au and Reece 2017; Hidalgo et al. 2009; Müller and Haag 2018). In this sample, we did not formally conduct a structured interview to assess sleep or psychiatric disorders during the screening process formally. However, we found that in this population, sadness tended to peak at the end of the day, independent of chronotype. An evening peak pattern in individuals with depressive symptoms was demonstrated (Pereira-Morales et al. 2019). We speculate that the evening peak in sadness could not differentiate depressive individuals from healthy subjects. A future study using the MRI to assess rhythmicity in individuals with the major depressive disorder would be informative about this subject.

We also found that 24-hour peaks of the MRI items self-esteem, sexual arousal, irritability, motivation to exercise and energy were different between the SJL groups, with these items occurring earlier in individuals with less than 2h of SJL when compared to individuals

with 2-4h of SJL. Sleepiness, irritability, and pessimism correlated negatively with SJL. These findings suggest that the duration of the misalignment between the internal/biological and the external/social time may differentially affect cognitive-affective and somatic-physiological variables (Roenneberg and Merrow 2016; Smarr and Schirmer 2018).

This study has some limitations. The data collection was made between September and October and, therefore, the potential influence of seasonality could not be ascertained. It is worth emphasizing that we evaluated a homogeneous convenience sample that is important for validation studies but limits the external validity. Besides, we do not have a similar distribution in the sample size according to age since we have few >40 years old participants. We intend to better explore age-related analyses in the future. Also, we did not formally assess sleep or physical conditions, nor did we assess the use of medications, shift work, and recent transmeridional travels that could interfere with circadian rhythms. Moreover, we did not assess when participants filled out the instrument, thus not being capable to evaluate if the answers vary according to the time of the day.

Spite these limitations, is essential to remark the necessity to evaluate the perceived rhythmicity of human behavior. Therefore, tools such as the MRI to evaluate mood rhythmicity in clinical practice are indispensable. As perspectives, we aim to evaluate if participants respond differently to MRI as function of the time of the day they fill it, with the possibility of updating the instrument accordingly. In addition, actimetry assessment would be applied to compare the MRI performance with an objective measure to address convergent validity. The application of the MRI in the transcultural context, i.e. Brazil, Spain and Canada, aligned to this scales for screening or diagnosis of mood disorders will be useful to help healthcare professionals. It is in line with the necessity to personalize the treatment detecting the most important predictive factors for mood-related disabilities. Concerning better patient response, this evaluation may be useful to guide therapeutic strategies that could be integrated with treatment and could complement the interpersonal and social rhythm therapy used for mood disorders (Haynes et al. 2016).

In conclusion, the MRI is a valid, user-friendly instrument that may be used to explore mood rhythmicity in cross-cultural studies. In a Spanish sample, we found that daily rhythms of sexual arousal were more frequent in females than males, and age groups showed significant peak differences in most of the MRI items. The frequency of rhythmicity of mood-related items was similar among chronotypes, although the peaks occurred during different periods of the

day when comparing morning and evening chronotypes. Investigating the peaks of mood variables over the course of 24-hours in psychiatric conditions in future studies might help to advance our understanding of the association between mood symptoms and circadian rhythm.

Acknowledgements

We would like to thank D. Costa and L.K. Pilz for the support with data management and analysis; W. Maich for the editorial support; AC has received grants from CAPES. MABO and MPH have received funding from CNPq.

Disclosure statement

The authors declare no competing interests.

Funding

Work supported by Fundo de Incentivo à Pesquisa – Hospital de Clínicas de Porto Alegre (FIPE-HCPA; #2015-0539), FAPERGS/MS/CNPq/SESRS (PPSUS-2017) and the Spanish Ministry of Economy, Industry and Competitiveness (PSI2015-65026-MINECO/FEDER/UE).

References

Adan A, Guàrdia J. 1993. Circadian variations of self-reported activation: a multidimensional approach. Chronobiologia. 20:233-244.

Adan A, Almirall H. 1991. Horne & Östberg morningness-eveningness questionnaire: A reduced scale. Pers. Individ. Diff. 12:241-253.

Adan A, Archer SN, Hidalgo MP, Di Milia L, Natale V, Randler C. 2012. Circadian typology: A comprehensive review. Chronobiol. Int. 29:1153-1175.

American Psychiatric Association. 2013. Diagnostic and statistical manual of mental disorders: DSM-5. 5th.ed. Washington: American Psychiatric Association.

Arbabi T, Vollmer C, Dörfler T, Randler C. 2015. The influence of chronotype and intelligence on academic achievement in primary school is mediated by conscientiousness, midpoint of sleep and motivation. Chronobiol. Int. 32:349-357.

Au J, Reece J. 2017. The relationship between chronotype and depressive symptoms: A meta-analysis. J. Affect. Disord. 218:93-104.

Bagayogo IP, Interian A, Escobar JI. 2013. Transcultural aspects of somatic symptoms in the context of depressive disorders. Adv. Psychosom. Med. 33:64-74.

Beauvalet JC, Quiles CL, Oliveira MAB de, Ilgenfritz CAV, Hidalgo MPL, Tonon AC. 2017. Social jetlag in health and behavioral research: a systematic review. ChronoPhysiology Ther. 7:19-31.

Biernacki P, Waldorf D. 1981. Snowball sampling: Problems and techniques of chain referral sampling. Sociol. Methods Res. **10:**141-163.

Boivin DB, Shechter A, Boudreau P, Begum EA, Ying-Kin NMKN. 2016. Diurnal and circadian variation of sleep and alertness in men vs. naturally cycling women. Proc. Natl. Acad. Sci. **113:**10980-10985.

Byrne JEM, Bullock B, Murray G. 2017. Development of a Measure of Sleep, Circadian Rhythms, and Mood: The SCRAM Questionnaire. Front Psychol. 1;8:2105.

Carpenter JS, Robillard R, Hermens DF, Naismith SL, Gordon C, Scott EM, Hickie IB. 2017. Sleep-wake profiles and circadian rhythms of core temperature and melatonin in young people with affective disorders. J. Psychiatr. Res. 94:131-138.

Carrier J, Paquet J, Morettini J, Touchette E. 2002. Phase advance of sleep and temperature circadian rhythms in the middle years of life in humans. Neurosci. Lett. 320:1-4.

Cornelissen G, Otsuka K. 2017. Chronobiology of aging: A mini-review. Gerontology. 63:118-128.

Daviss WB, Birmaher B, Melhem NA, Axelson DA, Michaels SM, Brent DA. 2006. Criterion validity of the Mood and Feelings Questionnaire for depressive episodes in clinic and non-clinic subjects. J Child Psychol Psychiatry. 47(9):927-34.

de Souza CM, Carissimi A, Costa D, Francisco AP, Medeiros MS, Ilgenfritz CA, et al. 2016. The Mood Rhythm Instrument: Development and preliminary report. Rev. Bras. Psiquiatr. 38:148-153.

Di Milia L, Adan A, Natale V, Randler C. 2013. Reviewing the psychometric properties of contemporary circadian typology measures. Chronobiol. Int. **30:**1261-1271.

Díaz-Morales JF, Escribano C, Jankowski KS. 2015. Chronotype and time-of-day effects on mood during school day. Chronobiol. Int. 32:37-42.

Dosseville F, Laborde S, Lericollais R. 2013. Validation of a chronotype questionnaire including an amplitude dimension. Chronobiol Int. 30(5):639-48.

Dzogang F, Lightman S, Cristianini N. 2018. Diurnal variations of psychometric indicators in Twitter content. PloS One. 13:e0197002.

Fernández-Mendoza, J., Ilioudi, C., Montes, M.I., Olavarrieta-Bernardino S., Aguirre-Berrocal A., Cruz-Troca J.J., Vela-Bueno A. 2010. Sleep Biol. Rhythms. 8: 52.

Francisco AP, Oliveira MAB de, Carissimi A, Fabris RC, Ilgenfritz CAV, Souza CM de, et al. 2017. Spanish translation of the mood rhythm instrument: A novel approach to mood evaluation. Clin. Biomed. Res. 37:41-47.

Golder SA, Macy MW. 2011. Diurnal and seasonal mood vary with work, sleep, and daylength across diverse cultures. Science. **333:**1878-1881.

Gupta SK, Lindemulder EA, Sathyan G. 2000 Modeling of circadian testosterone in healthy men and hypogonadal men. J. Clin. Pharmacol. 40:731-738.

Haynes PL, Gengler D, Kelly M. 2016. Social rhythm therapies for mood disorders: An update. Curr. Psychiatry. Rep. 18:75.

Hidalgo MP, Caumo W, Posser M, Coccaro SB, Camozzato AL, Chaves MLF. 2009. Relationship between depressive mood and chronotype in healthy subjects. Psychiatry. Clin. Neurosci. 63:283-290.

Jankowski KS, Díaz-Morales JF, Randler C. 2014. Chronotype, gender, and time for sex. Chronobiol. Int. 31**:**911-916.

Kronfeld-Schor N, Einat H. 2012. Circadian rhythms and depression: human psychopathology and animal models. Neuropharmacology. 62:101-114.

Levandovski R, Dantas G, Fernandes LC, Caumo W, Torres I, Roenneberg T, Allebrandt KV. 2011. Depression scores associate with chronotype and social jetlag in a rural population. Chronobiol. Int. 28:771-778.

Lim N. 2016. Cultural differences in emotion: Differences in emotional arousal level between the East and the West. Integr. Med. Res. **5:**105-109.

Logan RW, McClung CA. 2019. Rhythms of life: circadian disruption and brain disorders across the lifespan. Nat. Rev. Neurosci. 20:49-65.

Müller MJ, Haag A. 2018. The concept of chronotypes and its clinical importance for depressive disorders. Int. J. Psychiatry Med. 53:224-240.

Paine S-J, Gander PH, Travier N. 2006. The epidemiology of morningness/eveningness: influence of age, gender, ethnicity, and socioeconomic factors in adults (30-49 years). J. Biol. Rhythms. 21:68-76.

Pereira-Morales AJ, Casiraghi LP, Adan A, Camargo A. 2019. Mood rhythmicity is associated with depressive symptoms and caffeinated drinks consumption in South American young adults. Chronobiol. Int. 36:225-236.

Pilz LK, Carissimi A, Francisco AP, Oliveira MAB, Slyepchenko A, Epifano K, et al. 2018a. Prospective assessment of daily patterns of mood-related symptoms. Front. Psychiatry. 9:370.

Pilz LK, Carissimi A, Oliveira MAB, Francisco AP, Fabris RC, Medeiros MS, Scop M, Frey BN, Adan A, Hidalgo MP. 2018b. Rhythmicity of mood symptoms in individuals at risk for psychiatric disorders. Sci. Rep. 8:11402.

Randler C, Díaz-Morales JF, Rahafar A, Vollmer C. 2016. Morningness-eveningness and amplitude - development and validation of an improved composite scale to measure circadian preference and stability (MESSi). Chronobiol Int. 33(7):832-48.

Roenneberg T, Kuehnle T, Juda M, Kantermann T, Allebrandt K, Gordijn M, Merrow M. 2007. Epidemiology of the human circadian clock. Sleep. Med. Rev. 11:429-438.

Roenneberg T, Merrow M. 2016. The Circadian Clock and Human Health. Curr Biol. 26:R432-R443.

Roenneberg T, Wirz-Justice A, Merrow M. 2003. Life between clocks: daily temporal patterns of human chronotypes. J. Biol. Rhythms. **18:**80-90.

Smarr BL, Schirmer AE. 2018. 3.4 million real-world learning management system logins reveal the majority of students experience social jet lag correlated with decreased performance. Sci. Rep. **8:**4793.

Smolensky MH, Hermida RC, Reinberg A, Sackett-Lundeen L, Portaluppi F. 2016. Circadian disruption: New clinical perspective of disease pathology and basis for chronotherapeutic intervention. Chronobiol. Int. 33:1101-1119.

Vela-Bueno A, Fernandez-Mendoza J, Olavarrieta-Bernardino S, Vgontzas AN, Bixler EO, de la Cruz-Troca JJ, Oliván-Palacios, J. 2008. Sleep and behavioral correlates of napping among young adults: a survey of first-year university students in Madrid, Spain. J. Am. Coll. Health. 57:150-158.

Waterhouse J, Fukuda Y, Morita T. 2012. Daily rhythms of the sleep-wake cycle. J. Physiol. Anthropol. 31:5.

Watson D, Clark LA, Tellegen A. 1988. Development and validation of brief measures of positive and negative affect: the PANAS scales. J Pers Soc Psychol. 54(6):1063-70.

Wirz-Justice A. 2008. Diurnal variation of depressive symptoms. Dialogues. Clin. Neurosci. 10:337-343.

2.2 TRADUÇÃO E VALIDAÇÃO DO MRhI PARA A LÍNGUA INGLESA

Artigo publicado na revista *BMC Psychology* (IF: 2.59; Qualis A3 área Medicina II).

DOI: 10.1186/s40359-020-00397-2

Title: Validation of the English version of the Mood Rhythm Instrument

Running head: English Version of Mood Rhythm Instrument

Oliveira et al. BMC Psychology (2020) 8:35
https://doi.org/10.1186/s40359-020-00397-2

BMC Psychology

RESEARCH ARTICLE Open Access

Validation of the English version of the Mood Rhythm Instrument

Melissa A. B. Oliveira[1,2†], Kristina Epifano[3†], Salina Mathur[3], Felipe Gutiérrez Carvalho[1,4], Marina Scop[1], Alicia Carissimi[1,2], Ana Paula Francisco[1], Luciene L. S. Garay[1], Ana Adan[5,6], Maria Paz Hidalgo[1,2] and Benicio N. Frey[4,7*]

Abstract

Background: Disruption of biological rhythms has been linked to the pathophysiology of mental disorders. However, little is known regarding the rhythmicity of mood symptoms due to the lack of validated clinical questionnaires. A better understanding of the rhythmicity of mood symptoms can help identifying individuals whose severity of mood symptoms follows an altered circadian rhythm. The objective of this study was to validate the English version of the Mood Rhythm Instrument (MRhI), a self-reported measure of self-perceived rhythmicity of mood symptoms and behaviours, in a sample of the general population from Canada.

Methods: After the translation process, the final English version of the Mood Rhythm Instrument (MRhI-English) was applied on participants recruited at McMaster University and St. Joseph's Healthcare Hamilton campuses. Individuals were also asked to answer the Reduced Morningness-Eveningness Questionnaire (rMEQ).

Results: Four hundred one individuals completed the English version of the MRhI and the rMEQ. The MRhI-English presented a Cronbach's alpha of 0.75. The factorial analysis grouped the MRhI-15 items in 3 factors (cognitive, affective and somatic), with affective items having a lower frequency of self-reported 24-h peaks. Comparison between sexes showed that women reported a higher frequency of daily peaks in *irritability*, *anxiety*, *sadness* and *talking to friends*, while men exhibited peaks more frequently in *problem-solving*, *sexual arousal* and *motivation to exercise*.

Conclusions: Our findings suggest that the English version of the MRhI displayed good internal consistency. Future directions will include the use of the MRhI instrument in individuals with mood disorders, aiming to provide a better understanding of the relationship between daily patterns of mood variability and mental health outcomes.

Keywords: Circadian rhythm, Chronobiology, Sleep, Depression, Mood

Background

Mood disorders are chronic mental health conditions that cause a range of disabilities for patients, generating a negative impact on the individual, health systems and society [1]. Due to their multifactorial etiology, mood disorders are known to be influenced by genetic, personal and/or environmental factors [2, 3]. While mood disorders are prevalent, a significant proportion of individuals with mood disorders go undiagnosed due to the spectrum of severity and prognosis [4, 5]. In this context, it is important to find ways to improve the identification of its risk factors, leading to appropriate treatment management and consequently preventing unfavourable outcomes.

The etiology of mood disorders has been extensively studied, and some chronobiological factors have been found to play an essential role in the pathophysiology of

* Correspondence: freybn@mcmaster.ca
†Melissa A. B. Oliveira and Kristina Epifano contributed equally as first authors
[4]Mood Disorders Program and Women's Health Concerns Clinic, St. Joseph's Healthcare, Hamilton, ON, Canada
[7]Department of Psychiatry and Behavioural Neurosciences, McMaster University, 100 West 5th Street, Suite C124, Hamilton, ON L8N 3K7, Canada
Full list of author information is available at the end of the article

mood disorders [6]. For instance, previous studies have revealed that alterations in circadian rhythms are highly associated with major depression and bipolar disorder [7–9]. Specifically, altered variations of the clock genes, those involved with rhythmicity and timing of biological rhythms at a molecular level, have been found in individuals with bipolar disorder, as well as major depression [10]. Recent meta-analytic studies have concluded that the abnormal sleep rhythms are consistently observed in patients with these two major mood disorders [11, 12]. These disturbances can be a potential predictor of declining mental health, as they have been shown to contribute to escalated mood levels and the triggering of manic episodes in patients [13]. For instance, studies have shown that sleep deprivation and jet lag can trigger or aggravate depressive, hypomanic or manic episodes [14]. In addition, studies have shown that discrete patterns of daily activity rhythms can distinguish specific mood disorder subgroups, such as bipolar depression and mania, or non-melancholic and melancholic depression [15, 16]. Notably, lower stability and weakened amplitude in rest-activity rhythms have been associated with greater symptom severity (e.g. impulsivity and mood instability) in individuals with borderline personality disorder [17].

Therapies that target circadian rhythms synchronization might be useful in the management of mood disorders, such as bright light therapy and interpersonal and social rhythm therapy [18–20]. A better understanding of the rhythmicity of mood symptoms can help to identify individuals whose severity of mood symptoms follow an altered circadian rhythm. However, despite the increasing evidence linking mood disorders and circadian rhythms disruption, little is known regarding the rhythmicity of mood symptoms due to the lack of validated clinical questionnaires. In order to fill this gap, we have developed the Mood Rhythm Instrument (MRhI), a clinical tool aiming at assessing the self-perceived rhythmicity of mood symptoms.

The MRhI is a self-reported questionnaire developed to evaluate the presence and timing of daily patterns for mood-related symptoms over the last 15 days. Each of the 15 items comprises a categorical and a continuous question. The original version of this instrument was created in Brazilian Portuguese [21], which was then translated and validated in Spanish [22, 23]. In a large study with 708 participants that completed the MRhI, we found that the rhythmicity of specific mood-related symptoms and behaviors, such as pessimism and motivation to exercise were associated with higher risk for psychiatric disorders [24]. Notably, we also found specific cultural differences in comparing Spanish and Brazilian samples in terms of the daily patterns of mood-related symptoms [25]. These results are consistent with previous studies suggesting that cultural differences, as seen in different populations' sleep/wake habits [26–28], as well as ethnic differences [29], and as racial differences in tau and circadian phase shifting, are relevant factors in circadian rhythm research. Thus, this study aims to validate the MRhI English version in an English-speaking Canadian sample.

Methods

Step 1. Translation of the Mood Rhythm Instrument (MRhI)

The translation process is detailed in Fig. 1 and was composed by five steps, including forward translation, correctness, back translation, back translation review and harmonization.

The instructions on how to answer the questionnaire was updated with the removal of one sentence. In the translated version, the following sentence was written: "Answer the following questions according to the previous 15 days, taking into account how you have felt during most of the time, on the majority of the days and in the absence of any events that have caused you distress". In order to improve clarity, this sentence was changed

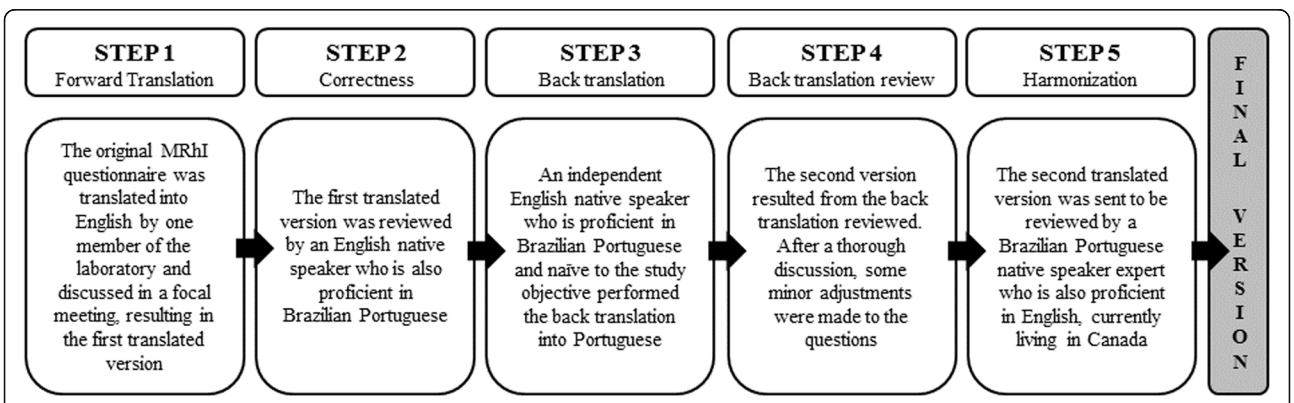


Fig. 1 Translation process of MRhI into English. The main steps for translation and cultural adaptation of Brazilian Portuguese into English are detailed

to: "Answer the following questions according to the previous 15 days, taking into account how you have felt during most of the time". All sentences had their conjugation changed to the Present Perfect Tense, as the MRhI intends to assess the self-perceived rhythmicity of mood-related symptoms in the last 15 days. For question 11, the word "prone" was changed to "motivated". For question 12, the sentence "you memorize" was changed to "your memory". For question 15, the sentence "when you feel your best" was changed to "when you have had more energy and motivation to do things". Furthermore, instead of a 24-h format scale as it stands in the Portuguese version, the English version has an am/pm format scale. The final English version of the MRhI can be found as Supplementary Methods.

Step 2. Validation of the Mood Rhythm Instrument

Participants and procedures

Data collection was conducted between January 2016 and September 2018. We recruited the study sample through poster advertisements at McMaster University and St. Joseph's Healthcare Hamilton campuses, and online research recruitment within the Department of Psychology, Neuroscience & Behaviour at McMaster University. The final study sample comprised 401 individuals (age: 18–60; mean age: 22. ±7), predominantly women (72%), with a mean of 15 ± 3 years of schooling. The study was approved by the Hamilton Integrated Research Ethics Board and was conducted in accordance with the Declaration of Helsinki. All study participants provided written informed consent before study entry.

Instruments

Mood Rhythm Instrument (MRhI) The MRhI is composed of 15 items referring to physical, psychological and behavioural aspects related to mood, and each item provides a categorical (presence or absence of a daily peak) and a continuous (peak time in a 24 h period) variable. Subjects answered if, in the last 15 days, there was a specific time of the day when they experienced a peak in mood-related symptoms. We have recently completed a study where we tested the agreement rates between the MRhI and a daily version of the MRhI, and we found high agreement rates between the two instruments, thus suggesting that the MRhI may not be significantly influenced by memory bias [25]. The MRhI displayed a satisfactory internal consistency (i.e. Cronbach's alpha) in Brazilian (0.73) and Spanish (0.70) populations [21, 23].

Reduced Morningness-Eveningness questionnaire (rMEQ) The rMEQ provides a self-evaluation of chronotype, which is a unidimensional construct and offers a classification that varies between evening and morning types. This questionnaire was developed by Adan and Almirall [30] and includes items 1, 7, 10, 18, and 19 of the original MEQ. These 5 questions comprise the smallest possible number of items that provides the maximum amount of information relative to the time when each individual feel to be more prone to perform daily activities and to sleep. Higher numbers indicate morning tendencies and lower numbers indicate evening tendencies (scores 4–11: evening type; 12–17: intermediate type; 18–25: morning type). The rMEQ has been widely used due to its practicality, allowing parallel recording of other variables, especially in large sample studies. The psychometric properties of the rMEQ has been evaluated in many countries of Europe, America, as well as in Kingdom of Saudi Arabia, China, India, Iraq and Iran. In most of these previous studies rMEQ showed similar values for internal consistency [31].

Reliability and validity process

Internal consistency was measured with Cronbach's alpha. A Cronbach's alpha value between 0.7 and 0.9 was considered acceptable [32]. Psychometric properties of MRhI-English were assessed through the exploratory factor analysis (EFA). The EFA was carried out using a tetrachoric correlation matrix since our data has a binary feature [33]. Maximum Likelihood and Varimax were the extraction and rotation methods, respectively. Compared to other commonly used extraction methods, Maximum Likelihood uses the full information solution of the 2p contingency table [34]. For practical implications, Maximum Likelihood is considered preferable for tests with few factors (stated as 1 to 3 factors), which is the main reason we opted for this specific extraction method [35]. Satorra-Bentler corrected model estimation algorithm was used to surmount biased estimates. Varimax is a widely used rotation technique, being suitable for the present data as it shows excellent results by differentiating groups in several simulation scenarios [36]. Factors extraction was initially performed through Velicer's minimum average partial (MAP) [37], Horn's Parallel Analysis (PA) [38], and Comparison Data (CD) [39], obtaining 2, 5, and 3 factors respectively. As supported by Ruscio and Roche [39], CD method performs better than MAP and PA in terms of accuracy and precision, with nearly unbiased results. Thus, the three-factor model was considered for the analysis. A confirmatory factor analysis (CFA) using Comparative Fit Index (CFI) > 0.95, Tucker-Lewis Index (TLI) > 0.95, Root mean square error of approximation (RMSEA) < 0.06, and Standardized Root Mean Square Residual (SRMR) < 0.08 as model fit indices were conducted [40]. The CFA model fit indices showed suitable or slightly less than

Table 1 Exploratory Factor Analysis of the English version of the Mood Rhythm Instrument items based on a three-factor solution

Items	Factor 1	Factor 2	Factor 3
1. Alertness	0.43	0.16	**0.45**
2. Sleepiness	0.26	0.2	**0.72**
3. Problem-Solving	**0.76**	0.09	0.28
4. Self-esteem	0.15	**0.45**	0.44
5. Concentration	**0.64**	0.1	0.31
6. Appetite	0.12	0.19	**0.54**
7. Sexual Arousal	0.11	0.26	**0.45**
8. Irritability	0.15	0.37	**0.49**
9. Anxiety	0.1	**0.66**	0.21
10. Sadness	0.1	**0.85**	0.01
11. Motivation to Exercise	0.2	−0.12	**0.45**
12. Memory	**0.72**	0.24	0
13. Pessimism	0.18	**0.63**	0.14
14. Talking to Friends	**0.32**	0.31	0.17
15. Energy	**0.59**	0.11	0.46
Eigenvalues	2.38	2.26	2.32
% of variance	0.16	0.15	0.15

the good fit values (χ^2 = 178.8, df = 87, CFI = 0.875, TLI = 0.850, RMSEA = 0.05, SRMR = 0.06).

Statistical analysis

Variables were tested for normality by the Shapiro-Wilk test. Comparisons of the frequency of the dichotomous MRhI-English according to sex were analyzed by Chi-square test (χ^2). Linear-circular correlations between time peaks of MRhI items and MEQ scores were performed [41]. The distribution of MRhI-English items peaks were shown as a circular mean and compared between sexes according to Mardia-Watson-Wheeler test, considering that the data do not follow a normal distribution for circular data [42]. R version 3.4.1 (package "Directional v3.3") and NCSS 12.0.9 were used for circular analysis. R version 3.4.1 (packages "psych", "lavaan" and "RGenData") and PASW Statistics Version 18 (SPSS Inc., Chicago, IL) were used for statistical analyses. Statistical significance was accepted at $p < 0.05$.

Results

Reliability and validity of the English version of the mood rhythm instrument

The MRhI-English presented a Cronbach's alpha of 0.75 in this sample, which suggests good internal consistency. Table 1 presents the three factors obtained with the factorial analysis of the categorical MRhI items. The first factor was predominantly composed by cognitive items such as *problem-solving, concentration, memory, talking to friends* and *energy*. Items related to affective aspects were in the second factor, e.g. *self-esteem, anxiety, sadness* and *pessimism*. Finally, the third factor grouped *alertness, sleepiness, irritability* and somatic items like *appetite, sexual arousal,* and *motivation to exercise*.

The frequency of self-reported rhythmicity for each MRhI item and the comparison between sexes is shown on Table 2. Items with the highest reported occurrence (> 70%) of a daily peak were *alertness*, *sleepiness*, *concentration*, *appetite* and *energy*. On the other hand, less than

Table 2 Frequency of self-reported rhythmicity of the Mood Rhythm Instrument (MRhI) items – English version

MRhI items	Total ($n = 401$) n (%)	Men ($n = 112$) n (%)	Women ($n = 289$) n (%)	χ^2, p value
Alertness	313 (78)	85 (76)	228 (79)	0.42, $p = 0.52$
Sleepiness	375 (94)	109 (97)	266 (92)	3.71, $p = 0.05$
Problem-solving	273 (68)	85 (76)	188 (65)	**4.36, $p < 0.05$***
Self-esteem	144 (36)	40 (36)	104 (36)	0.00, $p = 0.96$
Concentration	318 (79)	86 (77)	232 (80)	0.60, $p = 0.44$
Appetite	292 (73)	82 (73)	210 (73)	0.01, $p = 0.91$
Sexual Arousal	125 (31)	47 (42)	78 (27)	**8.44, $p < 0.01$****
Irritability	243 (61)	58 (52)	185 (64)	**5.06, $p < 0.05$***
Anxiety	172 (43)	34 (30)	138 (48)	**9.97, $p < 0.01$****
Sadness	157 (39)	30 (27)	127 (44)	**9.98, $p = 0.01$****
Motivation to exercise	248 (62)	79 (70)	169 (58)	**4.97, $p = 0.05$***
Memory	127 (32)	37 (33)	90 (31)	0.13, $p = 0.72$
Pessimism	122 (30)	30 (27)	92 (32)	0.97, $p = 0.32$
Talking to Friends	205 (51)	43 (38)	162 (56)	**10.08, $p < 0.01$****
Energy	310 (77)	86 (77)	224 (78)	0.02, $p = 0.88$

Chi-square test; *$p < 0.05$; **$p \leq 0.01$

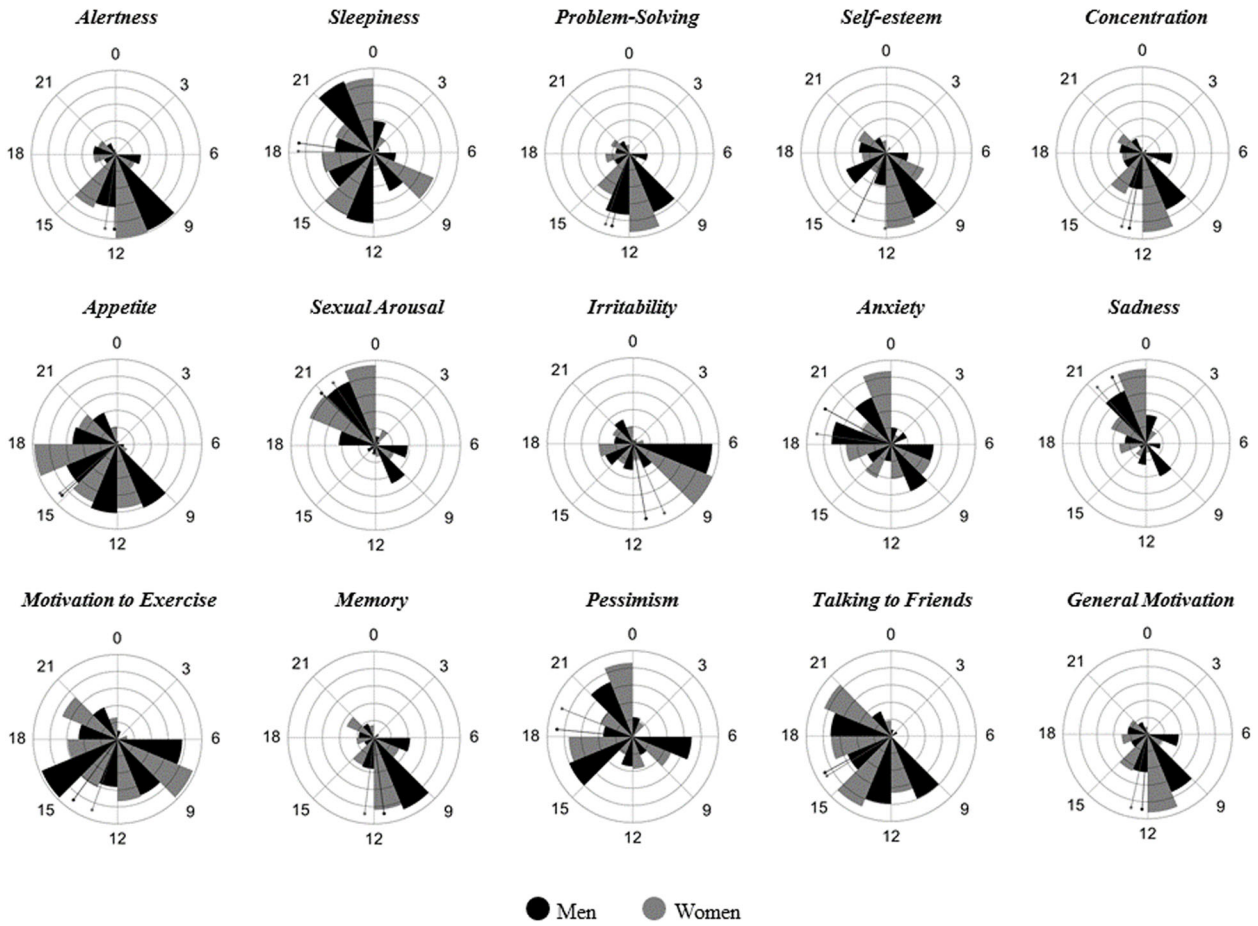


Fig. 2 Rose plots for the Mood Rhythm Instrument (MRhI) time variables divided by groups of sex. The circle represents 24 h and each bin represents a 3-h period. The petals magnitudes are based on the proportion of participants of each group presenting a daily peak in the corresponding 3-h period. The bin width is divided equally by the two groups and the petals are laid out sequentially in the bin. The lines represent the circular mean for each group. Men and women do not differ in any MRhI item considering a level of significance of $\alpha = 0.05$ (Mardia-Watson-Wheeler test for equal distributions)

40% of subjects reported a daily peak in *self-esteem*, *sexual arousal*, *sadness*, *memory* and *pessimism*. The comparison between sexes showed that women reported a higher frequency of daily patterns in *irritability*, *anxiety*, *sadness* and *talking to friends*, while men reported in *problem-solving*, *sexual arousal* and *motivation to exercise*.

The comparison of MRhI time variables distribution according to sex is displayed in Rose plots (Fig. 2). The items did not vary between men and women (all $p > 0.05$). Notably, *sleepiness*, *appetite*, *anxiety*, *motivation to exercise*, *pessimism* and *talking to friends* seemed to have a multimodal (i.e. more than one peak time in a 24 h period) pattern of occurrence.

According to linear-circular correlation, all MRhI timing items were significantly correlated with rMEQ scores (Table 3). However, we cannot establish if items are positively or negatively correlated to rMEQ scores due to the nature of a circular measure. Figure 3 presents the frequency of which subjects responded with having a peak for MRhI items and the circular means of the reported peaks according to chronotype. The later the circular means appeared for cognitive and somatic items, the more eveningness the chronotype became (e.g. *alertness, problem-solving, concentration, appetite, motivation to exercise, memory, talking to friends* and *energy*). The opposite occurred with *irritability*, *sleepiness*, *anxiety* and *pessimism*. *Sexual arousal* and *sadness* did not seem to vary among the different chronotypes and showed little rhythmicity.

Discussion

The translation of the MRhI to English was adjusted to language and clarity. Importantly, the time scale was modified into the 12-h clock format for a cultural adaptation for the Canadian and most English-speaking countries. The Cronbach's alpha was 0.75, meaning that the items had an acceptable internal consistency and were adequate, similar to previous validation studies of the MRhI [21, 23].

Table 3 Linear-circular correlations between time of peak of MRhI items and rMEQ scores

MRhI items (n)	R-squared	*p*-value
Alertness (309)	0.290	≤0.001
Sleepiness (370)	0.106	≤0.001
Problem-Solving (270)	0.147	≤0.001
Self-esteem (142)	0.206	≤0.001
Concentration (315)	0.247	≤0.001
Appetite (289)	0.028	≤0.001
Sexual Arousal (123)	0.066	≤0.001
Irritability (241)	0.203	≤0.001
Anxiety (170)	0.089	≤0.001
Sadness (157)	0.046	≤0.001
Motivation to Exercise (246)	0.162	≤0.001
Memory (126)	0.231	≤0.001
Pessimism (120)	0.107	≤0.001
Talking to friends (203)	0.020	0.018
Energy (305)	0.221	≤0.001

Abbreviations: *rMEQ* Reduced Morningness-Eveningness Questionnaire

The three factors solution grouped the items based on the nature of their features. Considering that the first factor explains the greatest percentage of the variance, items in this factor are considered to have an important contribution to the explained variance [43]. It seems that in this Canadian sample, cognitive items are more important in assessing the profile of mood rhythmicity than affective and somatic ones. Yet, in the recent Spanish validation of MRhI, psychometric analysis showed that the first factor grouped somatic items except for *problem-solving* [23].

In line with previous MRhI studies, cognitive and somatic items had more reported peaks than affective items [21, 23]. With the exception of *anxiety*, the affective items exhibited perceived peaks on less than 40% of subjects from the whole sample. When considering sex to compare the frequency of daily peaks, we found significant differences in *irritability*, *anxiety* and *sadness*, which were more frequently reported by women. Notably, these results are consistent with epidemiological data pointing to a higher prevalence of mood and anxiety disorders in women, which is corroborated by biological [44] and socioeconomic [45] contributors. Another factor that may be related to the sex differences observed are alexithymic behaviors, which are more prevalent in men, resulting in a lack of report of negative emotions in this population [46].

Women also reported a higher frequency of daily peaks for *talking to friends*, which is in accordance to a previous British study that evaluated social interaction components of circadian rhythms through phone calls monitoring. Results showed that, comparatively to men, women spent more time on calls with friends in the evening and at night [47]. Interestingly, in terms of sexual arousal, men reported more frequency of sexual arousal peaks than women, which is exactly the opposite from what we observed in Spanish population [23] and also distinct from Brazilians that did not display differences in sexual arousal peak between women and men [21]. Moreover, *problem-solving* and *motivation to exercise* were more frequently pointed as having a daily peak in men. This result can be in part supported by the fact that women are more prone to be extrinsically motivated (with expectations to gain rewards or outcomes) to exercise than intrinsically motivated (aiming personal satisfaction and/or enjoyment), resulting in less motivation for regular physical activity in comparison to men [48]. In contrast to the Brazilian sample, where *alertness* was the only item that differed between sexes, with women reporting more frequently to have a peak than men [21], no differences with regards to *alertness* were found in the Canadian sample.

Overall, we observed that the Canadians reported more sex differences with regards to frequency of perceived peaks than the Brazilian sample. Regarding negative mood and somatic symptomatology, women reported more frequent peaks than men (irritability, anxiety and sadness), while for positive cognitive and somatic activity behaviors men reported more frequent peaks than women (sexual arousal, problem solving and motivation to exercise). As aforementioned, higher prevalence of mood symptoms in women possibly contrasts observations related to the affective items in a sample mostly composed by them. Thus, future research exploring these factors which controls for the relationship between sex and psychiatric symptoms shall bring valuable insights related to the sex differences observed.

The time when items peak did not differ between men and women in any of the MRhI items. Considering that participants could only choose one time peak, even though men reported to have a *sexual arousal* peak in the morning, pointed morning peaks were much more frequent as among women and, therefore, circular means were similar between women and men. A Polish study reported that women had evening peaks of "greatest need for sex", whereas men had both morning and evening peaks [49]. This multimodal pattern of occurrence was also identified in *appetite* and usually varies among breakfast, lunch and dinner [50]. Subjects also reported peaks of *sleepiness* in the morning, right after midday and at night which is in line with previous analyses of sleepiness expression [51].

As we expected, the circadian typology, measured by means of chronotype, is significantly correlated to all MRhI items time peaks [52]. Participants classified as morning types reported earlier peak times for *concentration*, *alertness*, *problem-solving*, *energy*, *memory*, *motivation to exercise* and *self-esteem*. In contrast, individuals classified as evening types reported that these items

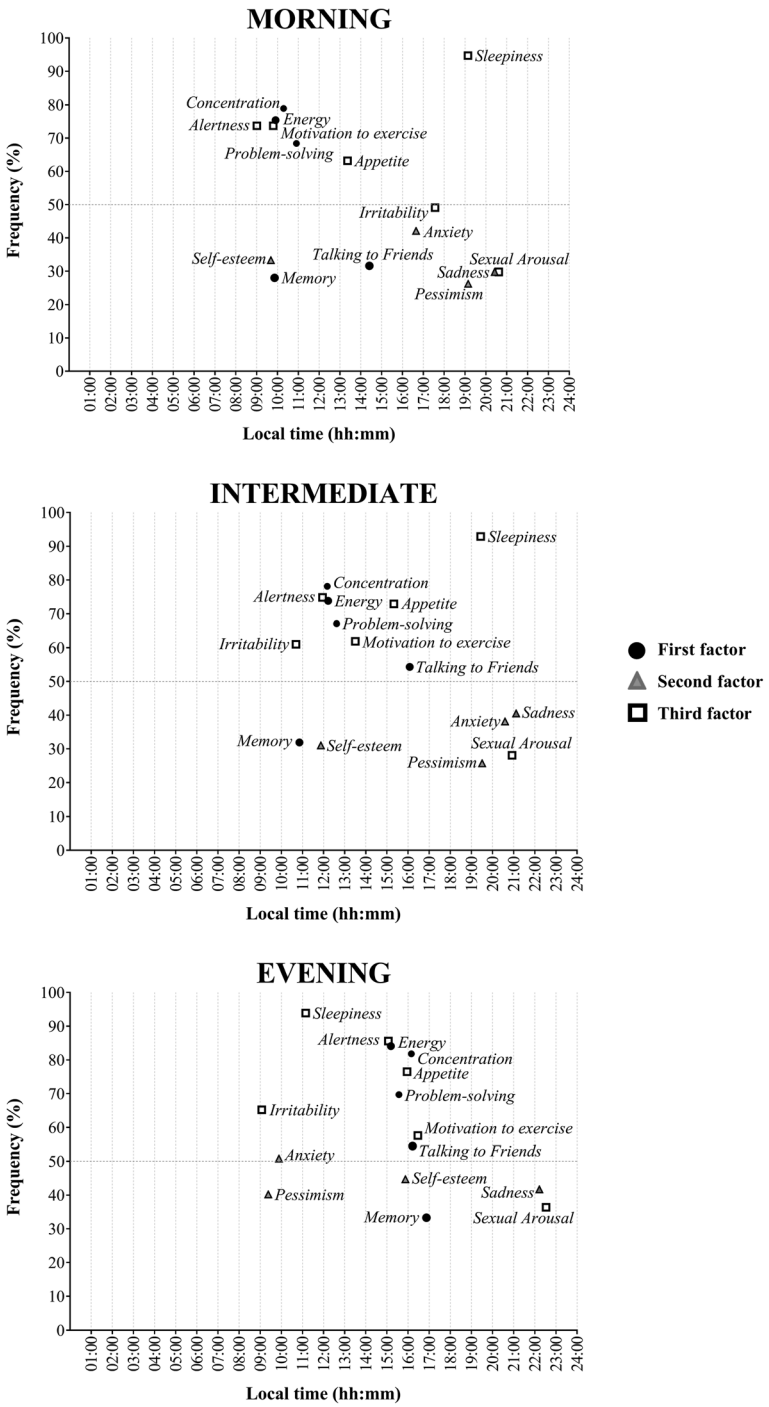


Fig. 3 Frequency and peak of each MRhI item. The circular mean of each 24-h peak for mood symptoms is depicted on the x-axis and frequency (%) is depicted on the y-axis

peaked later in the day. These results are consistent with previous studies from Europe and United States showing that individuals with morning chronotype performed better in terms of attention, alertness and working memory in the morning and afternoon when compared to individuals with an evening chronotype [53, 54]. Also, depending on the type of problem to solve (e.g. insight or analytic), individuals classified as having a later circadian arousal perform better during later afternoon sessions (between 4 pm and 5:30 pm) [55].

Cognitive performance has been shown to be correlated with individual's body temperature rhythm. Wright et al.

[56] found that cognitive tasks were performed better when body temperature is high and near its circadian peak. Alongside these findings, another study found that individuals with a morning chronotype have earlier temperature rhythms, thus their peaks in cognitive performance such as memory and alertness would also occur earlier than those with evening chronotypes [57]. Our data revealed that individuals with evening chronotype reported earlier peaks of *sleepiness* compared to individuals with morning and intermediate chronotypes. However, it is possible that this finding does not reflect spontaneous behaviors, but rather the consequences of sleep disruption related to diurnal social demands, a hypothesis that is endorsed by the same finding regarding *irritability, pessimism* and *anxiety* (earlier in evening chronotypes). Due to the mean age of 22.2 ± 7 years of our sample, which in general is related to later chronotypes [52], the opposite maladaptation for nocturnal activities could be observed in *talking to friends*. This item peaked at a similar time for intermediate and evening types, and earlier in morning types. Finally, in our sample *sexual arousal, appetite* and *sadness* displayed little variation between chronotypes.

This study has some limitations. We are aware that the MRhI does not reflect mood rhythms independently of external or social factors, as responsibilities and schedules of participants probably influence their responses. However, it is of our interest to evaluate individuals' self-perception of rhythmicity of mood-related symptoms when inserted in a real-life setting, rather than assessing internal rhythm alone. External factors that exist in a person's environment are intricate experiences that can also influence how symptoms of mood disorders present themselves. Another limitation is that only one external validation measure was used, albeit it is a well-established questionnaire to evaluate chronotype [30]. Longitudinal monitoring of cognitive, affective, and somatic symptoms using Ecological Momentary Assessment methods should also be considered when validating instruments like the MRhI.

Conclusions

In conclusion, the results obtained with the English version of the MRhI are consistent with previous chronobiology studies, suggesting that this instrument might be useful to enhance the knowledge of self-perceived daily patterns of mood-related symptoms. The Cronbach's alpha analysis suggests good internal consistency of this instrument. Cognitive, affective and somatic items presented different frequency of reported peaks and regarding its timing, they seemed to behave accordingly to chronotype. The future directions will be the use of the MRhI instrument in a large sample of individuals with mood disorders, aiming to provide a better understanding of the relationship between daily patterns of mood variability and mental disorders.

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s40359-020-00397-2.

Additional file 1.

Abbreviations

MRhI: Mood Rhythm Instrument; rMEQ: Reduced Morningness-Eveningness Questionnaire; MRhI-English: English version of the Mood Rhythm Instrument; MAP: Minimum average partial; CD: Comparison Data; CFA: Confirmatory factor analysis; TLI: Tucker-Lewis Index; RMSEA: Root mean square error of approximation

Authors' contributions

M.A.B.O., A.C., A.P.F., A.A., M.P.H. and B.N.F. designed the study. M.A.B.O., K.E., M.S., S.M., F.G.C., A.C., A.P.F. and L.L.S.G. collected and organized the data. M.A.B.O, K.E., M.S., F.G.C., A.C. and M.P.H. analyzed the data. M.A.B.O., K.E., M.S., A.C., L.L.S.G., M.P.H and B.N.F. wrote the first draft of the manuscript. All authors have read, revised and approved the final manuscript.

Funding

Financial support was provided by CAPES (MO, FC), Propesq-UFRGS (MS), PQ-CNPq (MH), PVE-CNPq and PRINT-CAPES (BF), the Spanish Ministry of Economy, Industry, and Competitiveness (grant PSI2015–65026; MINECO / FEDER / UE) (AA). The funding bodies had no role in the study design, data collection, analysis, or interpretation, or in the writing of the manuscript.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Ethics approval and consent to participate

All individuals who agreed to participate provided written informed consent. The study was approved by the Hamilton Integrated Research Ethics Board and was conducted in accordance with the Declaration of Helsinki.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

[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, Brazil. [2]Programa de Pós-Graduação em Psiquiatria e Ciências do Comportamento – Faculdade de Medicina, UFRGS, Porto Alegre, Rio Grande do Sul, Brazil. [3]Department of Psychology, Neuroscience & Behaviour, McMaster University, Hamilton, ON, Canada. [4]Mood Disorders Program and Women's Health Concerns Clinic, St. Joseph's Healthcare, Hamilton, ON, Canada. [5]Department of Clinical Psychology and Psychobiology, School of Psychology, University of Barcelona, Barcelona, Spain. [6]Institute of Neurosciences, University of Barcelona, Barcelona, Spain. [7]Department of Psychiatry and Behavioural Neurosciences, McMaster University, 100 West 5th Street, Suite C124, Hamilton, ON L8N 3K7, Canada.

Received: 27 June 2019 Accepted: 23 March 2020
Published online: 17 April 2020

References

1. Vos T, Barber RM, Bell B, Bertozzi-Villa A, Biryukov S, Bolliger I, et al. Global, regional, and national incidence, prevalence, and years lived with disability for 301 acute and chronic diseases and injuries in 188 countries, 1990-2013: A systematic analysis for the Global Burden of Disease Study 2013. Lancet. 2015;386(9995):743–800 Available from: http://www.ncbi.nlm.nih.gov/pubmed/26063472. [cited 2019 may 21].
2. Pittenger C, Duman RS. Stress, depression, and neuroplasticity: a convergence of mechanisms. Neuropsychopharmacology. 2008;33(1):88–109 Epub 2007 Sep 12. Review. PubMed PMID: 17851537.

3. Krishnan V, Nestler EJ. Linking molecules to mood: new insight into the biology of depression. Am J Psychiatry. 2010;167(11):1305–20. https://doi.org/10.1176/appi.ajp.2009.10030434 Epub 2010 Sep 15. Review. PubMed PMID: 20843874; PubMed Central PMCID: PMC3031089.
4. Angst J, Azorin JM, Bowden CL, Perugi G, Vieta E, Gamma A, Young AH, BRIDGE Study Group. Prevalence and characteristics of undiagnosed bipolar disorders in patients with a major depressive episode: the BRIDGE study. Arch Gen Psychiatry. 2011;68(8):791–8. https://doi.org/10.1001/archgenpsychiatry.2011.87 PubMed PMID: 21810644.
5. Shefer G, Henderson C, Howard LM, Murray J, Thornicroft G. Diagnostic overshadowing and other challenges involved in the diagnostic process of patients with mental illness who present in emergency departments with physical symptoms--a qualitative study. PLoS One. 2014;9(11):e111682. https://doi.org/10.1371/journal.pone.0111682 eCollection 2014. PubMed PMID: 25369130; PubMed Central PMCID: PMC4219761.
6. McClung CA. How might circadian rhythms control mood? Let me count the ways. Biol Psychiatry. 2013;74(4):242–9. https://doi.org/10.1016/j.biopsych.2013.02.019 Epub 2013 Apr 1. Review. PubMed PMID: 23558300; PubMed Central PMCID: PMC3725187.
7. Slyepchenko A, Allega OR, Leng X, Minuzzi L, Eltayebani MM, Skelly M, Sassi RB, Soares CN, Kennedy SH, Frey BN. Association of functioning and quality of life with objective and subjective measures of sleep and biological rhythms in major depressive and bipolar disorder. Aust N Z J Psychiatry. 2019;53(7):683–96. https://doi.org/10.1177/0004867419829228 Epub 2019 Feb 13. PubMed PMID: 30759998.
8. Ávila Moraes C, Cambras T, Diez-Noguera A, Schimitt R, Dantas G, Levandovski R, Hidalgo MP. A new chronobiological approach to discriminate between acute and chronic depression using peripheral temperature, rest-activity, and light exposure parameters. BMC Psychiatry. 2013;13:77. https://doi.org/10.1186/1471-244X-13-77 PubMed PMID: 23510455; PubMed Central PMCID: PMC3599978.
9. Walker WH, Walton JC, DeVries AC, et al. Circadian rhythm disruption and mental health. Transl Psychiatry. 2020;10:28. https://doi.org/10.1038/s41398-020-0694-0.
10. Charrier A, Olliac B, Roubertoux P, Tordjman S. Clock genes and altered sleep–wake rhythms: Their role in the development of psychiatric disorders. Int J Mol Sci. 2017;18(5):938 Available from: http://www.ncbi.nlm.nih.gov/pubmed/28468274. [cited 2019 May 21].
11. Geoffroy PA, Micoulaud Franchi JA, Lopez R, Poirot I, Brion A, Royant-Parola S, et al. Comment caractériser et traiter les plaintes de sommeil dans les troubles bipolaires ? Encephale. 2017;43(4):363–73 Available from: https://linkinghub.elsevier.com/retrieve/pii/S0013700616301804. [cited 2019 May 21].
12. Hertenstein E, Feige B, Gmeiner T, Kienzler C, Spiegelhalder K, Johann A, et al. Insomnia as a predictor of mental disorders: A systematic review and meta-analysis. Sleep Med Rev. 2019;43:96–105 Available from: https://linkinghub.elsevier.com/retrieve/pii/S1087079218301138. [cited 2019 May 21].
13. Boivin DB. Influence of sleep-wake and circadian rhythm disturbances in psychiatric disorders. J Psychiatry Neurosci. 2000;25(5):446–58 Available from: http://www.ncbi.nlm.nih.gov/pubmed/11109296. [cited 2019 may 21].
14. Proudfoot J, Whitton A, Parker G, Doran J, Manicavasagar V, Delmas K. Triggers of mania and depression in young adults with bipolar disorder. J Affect Disord. 2012;143(1–3):196–202 Available from: http://www.ncbi.nlm.nih.gov/pubmed/22884233. [cited 2019 may 21].
15. Tonon AC, Fuchs DFP, Barbosa Gomes W, Levandovski R, Pio de Almeida Fleck M, MPL H, et al. Nocturnal motor activity and light exposure: Objective actigraphy-based marks of melancholic and non-melancholic depressive disorder. Brief report. Psychiatry Res. 2017;258:587–90 Available from: https://linkinghub.elsevier.com/retrieve/pii/S016517811730608X. [cited 2019 May 21].
16. Krane-Gartiser K, Vaaler AE, Fasmer OB, Sørensen K, Morken G, Scott J. Variability of activity patterns across mood disorders and time of day. BMC Psychiatry. 2017;17(1):404.
17. McGowan NM, Goodwin GM, Bilderbeck AC, Saunders KEA. Actigraphic patterns, impulsivity and mood instability in bipolar disorder, borderline personality disorder and healthy controls. Acta Psychiatr Scand. 2020. https://doi.org/10.1111/acps.13148 [Epub ahead of print] PubMed PMID: 31916240.
18. Bottai T, Biloa-Tang M, Christophe S, Dupuy C, Jacquesy L, Kochman F, et al. Thérapie interpersonnelle et aménagement des rythmes sociaux (TIPARS) : du concept anglo-saxon l'expérience franaise. Encephale. 2010;36(SUPPL. 6):S206–17 Available from: http://www.ncbi.nlm.nih.gov/pubmed/21237358. [cited 2019 May 21].
19. Haynes PL, Gengler D, Kelly M. Social Rhythm Therapies for Mood Disorders: an Update. Curr Psychiatry Rep. 2016;18(8):75 Available from: http://link.springer.com/10.1007/s11920-016-0712-3. [cited 2019 May 21].
20. Pail G, Huf W, Pjrek E, Winkler D, Willeit M, Praschak-Rieder N, et al. Bright-light therapy in the treatment of mood disorders. Neuropsychobiology. 2011;64(3):152–62 Available from: http://www.ncbi.nlm.nih.gov/pubmed/21811085. [cited 2019 may 21].
21. De Souza CM, Carissimi A, Costa D, Francisco AP, Medeiros MS, Ilgenfritz CA, et al. The mood rhythm instrument: Development and preliminary report. Rev Bras Psiquiatr. 2016;38(2):148–53 Available from: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1516-44462016000200148&lng=en&tlng=en. [cited 2019 May 21].
22. Francisco AP, de Oliveira MAB, Carissimi A, Fabris RC, Ilgenfritz CAV, de Souza CM, et al. Spanish Translation of the Mood Rhythm Instrument: a Novel Approach To Mood Evaluation. Clin Biomed Res. 2017;37(1):41–7 Available from: doi: 10.4322/2357-9730.69877. [cited 2019 May 21].
23. Carissimi A, Oliveira MAB, Frey BN, Navarro JF, Hidalgo MP, Adan A. Validation and psychometric properties of the Spanish mood rhythm instrument. Biol Rhythm Res. 2019. https://doi.org/10.1080/09291016.2019.1675023.
24. Pilz LK, Carissimi A, Oliveira MAB, Francisco AP, Fabris RC, Medeiros MS, et al. Rhythmicity of Mood Symptoms in Individuals at Risk for Psychiatric Disorders. Sci Rep. 2018;8(1):11402 Available from: http://www.ncbi.nlm.nih.gov/pubmed/30061722. [cited 2019 May 21].
25. Pilz LK, Carissimi A, Francisco AP, Oliveira MAB, Slyepchenko A, Epifano K, et al. Prospective assessment of daily patterns of mood-related symptoms. Front Psychiatry. 2018;9 (AUG). Available from: https://www.frontiersin.org/article/10.3389/fpsyt.2018.00370/full. [cited 2019 May 21].
26. Mistlberger RE, Skene DJ. Social influences on mammalian circadian rhythms: animal and human studies. Biol Rev Camb Philos Soc. 2004;79(3):533–56 Available from: http://www.ncbi.nlm.nih.gov/pubmed/15366762. [cited 2019 may 21].
27. Gradisar M, Gardner G, Dohnt H. Recent worldwide sleep patterns and problems during adolescence: A review and meta-analysis of age, region, and sleep. Sleep Med. 2011;12(2):110–8 Available from: https://linkinghub.elsevier.com/retrieve/pii/S1389945710004326. [cited 2019 May 21].
28. Soldatos CR, Allaert FA, Ohta T, Dikeos DG. How do individuals sleep around the world? Results from a single-day survey in ten countries. Sleep Med. 2005;6(1):5–13 Available from: https://linkinghub.elsevier.com/retrieve/pii/S1389945704001935. [cited 2019 May 21].
29. Smith MR, Burgess HJ, Fogg LF, Eastman CI. Racial differences in the human endogenous circadian period. Yamazaki S, editor. PLoS One. 2009;4(6):e6014 Available from: https://dx.plos.org/10.1371/journal.pone.0006014. [cited 2019 May 21].
30. Adan A, Almirall H. Horne & Östberg morningness-eveningness questionnaire: A reduced scale. Pers Individ Dif. 1991;12(3):241–53 Available from: https://www.sciencedirect.com/science/article/pii/019188699190110W. [cited 2019 May 21].
31. Danielsson K, Sakarya A, Jansson-Fröjmark M. The reduced Morningness-Eveningness questionnaire: psychometric properties and related factors in a young Swedish population. Chronobiol Int. 2019;36(4):530–40. https://doi.org/10.1080/07420528.2018.1564322 Epub 2019 Jan 7. PubMed PMID: 30614272.
32. Tavakol M, Dennick R. Making sense of Cronbach's alpha. Int J Med Educ. 2011;2:53–5 Available from: http://www.ncbi.nlm.nih.gov/pubmed/28029643. [cited 2019 May 21].
33. Bartholomew DJ, Steele F, Moustaki I, Galbraith J. Analysis of Multivariate Social Science Data, Second Edition. Int Stat Rev. 2008;76(3):456 Available from: http://doi.wiley.com/10.1111/j.1751-5823.2008.00062_21.x. [cited 2019 May 21].
34. Mislevy R. Recent developments in the factor analysis of categorical variables. J Educ Stat. 1986;11(1):3–31. https://doi.org/10.2307/1164846.
35. Bock RD, Gibbons R, Muraki E. Full-information item factor analysis. Appl Psychol Meas. 1988;12(3):261–80. https://doi.org/10.1177/014662168801200305.
36. Finch WH. A Comparison of Factor Rotation Methods for Dichotomous Data. J Mod Appl Stat Methods. 2011;10(2):Article 14. https://doi.org/10.22237/jmasm/1320120780.
37. Velicer WF. Determining the number of components from the matrix of partial correlations. Psychometrika. 1976;41(3):321–7 Available from: http://link.springer.com/10.1007/BF02293557. [cited 2019 May 21].
38. Horn JL. A rationale and test for the number of factors in factor analysis. Psychometrika. 1965;30(2):179–85 Available from: http://link.springer.com/10.1007/BF02289447. [cited 2019 May 21].

39. Ruscio J, Roche B. Determining the number of factors to retain in an exploratory factor analysis using comparison data of known factorial structure. Psychol Assess. 2012;24(2):282–92 Available from: http://www.ncbi.nlm.nih.gov/pubmed/21966933. [cited 2019 May 21].
40. Brown TA. Methodology in the social sciences. Confirmatory factor analysis for applied research. 2nd ed. New York: The Guilford press; 2015.
41. Mardia KV, Jupp PE. Directional Statistics. Hoboken: Wiley; 1999. (Wiley Series in Probability and Statistics). Available from: http://doi.wiley.com/10.1002/9780470316979. [cited 2019 May 21].
42. Mardia KV. Statistics of directional data. J R Stat Soc Ser B Methodol. 1975; 37:349–93.
43. Nias DKB. The handbook of psychological testing. Vol. 20, personality and individual differences. Florence: Taylor & Frances/Routledge; 2003. p. 128.
44. Kuehner C. Why is depression more common among women than among men? Lancet Psychiatry. 2017;4(2):146–58.
45. Lim GY, Tam WW, Lu Y, Ho CS, Zhang MW, Ho RC. Prevalence of Depression in the Community from 30 Countries between 1994 and 2014 /692/699/476/1414 /692/499 article. Sci Rep. 2018;8(1):2861 Available from: http://www.nature.com/articles/s41598-018-21243-x. [cited 2019 May 21].
46. Sullivan L, Camic PM, JSL B. Masculinity, alexithymia, and fear of intimacy as predictors of UK men's attitudes towards seeking professional psychological help. Br J Health Psychol. 2015;20(1):194–211 Available from: http://doi.wiley.com/10.1111/bjhp.12089. [cited 2019 May 21].
47. Aledavood T, López E, Roberts SGB, Reed-Tsochas F, Moro E, Dunbar RIM, et al. Daily rhythms in mobile telephone communication. Lambiotte R, editor. PLoS One. 2015;10(9):e0138098 Available from: https://dx.plos.org/10.1371/journal.pone.0138098. [cited 2019 May 21].
48. Ryan RM. Intrinsic motivation and exercise adherence. Int J Sport Psychol. 1997;28(4):335–54 Available from: https://www.researchgate.net/publication/279910235_Intrinsic_Motivation_and_Exercise_Adherence.
49. Jankowski KS, Díaz-Morales JF, Randler C. Chronotype, gender, and time for sex. Chronobiol Int. 2014;31(8):911–6 Available from: http://www.ncbi.nlm.nih.gov/pubmed/24927370. [cited 2019 May 21].
50. Scheer FAJL, Morris CJ, Shea SA. The internal circadian clock increases hunger and appetite in the evening independent of food intake and other behaviors. Obesity. 2013;21(3):421–3 Available from: http://doi.wiley.com/10.1002/oby.20351. [cited 2019 May 21].
51. Campbell SS, Murphy PJ. The nature of spontaneous sleep across adulthood. J Sleep Res. 2007;16(1):24–32 Available from: http://doi.wiley.com/10.1111/j.1365-2869.2007.00567.x. [cited 2019 May 21].
52. Adan A, Archer SN, Hidalgo MP, Di Milia L, Natale V, Randler C. Circadian typology: A comprehensive review. Chronobiol Int. 2012;29(9):1153–75 Available from: http://www.tandfonline.com/doi/full/10.3109/07420528.2012.719971. [cited 2019 May 21].
53. Matchock RL, Toby Mordkoff J. Chronotype and time-of-day influences on the alerting, orienting, and executive components of attention. Exp Brain Res. 2009;192(2):189–98 Available from: http://link.springer.com/10.1007/s00221-008-1567-6. [cited 2019 May 21].
54. Schmidt C, Collette F, Reichert CF, Maire M, Vandewalle G, Peigneux P, et al. Pushing the limits: Chronotype and time of day modulate working memory-dependent cerebral activity. Front Neurol. 2015;6(SEP):199 Available from: http://journal.frontiersin.org/Article/10.3389/fneur.2015.00199/abstract. [cited 2019 May 21].
55. Wieth MB, Zacks RT. Time of day effects on problem solving: when the non-optimal is optimal. Think Reason. 2011;17(4):387–401.
56. Wright KP, Hull JT, Czeisler CA. Relationship between alertness, performance, and body temperature in humans. Am J Physiol Integr Comp Physiol. 2015; 283(6):R1370–7 Available from: http://www.physiology.org/doi/10.1152/ajpregu.00205.2002. [cited 2019 May 21].
57. Lack L, Bailey M, Lovato N, Wright H. Chronotype differences in circadian rhythms of temperature, melatonin, and sleepiness as measured in a modified constant routine protocol. Nat Sci Sleep. 2009;1:1–8 Available from: http://www.ncbi.nlm.nih.gov/pubmed/23616692%0A. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC3630920. [cited 2019 May 21].

Publisher's Note

2.3 MRhI VERSÃO REVISADA

Artigo publicado na revista *Journal do Clinical Medicine* (IF 4.96; Qualis A2 área Medicina II).

DOI: 10.3390/jcm10030388

Title: The Revised Mood Rhythm Instrument: A Large Multicultural Psychometric Study

Melissa A. B. Oliveira[1, 2], Euclides José de Mendonça Filho[3], Alicia Carissimi[1], Luciene L. S. Garay[1,2], Marina Scop[1], Denise Ruschel Bandeira[3], Felipe Gutiérrez Carvalho[1,2], Salina Mathur[4], Kristina Epifano[5], Ana Adan[6,7, +], Benicio N. Frey [8,9, +] and Maria Paz Hidalgo[1, 2, +, *]

[1] Laboratório de Cronobiologia e Sono do Hospital de Clínicas de Porto Alegre, Universidade Federal do Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil;

[2] Graduate Program in Psychiatry and Behavioral Sciences, Faculty of Medicine, Universidade Federal do Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil;

[3] Graduate Program in Psychology, Universidade Federal do Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil;

[4] Department of Occupational Science and Occupational Therapy, University of Toronto, Mississauga, ON, Canada;

[5] Department of Psychology, Neuroscience & Behaviour, McMaster University, Hamilton, ON, Canada;

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

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

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

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

+ Contributed equally as senior authors.

***** Correspondence: Maria Paz Hidalgo, Phone: +5551 3359-6339; email: labcronoesono@hcpa.edu.br.

Abstract

Background: Recent studies with the Mood Rhythm Instrument (MRhI) have shown that the presence of recurrent daily peaks in specific mood symptoms are significantly associated with increased risk of psychiatric disorders. Using a large sample collected in Brazil, Spain and Canada, we aimed to analyze which MRhI items maintained good psychometric properties across cultures. As a secondary aim, we used network analysis to visualize the strength of the association between the MRhI items. **Methods:** Adults (n= 1275) between 18-60 years old from Spain (n= 458), Brazil (n= 415) and Canada (n= 401) completed the MRhI and the Self-Reporting Questionnaire (SRQ-20). Psychometric analyses followed three steps: factor analysis, item response theory and network analysis. **Results:** The factor analysis indicated the retention of three factors that grouped the MRhI items into cognitive, somatic and affective domains. The item response theory analysis suggested the exclusion of items that displayed a significant divergence in difficulty measures between countries. Finally, the network analysis revealed a structure where sleepiness plays a central role in connecting the three domains. These psychometric analyses enabled a psychometric-based refinement of the MRhI, where the 11 items with good properties across cultures were kept in a shorter, revised MRhI version (MRhI-r). **Limitations:** Participants were mainly university students and, as we did not conduct a formal clinical assessment, any potential correlations (beyond the validated SRQ) cannot be ascertained. **Conclusions:** The MRhI-r is a novel tool to investigate self-perceived rhythmicity of mood-related symptoms and behaviours, with good psychometric properties across multiple cultures.

Keywords: mood symptoms; depressive symptoms; circadian rhythms; mood disorders; network analysis

1.Introduction

Several lines of research highlight the presence of alterations in circadian rhythm and sleep regulation in psychiatric and neurocognitive disorders (Adan et al., 2012; Allega et al., 2018; Ávila Moraes et al., 2013; Logan and McClung, 2019; McClung, 2013; Wulff et al., 2010). Characterizing circadian functioning may optimize the management of mood disorders and promote preventive strategies in those who are at risk of developing mental disorders (Hickie et al., 2013; Hühne et al., 2018; Krawczak et al., 2016; McCarthy et al., 2019; Tonon et al., 2017). Biological rhythms are regulated by endogenous networks of gene activity and can be modulated by changes in the environment. Proper synchronization between light, the circadian clock and output behaviours is essential for survival (Logan and McClung, 2019). Irregular exposure to light – by means of light pollution, lack of natural light during the day, night shift work, easy access to electronic devices –, can disrupt circadian rhythms and sleep. Eventually, these unhealthy behaviors can lead to depressed mood (Bedrosian and Nelson, 2013; Karatsoreos and McEwen, 2011).

Given the strong link between disturbances in biological rhythms and mood-related symptoms (McClung, 2013), it is important to better understand the symptoms and the phenotype of psychiatric disorders considering the temporal context of their clinical symptoms. Therefore, clinical assessment tools to evaluate the daily variability of mood are needed. We developed the Mood Rhythm Instrument (MRhI), a 15-item self-reported questionnaire that assesses self-perceived rhythmicity of somatic, cognitive and affective symptoms, to measure the rhythmicity of mood symptoms within the 24-hour cycle (de Souza et al., 2016).

The MRhI was initially created in Brazilian Portuguese (de Souza et al., 2016) and was subsequently translated and validated in Spanish (Carissimi et al., 2019; Francisco et al., 2017) and English (Oliveira MAB et al., 2020) languages. Further investigation of this instrument revealed that it is not affected by recency or recall biases and it is a valid tool to investigate daily patterns of mood symptoms over 24 hours (Pilz et al., 2018a). Moreover, recent studies with the MRhI have shown that the presence of recurrent daily peaks in specific items are significantly associated with increased risk of psychiatric disorders, evaluated by Self-Reporting Questionnaire-20 (Pilz et al., 2018b). Another study showed that mood-related symptoms in individuals with depressive symptoms tend to peak more frequently in the evening (Pereira-Morales et al., 2019).

The main objective of the present study was to use a large dataset collected in Brazil, Spain and Canada to provide complementary sources of validity evidence. Thus, we examined the MRhI's factor structure, internal consistency, item fit to the measurement model and invariance in relation to

participants' country of origin. As secondary aims, (1) network analyses were used to visualize the strength of the association of the rhythmicity of mood-related symptoms and behaviors; and (2) we investigated the association between MRhI-r and the Self-Reporting Questionnaire-20.

2.Methods

2.1. Sample characterization

The study sample (n=1275) was composed of 458 (35.9%) Spanish, 415 (32.5%) Brazilian, and 401 (31.4%) Canadian responders between 18 and 60 years old. Participants were recruited through snowball or convenience sampling, poster advertisements and online research recruitment. All study participants provided written informed consent before study entry. The study was approved by the University of Barcelona (#IRB00003099), Ethics Committee of Hospital de Clínicas de Porto Alegre (#15-0539 GPPG/HCPA) and Hamilton Integrated Research Ethics Board (#2015-0619), and was conducted in accordance with the Declaration of Helsinki.

2.2. Mood Rhythm Instrument (MRhI)

The Spanish, Brazilian and Canadian participants were requested to complete the MRhI. The MRhI questionnaire is composed of 15 self-reported items that are grouped into three domains: cognitive, somatic and affective. Each item provides a categorical question (yes/no) assessing the presence or absence of a daily peak (e.g. "Is there a specific time of the day when you have felt more sad?"). If the participants answer "yes", they indicate on a visual analog scale (VAS) the peak time within a 24-hour period (time variable, e.g. "If you answer yes, indicate below the approximate hour"). The sum of the categorical variables provides a total score, which ranges from 0 to 15, with 0 being the lowest and 15 the highest perceived rhythmicity. In short, individuals answered if there was a specific time of the day when they perceived a variety of mood-related symptoms in the last 15 days.

2.3. Self-Reporting Questionnaire (SRQ-20)

The SRQ-20 consists of 20 self-reported items to screen for non-psychotic psychiatric disorders. Items have a categorical (yes/no) answer format, representing the presence or absence of a symptom. The validity, reliability and cut-off of the SRQ-20 vary in different settings across a variety of populations (Beusenberg et al., 1994; Cherian et al., 1998; Iacoponi and Mari, 1989; van der Westhuizen et al., 2016). In this study, we used the validated Brazilian Portuguese and Spanish versions and their corresponding validated screening cut-offs to detect psychiatric disorders. In the Canadian sample, the standard cut-off was applied for the English version following the developer's suggestion

(Upadhyaya et al., 1990). Thus, scores higher than 7 were considered SRQ positive in Brazil and Canada, while scores higher than 3 were considered SRQ positive in Spain, meaning high risk for common mental disorders (Gonçalves et al., 2008; Livianos Aldana et al., 1990).

2.4. Data analysis procedures

Psychometric analysis of the MRhI followed three steps: factor analysis, item response theory (IRT; Rasch Analysis) and network analysis. First, we investigated the factorial structure of the inventory using exploratory factor analysis (Izquierdo et al., 2014). At this stage, we considered the entire sample across all countries. The polychoric correlation matrix of the data was submitted to robust weighted least squares (WLS) estimation method with GEOMIN oblique rotation in order to obtain results representative of the general population and appropriate correlation from categorical dichotomous variables (Muthén and Muthén, 2010). The number of extracted factors were determined using the scree plot criteria (Cattell, 1966) and Horn's parallel analysis (Horn, 1965). Scree plot often suggests a low number of factors and the Horn's parallel analysis suggests a large number of factors that might overfit the model. Therefore, we evaluated the fit of a variety of dimensional models considering the following: (1) Comparative Fit Index and Tucker-Lewis Index (TLI) > 0.9 and (2) root mean square error of approximation (RMSEA) and standardized root mean square residual (SRMR) < 0.08 (Beaujean, 2014), provided by the MPLUS package (Muthén and Muthén, 2010). Other fit indexes such as Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC) were used to assess factorial models - smaller values indicating better fit (Hu and Bentler, 1999).

Next, each dimension was analyzed separately using IRT via Rasch modeling. This framework allows for comparing participants' parameters in relation to item properties. The IRT enables the comparison between items and people's mood perception levels along a latent continuum (Bond and Fox, 2015). Furthermore, it is possible to assess the fit of each item to the measurement model using infit mean-square statistics. This index indicates the discrepancy between the predicted patterns of response for a given item against the observed pattern. Since this model has an approximate chi-square distribution, it was possible to determine cut off values that indicate item misfit. According to Linacre, items with infit close to one are considered perfectly fitting, items with "values above 2 distort or degrade the measurement system, and items with values between 1.5 and 2 are unproductive for measurement development, but not degrading" (Linacre, 2002). Since infit problems are more problematic for measurement than the outfit, items with infit above 1.5 or below 0.5, or outfit above 2.0, were deemed as not contributing adequately to the revised scale (Bond and Fox, 2015; Linacre, 2002). Subsequently, Rasch analysis was used to identify items with differential functioning (DIF) as a function of participants' country. This analysis allows verifying if the scales are invariant across

nationalities. Following Boone et al. (2014) recommendations, an item difficulty contrast between the two investigated groups larger than |0.64| logits were considered evidence of a large DIF effect size.

Thus, items with DIF values > |0.64| or a significant Welch test adjusted for multiple comparisons were flagged as differential functioning items. It should be highlighted that an item with DIF does not necessarily need to be removed or represents a problematic item (Boone et al., 2014). In this case, we followed the recommendation to assess the construct-content importance of the flagged to assure that its exclusion is not detrimental to the instrument. A team of experts composed by psychiatrists, psychologists, a biomedical professional and a medical doctor reviewed the results from the DIF and consensually agreed on the items that were redundant and could be deleted and the items that were kept in the MRhI-r.

Finally, network analysis was used to visualize the strength of the association of the MRhI items. More specifically, the nodes of the networks consist of “mood symptoms” and the edges are “strength of the association between the symptoms”. Gray lines in the network indicate positive partial or bivariate correlations, and the wider and more saturated the line, the stronger the correlation (Epskamp et al., 2018). After item removal indicated by previous steps, a machine learning graph technique was used to visualize associations and patterns of MRhI data (Schmittmann et al., 2013). A graphical lasso algorithm was applied to make the network “parsimonious” and avoid the estimation of false-positive edges (Friedman et al., 2008). To assess the importance of nodes in the network, we computed the node strength, which is a common metric to evaluate centrality indices of a network structure and is defined as the sum of all associations a given symptom displays with all other nodes. We also investigated the quality of the network by calculating stability of centrality estimates and analyzing the accuracy of edge-weights using bootstrapping routines according to Epskamp, Borsboom, & Fried, 2018 (Epskamp et al., 2018).

The SRQ-20 was used in our study also as a tool for convergent validity. The correlation between the MRhI-r sum and SRQ-20 total scores was tested using Spearman's correlation coefficient according to country and MRhI-r domains. Only participants who completed both questionnaires were included in this analysis (n= 1195).

Data were analyzed in R Studio (R Core Team, 2017). Functions implemented by the package *psych* (Revelle, 2019) were used to determine the number of factors; the package *qgraph* (Epskamp et al., 2015) enabled the network estimation and the package *bootnet* the bootstrapping (Epskamp et al., 2018). The factorial structure of MRhI was further investigated in *Mplus* and Winsteps was used for Rasch and DIF analyzes. Correlation graphs were plotted using the R package ggplot2 (Wickham et al., 2020).

3. Results

3.1. Factor analysis

The inspection of the scree plot visualization indicated the retention of three factors, computation of eigenvalues of the tetrachoric correlation matrix suggested four factors with eigenvalues greater than one, and the parallel analysis determined five factors (Table 1 and Supplementary tables 1 to 3). Given that the considered criteria did not agree and the fact that parallel analyses often suggest too many factors, three to five factors were extracted, and the resulting matrices of factor loadings were inspected. The four-factor model added only 7.1% of explained shared variance compared to the three-factor model, and the five-factor model added only 5.1% of explained shared variance compared to the four-factor model. Therefore, we opted to retain three factors for subsequent analysis since it presented adequate fit indexes – χ^2 (63) = 115.4, $p < 0.001$; CFI = 0.98; TLI = 0.97; SRMR = 0.04 and RMSEA = 0.02 – explaining 53.3% of items' shared variance. More importantly, the three-factor model presented the highest content validity with regards to their meaning when grouped in each factor, thus proving to be the most interpretable in all solutions examined. The factor that explains most of the variance refers to the cognitive domain, followed by a factor containing somatic items, and lastly, the factor encompassing the affective domain.

The item *physical exercise* showed significant values in all three factors, despite a high percentage of variance not explained by the three-factor model (79%; Table 1). Due to its multidimensionality, this item was, therefore, excluded. Moreover, because *talking to friends* presented significant values in two of three factors and did not seem to agree in terms of meaning/construct to the other items in the same factor, this item was also excluded.

Table 1. Factor analysis considering the whole sample.

	Cognitive	Somatic	Affective	*U*
Q5 Concentration	0.83*	-0.02	-0.03	0.34
Q1 Alertness	0.64*	-0.05	0.03	0.60
Q15 Energy	0.59*	0.02	0.13	0.55
Q3 Problem-solving	0.53*	0.22*	0.01	0.54
Q12 Memory	0.45*	0.21*	0.04	0.62
Q11 Physical Exercise	0.28*	0.28*	-0.26*	0.79
Q4 Self-esteem	-0.01	0.57*	0.29	0.48
Q7 Sexual Arousal	-0.07	0.56*	0.10	0.67
Q2 Sleepiness	0.25	0.49*	-0.03	0.58
Q6 Appetite	0.07	0.37*	0.08	0.79
Q14 Talking to Friends	0.06	0.31*	0.21*	0.78
Q13 Pessimism	0.01	-0.06	0.87*	0.27
Q10 Sadness	-0.05	0.01	0.83*	0.32
Q9 Anxiety	0.10	0.01	0.62*	0.55
Q8 Irritability	0.06	0.22*	0.44*	0.65
Eigenvalues	*4.90*	*1.89*	*1.19*	
% variance	*32.7*	*12.7*	*8.0*	

*$p < 0.05$

3.2. Rasch Analysis

IRT analysis using the Rasch modeling indicated that all the MRhI items were adequate, relative to the measuring model, presenting infit values within an appropriate range. The cognitive items presented a reliability of 0.9 and a separation reliability index of 13.5, with a mean infit of 0.99 (SD = 0.12), the somatic items presented a reliability of 0.99, a separation reliability of 20.2 and a mean infit of 0.98 (SD = 0.07), and the affective items presented a reliability of 0.89, a separation reliability of 9.8 and a mean infit of 1.00 (SD = 0.13).

Six items exhibited considerable DIF based on their respective country of data collection (Table 2). *Alertness*, *energy, memory, irritability* and *self-esteem* yielded higher tendency to be percieved as rhythmic by Spain responses than in Brazil and Canada. The item *sexual arousal* demonstrated higher tendency to have a daily peak in Spain in relation to Canada. Although the items *memory* and *irritability* did not perform well in the difficulty parameters analysis, these items displayed good factor load (0.45

and 0.44) and location in the cognitive and affective domains, respectively. Also, *memory* and *irritability* are key clinical features of several psychiatric disorders (e.g. memory/cognitive impairment, mixed states/mixed features); therefore, these two items were kept. Based on the clinical importance of *energy* in improving the detection and accuracy of bipolar disorder diagnosis (Yatham et al., 2018), this item was kept in the final version due to its clinical relevance. Figure 1 shows the process of the reduction of the number of items from MRhI to MRhI-r and Table 3 shows the final Rasch analysis. The final version of the MRhI-r is available in the Supplemental Materials.

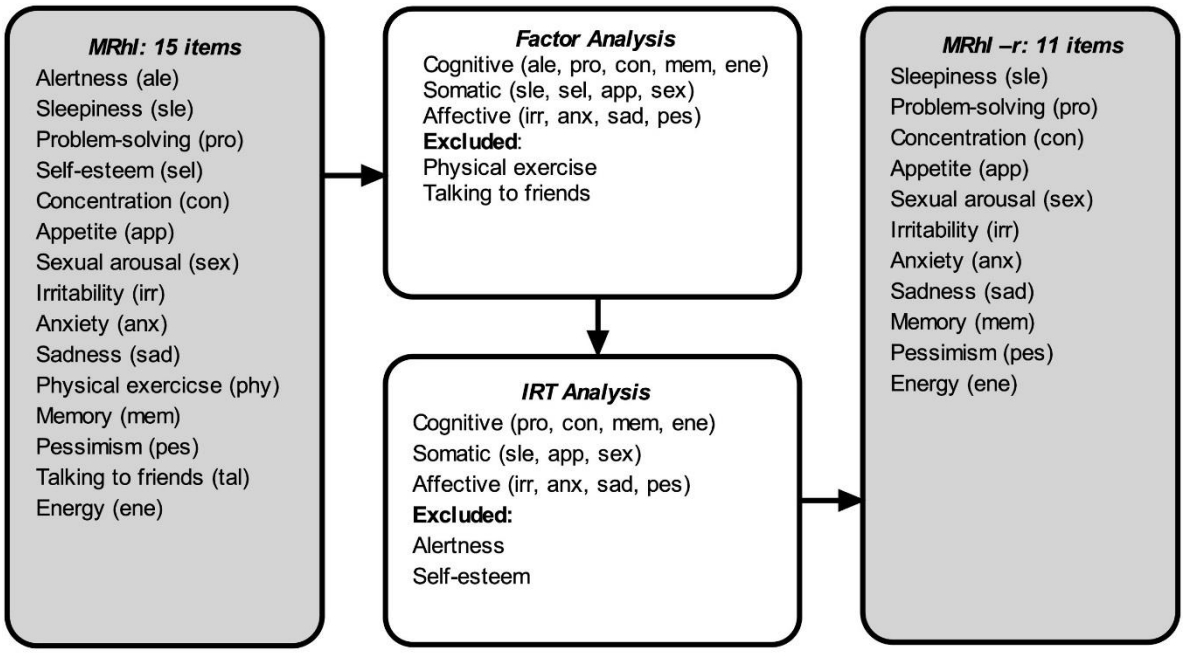


Fig. 1. Flowchart showing the various steps in the development of the MRhI-r.

Table 2. Item difficulty, fit measures and differential item functioning of subscales (MRhI - 13 items).

				Difficulty measure			Differential Item functioning contrast		
		Difficulty	Infit	SP	BR	CA	SP-BR	SP-CA	BR-CA
Cognitive	Memory	2.10	1.02	1.48	2.45	2.55	-0.97*	-1.07*	-0.10
	Concentration	-1.33	0.84	-1.67	-1.33	-1.01	-0.34	-0.66	-0.32
	Alertness	-0.64	1.00	-0.02	-1.39	-0.90	1.37*	0.88*	-0.49
	Energy	-0.16	0.94	0.71	-0.72	-0.83	1.43*	1.54*	0.11
	Problem-solving	0.04	0.91	-0.10	0.34	-0.10	-0.44	0.00	0.44
Somatic	Sleepiness	-3.97	1.02	-3.71	-3.60	-4.39	-0.11	0.68	0.79
	Self-esteem	2.75	0.99	3.35	2.53	2.30	0.82*	1.05*	0.23
	Sexual Arousal	2.46	0.94	2.12	2.59	2.81	-0.47	-0.69*	-0.22
	Appetite	-1.25	0.96	-1.63	-1.20	-0.98	-0.42	-0.64	-0.22
Affective	Irritability	-1.65	1.16	-2.16	-1.35	-1.39	-0.81*	-0.77*	0.04
	Pessimism	1.00	0.88	0.80	1.14	1.08	-0.34	-0.28	0.06
	Sadness	0.57	0.91	0.88	0.50	0.32	0.38	0.56	0.18
	Anxiety	0.08	1.02	0.40	-0.22	0.03	0.62	0.37	-0.25

* Welch significance test, $p < 0.016$. SP: Spain, BR: Brazil, CA: Canada.

Table 3. Item difficulty, fit measures and differential item functioning of subscales (MRhI - 11 items).

				Difficulty measure			Differential Item functioning contrast		
		Difficulty	Infit	SP	BR	CA	SP-BR	SP-CA	BR-CA
Cognitive	Memory	2.45	1.09	1.60	2.78	3.28	-1.18*	-1.68*	-0.51
	Concentration	-1.87	0.97	-2.31	-1.87	-1.43	-0.44	-0.88*	-0.45
	Energy	-0.40	1.00	0.61	-1.12	-1.19	1.73*	1.79*	0.06
	Problem-solving	-0.17	0.91	-0.36	0.17	-0.27	-0.53	-0.09	0.44
Somatic	Sleepiness	-3.57	1.05	-3.19	-3.26	-3.96	0.08	0.77	0.69
	Sexual Arousal	4.04	1.00	4.04	3.98	4.14	0.07	-0.09	-0.16
	Appetite	-0.47	0.88	-0.57	-0.52	-0.36	-0.04	-0.21	-0.16
Affective	Irritability	-1.65	1.16	-2.16	-1.35	-1.39	-0.81*	-0.77*	0.04
	Pessimism	1.00	0.88	0.80	1.14	1.08	-0.34	-0.28	0.06
	Sadness	0.57	0.91	0.80	0.50	0.32	0.38	0.56	0.18
	Anxiety	0.08	1.02	0.40	-0.22	0.03	0.62	0.37	-0.25

* Welch significance test, $p < 0.016$. SP: Spain, BR: Brazil, CA: Canada.

3.3. Network analysis

Stability estimates confirm the quality of the MRhI-r network. It displayed satisfactory accuracy indicated by small confidence intervals around the edge weights and stable strength centrality with a *CS-coefficient* of 0.28 (Figure S1).

The intercorrelations between all MRhI items are shown through its bivariate and regularized regressions correlations (Table S4). Consistent with the factor analysis, the bivariate network illustrates how items clustered in dimensions of cognitive, somatic and affective mood-related symptoms form the MRhI hypothetical structure. After controlling for the mutual effects using the lasso algorithm, *sleepiness* plays a central role in connecting the cognitive, somatic and affective dimensions (Figure 2a).

Node strength quantifies how well a node is directly connected to others. For this reason, being the most central node, *sleepiness* possesses the highest value, followed by *pessimism* and *concentration* (Figure 2b).

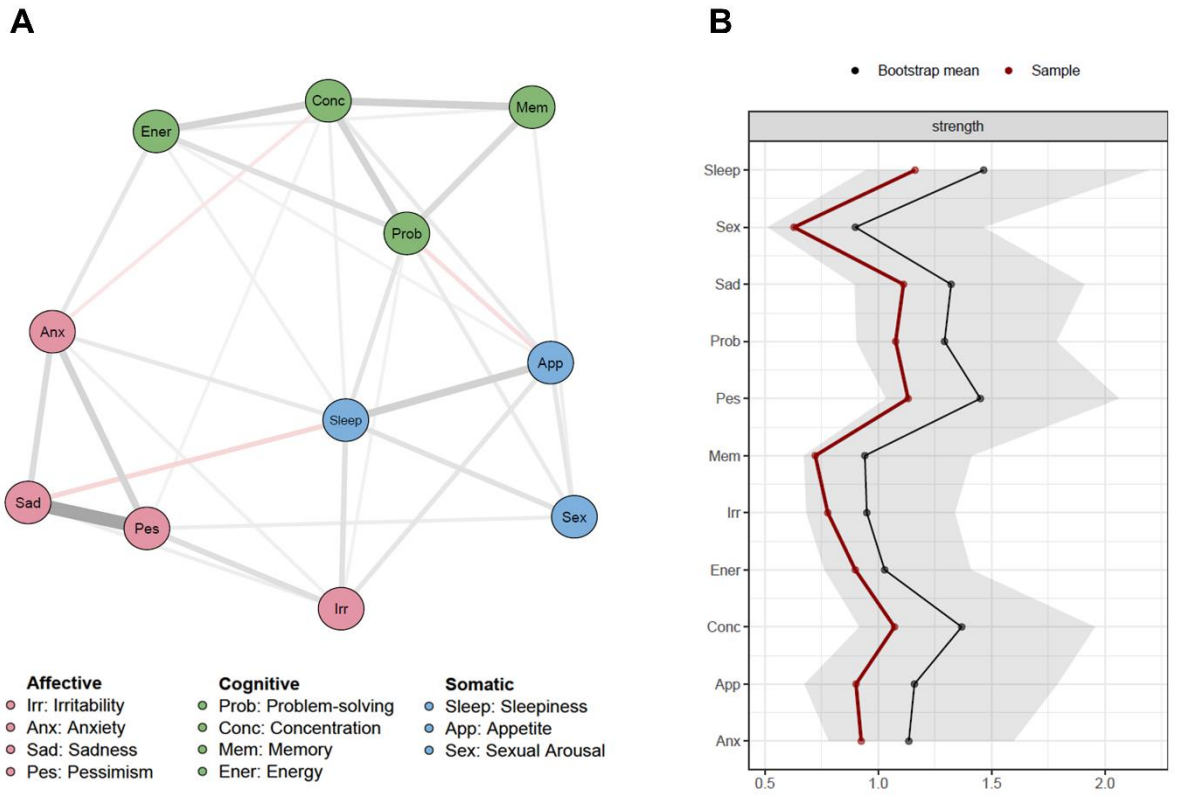


Fig. 2. MRhI-r network. A: Lasso (least absolute shrinkage and selection operator) correlations network containing the 11 items that compose the MRhI-r. Thicker lines represent stronger correlations. Gray lines stand for positive correlations and red lines for negatives correlations. B: Node strength estimates (n = 1275), including bootstrapped 95% confidence intervals.

3.4. Correlation with psychiatric disorders screening

The correlation between the total sum of MRhI-r dichotomous variables and SRQ-20 total scores showed that, independent from the country, the more individuals perceive the presence of daily peaks of mood symptoms, the higher the risk for psychiatric disorders (Figure 3), an association which is driven primarily by the affective dimension.

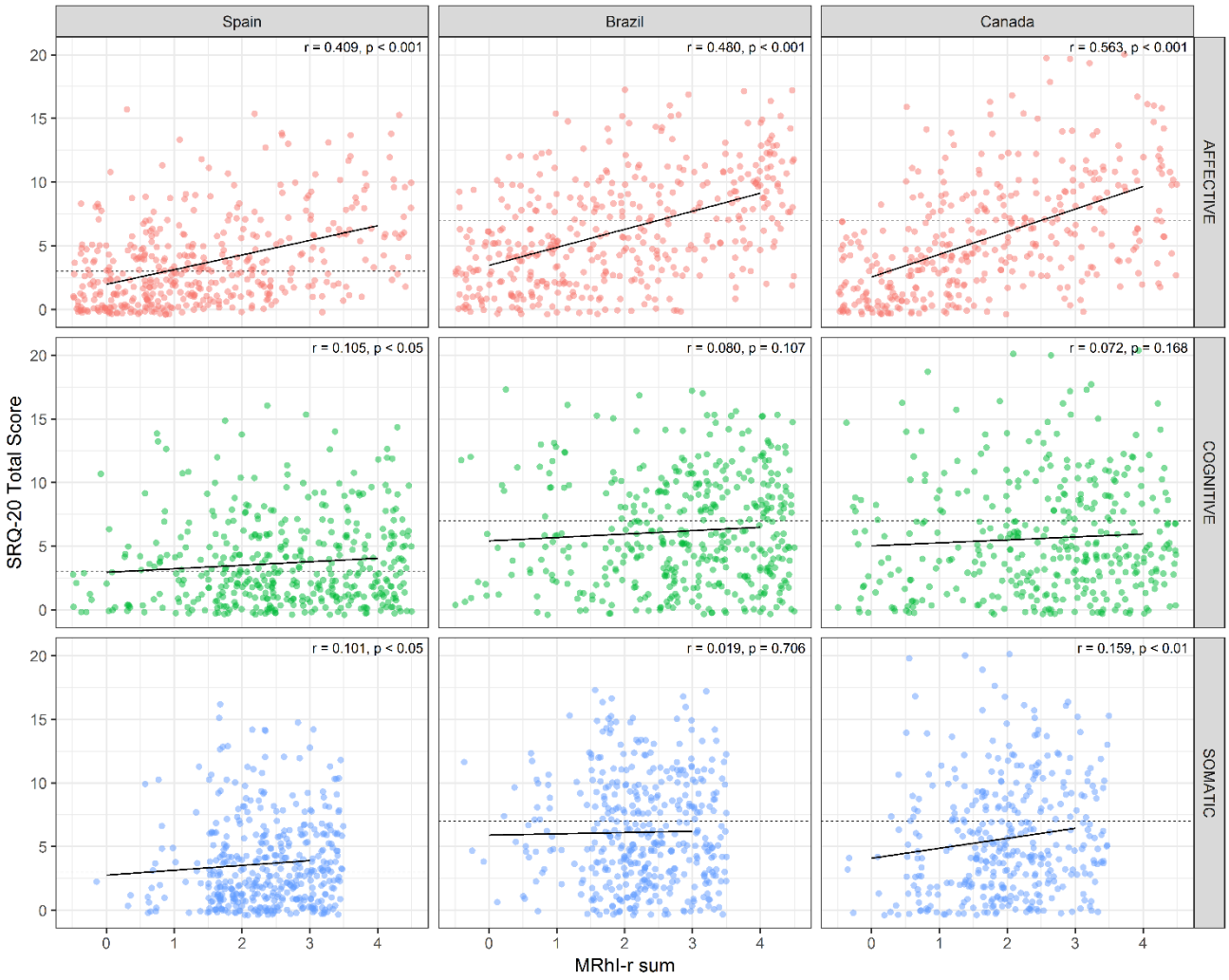


Fig. 3. Correlations between the total sum of MRhI-r dichotomous variables (MRhI-r sum) and SRQ-20 total scores (SRQ-20 score) separately for domain and country. SRQ cutoffs, which are distinct according to country, are displayed as dashed lines. Only data from participants that completed the entire SRQ were included (Spain-cognitive, n= 417; Spain-affective, n= 418; Spain-somatic, n= 419; Brazil-cognitive, n= 411; Brazil-affective, n= 412; Brazil-somatic, n= 412; Canada-cognitive, n= 367; Canada-affective, n= 367; Canada-somatic, n= 367). The significant correlations were in affective domais for all countries, in cognitive domain for Spain and somatic domain for Spain and Canada.

4. Discussion

In this large (N=1275) multicenter cross-cultural study, we used multiple psychometric analyses including the MRhI's factor structure, internal consistency, item fit to the measurement model and its invariance in relation to participants' country of origin. The factor analysis supported the retention of three factors, grouping the MRhI items into cognitive, somatic and affective domains. Results from factor analysis, item response theory analysis and clinical relevance were used to refine the MRhI into a more concise and psychometrically sound version, the MRhI-r. Finally, after controlling for the mutual effects, the network analysis showed a structure where *sleepiness* plays a central role in connecting the cognitive, somatic and affective dimensions.

In the development of clinical instruments measuring self-perceived outcomes that might be modulated by differences in local/cultural perspectives, it is critical to test its psychometric properties across different countries. For instance, we found in the IRT analyses that Brazilian and Canadian subjects had similar tendencies to endorse the MRhI items; in contrast, the Spanish population had a higher tendency to report daily peaks of memory, irritability and concentration, and a lower tendency to report daily peaks of energy. We have recently found that the self-perception of daily peaks of *pessimism* and *motivation to exercise* were associated with risk for psychiatric disorders in Spanish and Brazilian individuals (Pilz et al., 2018b). In the present study, when we examined this association in a larger sample across three culturally diverse countries using the revised version of the MRhI. We found that the association between self-perceived daily peaks of mood symptoms and risk of psychiatric disorders is maintained and is primarily driven by affective items like *irritability*, *anxiety*, *sadness* and *pessimism*. The affective dimension was the only domain that maintained a significant correlation with SRQ scores across all three countries. These results are consistent with another recent study in a non-clinical sample of young adults from Colombia showing that higher self-perceived mood rhythmicity of self-esteem, irritability, anxiety, sadness and pessimism were associated with higher scores in the Hospital Anxiety and Depression Scale (n=352) compared to individuals with lower depressive scores (n=114) (Pereira-Morales et al., 2019).

The use of network analysis to study psychopathological states is an innovative analytical approach that has been recently used to identify symptoms with the greatest importance in the network structure, in terms of centrality and strength of associations within the network (Boschloo et al., 2016; Contreras et al., 2019). This approach has been applied to identify symptoms that can predict the onset of depression (Borsboom, 2017), to distinguish individuals with and without bipolar disorder through different activation patterns of affect and physical activity (Curtiss et al., 2019) and to uncover

specific bridge symptoms connecting two co-morbid psychiatric disorders (Vanzhula et al., 2019). In the present study, the network analysis was consistent with the factor analyses showing that the structure of the three dimensions was preserved. Regarding the edges, the cognitive dimension had the strongest connections, followed by the affective dimension. An interesting result from the network analysis was that *sleepiness* was positioned with high centrality, which reinforces its importance as a core construct of mood states from a self-perceived rhythmicity perspective (Fried et al., 2016). This result is consistent with clinical studies in depression reporting a bidirectional association between sleep disturbance and depressed mood, where insomnia has been described as a predictor or a residual symptom of depression (Fang et al., 2019; Thorpy, 2005). Future studies applying the MRhI-r in clinical samples of individuals with major depression will allow us to deconstruct the heterogeneous phenotypes of depression from a different angle.

The present results must be considered according to the limitations of our study. First, most individuals who participated in this study were young university students, so our results might not reflect the self-perceived rhythmicity of mood-related symptoms in older populations. Another limitation is that we did not conduct a formal psychological/psychiatric assessment with these individuals, so any potential clinical correlations (beyond the validated SRQ) cannot be ascertained. Currently, to address this concern, we are using the MRhI and MRhI-r in well-characterized clinical samples of individuals with major depression.

In conclusion, using multiple psychometric analyses, we were able to refine the MRhI instrument into a more psychometrically sound 11-item revised version. A better understanding of self-perceived daily peaks of mood-related symptoms may help advance the knowledge of the role of biological rhythms in mood and related disorders.

References

Adan, A., Archer, S.N., Hidalgo, M.P., Di Milia, L., Natale, V., Randler, C., 2012. Circadian typology: a comprehensive review. Chronobiol. Int. 29, 1153–1175. https://doi.org/10.3109/07420528.2012.719971

Allega, O.R., Leng, X., Vaccarino, A., Skelly, M., Lanzini, M., Hidalgo, M.P., Soares, C.N., Kennedy, S.H., Frey, B.N., 2018. Performance of the biological rhythms interview for assessment in neuropsychiatry: An item response theory and actigraphy analysis. J Affect Disord 225, 54–63. https://doi.org/10.1016/j.jad.2017.07.047

Ávila Moraes, C., Cambras, T., Diez-Noguera, A., Schimitt, R., Dantas, G., Levandovski, R., Hidalgo, M.P., 2013. A new chronobiological approach to discriminate between acute and chronic depression using peripheral temperature, rest-activity, and light exposure parameters. BMC Psychiatry 13, 77. https://doi.org/10.1186/1471-244X-13-77

Beaujean, A.A., 2014. Latent Variable Modeling Using R. Routledge, New York. https://doi.org/10.4324/9781315869780

Bedrosian, T.A., Nelson, R.J., 2013. Influence of the modern light environment on mood. Mol. Psychiatry 18, 751–757. https://doi.org/10.1038/mp.2013.70

Beusenberg, M., Orley, J.H., Health, W.H.O.D. of M., 1994. A User's guide to the self reporting questionnaire (SRQ.

Bond, T.G., Fox, C.M., 2015. Applying the Rasch Model: Fundamental measurement in the human sciences, 3rd. ed. Routledge, New York, NY.

Boone, W.J., Staver, J.R., Yale, M.S., 2014. Rasch analysis in the human sciences. Springer, New York, NY.

Borsboom, D., 2017. A network theory of mental disorders. World Psychiatry 16, 5–13. https://doi.org/10.1002/wps.20375

Boschloo, L., Borkulo, C.D. van, Borsboom, D., Schoevers, R.A., 2016. A Prospective Study on How Symptoms in a Network Predict the Onset of Depression. PPS 85, 183–184. https://doi.org/10.1159/000442001

Carissimi, A., Oliveira, M.A.B., Frey, B.N., Navarro, J.F., Hidalgo, M.P., Adan, A., 2019. Validation and psychometric properties of the Spanish Mood Rhythm Instrument. Biological Rhythm Research 0, 1–13. https://doi.org/10.1080/09291016.2019.1675023

Cattell, R.B., 1966. The Scree Test For The Number Of Factors. Multivariate Behavioral Research 1, 245–276. https://doi.org/10.1207/s15327906mbr0102_10

Cherian, V.I., Peltzer, K., Cherian, L., 1998. The factor-structure of the Self Reporting Questionnaire (SRQ-20) in South Africa. East Afr Med J 75, 654–656.

Contreras, A., Nieto, I., Valiente, C., Espinosa, R., Vazquez, C., 2019. The Study of Psychopathology from the Network Analysis Perspective: A Systematic Review. Psychother Psychosom 88, 71–83. https://doi.org/10.1159/000497425

Curtiss, J., Fulford, D., Hofmann, S.G., Gershon, A., 2019. Network dynamics of positive and negative affect in bipolar disorder. J Affect Disord 249, 270–277. https://doi.org/10.1016/j.jad.2019.02.017

de Souza, C.M., Carissimi, A., Costa, D., Francisco, A.P., Medeiros, M.S., Ilgenfritz, C.A., de Oliveira, M.A., Frey, B.N., Hidalgo, M.P., de Souza, C.M., Carissimi, A., Costa, D., Francisco, A.P., Medeiros, M.S., Ilgenfritz, C.A., de Oliveira, M.A., Frey, B.N., Hidalgo, M.P., 2016. The Mood Rhythm Instrument: development and preliminary report. Brazilian Journal of Psychiatry 38, 148–153. https://doi.org/10.1590/1516-4446-2015-1763

Epskamp, S., Borsboom, D., Fried, E.I., 2018. Estimating psychological networks and their accuracy: A tutorial paper. Behav Res Methods 50, 195–212. https://doi.org/10.3758/s13428-017-0862-1

Epskamp, S., Cramer, A.O.J., Waldorp, L.J., Schmittmann, V.D., Borsboom, D., 2015. qgraph : Network Visualizations of Relationships in Psychometric Data. Journal of Statistical Software 48. https://doi.org/10.18637/jss.v048.i04

Fang, H., Tu, S., Sheng, J., Shao, A., 2019. Depression in sleep disturbance: A review on a bidirectional relationship, mechanisms and treatment. J Cell Mol Med 23, 2324–2332. https://doi.org/10.1111/jcmm.14170

Francisco, A.P., Oliveira, M.A.B. de, Carissimi, A., Fabris, R.C., Ilgenfritz, C.A.V., Souza, C.M. de, Medeiros, M.S., Adan, A., Hidalgo, M.P.L., 2017. Spanish translation of the mood rhythm instrument: a novel approach to mood evaluation. Clinical & Biomedical Research 37.

Fried, E.I., Epskamp, S., Nesse, R.M., Tuerlinckx, F., Borsboom, D., 2016. What are "good" depression symptoms? Comparing the centrality of DSM and non-DSM symptoms of depression in a network analysis. J Affect Disord 189, 314–320. https://doi.org/10.1016/j.jad.2015.09.005

Friedman, J., Hastie, T., Tibshirani, R., 2008. Sparse inverse covariance estimation with the graphical lasso. Biostatistics 9, 432–441. https://doi.org/10.1093/biostatistics/kxm045

Gonçalves, D.M., Stein, A.T., Kapczinski, F., 2008. Performance of the Self-Reporting Questionnaire as a psychiatric screening questionnaire: a comparativestudy with Structured Clinical Interview for DSM-IV-TR. Cadernos de Saúde Pública 24, 380–390. https://doi.org/10.1590/S0102-311X2008000200017

Hickie, I.B., Naismith, S.L., Robillard, R., Scott, E.M., Hermens, D.F., 2013. Manipulating the sleep-wake cycle and circadian rhythms to improve clinical management of major depression. BMC Med 11, 79. https://doi.org/10.1186/1741-7015-11-79

Horn, J.L., 1965. A rationale and test for the number of factors in factor analysis. Psychometrika 30, 179–185. https://doi.org/10.1007/BF02289447

Hu, L., Bentler, P.M., 1999. Cutoff criteria for fit indexes in covariance structure analysis: Conventional criteria versus new alternatives. Structural Equation Modeling: A Multidisciplinary Journal 6, 1–55. https://doi.org/10.1080/10705519909540118

Hühne, A., Welsh, D.K., Landgraf, D., 2018. Prospects for circadian treatment of mood disorders. Ann. Med. 50, 637–654. https://doi.org/10.1080/07853890.2018.1530449

Iacoponi, E., Mari, J.J., 1989. Reliability and factor structure of the Portuguese version of Self-Reporting Questionnaire. Int J Soc Psychiatry 35, 213–222. https://doi.org/10.1177/002076408903500301

Izquierdo, I., Olea, J., Abad, F.J., 2014. Exploratory factor analysis in validation studies: uses and recommendations. Psicothema 26, 395–400. https://doi.org/10.7334/psicothema2013.349

Karatsoreos, I.N., McEwen, B.S., 2011. Psychobiological allostasis: resistance, resilience and vulnerability. Trends in Cognitive Sciences 15, 576–584. https://doi.org/10.1016/j.tics.2011.10.005

Krawczak, E.M., Minuzzi, L., Hidalgo, M.P., Frey, B.N., 2016. Do changes in subjective sleep and biological rhythms predict worsening in postpartum depressive symptoms? A prospective study across the perinatal period. Arch Womens Ment Health 19, 591–598. https://doi.org/10.1007/s00737-016-0612-x

Linacre, J.M., 2002. What do Infit and Outfit, Mean-square and Standardized mean? Rasch Measurement Transactions 16, 878.

Livianos Aldana, L., Rojo Moreno, L., Domínguez Carabantes, A., Bofill Moscardó, I., 1990. Validación del SRQ en los exámenes de salud mental en la población general. Actas Luso Esp Neurol Psiquiatr Cienc Afines 286–9.

Logan, R.W., McClung, C.A., 2019. Rhythms of life: circadian disruption and brain disorders across the lifespan. Nat. Rev. Neurosci. 20, 49–65. https://doi.org/10.1038/s41583-018-0088-y

McCarthy, M.J., Wei, H., Nievergelt, C.M., Stautland, A., Maihofer, A.X., Welsh, D.K., Shilling, P., Alda, M., Alliey-Rodriguez, N., Anand, A., Andreasson, O.A., Balaraman, Y., Berrettini, W.H., Bertram, H., Brennand, K.J., Calabrese, J.R., Calkin, C.V., Claasen, A., Conroy, C., Coryell, W.H., Craig, D.W., D'Arcangelo, N., Demodena, A., Djurovic, S., Feeder, S., Fisher, C., Frazier, N., Frye, M.A., Gage, F.H., Gao, K., Garnham, J., Gershon, E.S., Glazer, K., Goes, F., Goto, T., Harrington, G., Jakobsen, P., Kamali, M., Karberg, E., Kelly, M., Leckband, S.G., Lohoff, F., McInnis, M.G., Mondimore, F., Morken, G., Nurnberger, J.I., Obral, S., Oedegaard, K.J., Ortiz, A., Ritchey, M., Ryan, K., Schinagle, M., Schoeyen, H., Schwebel, C., Shaw, M., Shekhtman, T., Slaney, C., Stapp, E., Szelinger, S., Tarwater, B., Zandi, P.P., Kelsoe, J.R., 2019. Chronotype and cellular circadian rhythms predict the clinical response to lithium maintenance treatment in patients with bipolar disorder. Neuropsychopharmacology 44, 620–628. https://doi.org/10.1038/s41386-018-0273-8

McClung, C.A., 2013. How might circadian rhythms control mood? Let me count the ways.. Biol. Psychiatry 74, 242–249. https://doi.org/10.1016/j.biopsych.2013.02.019

Muthén, L.K., Muthén, B.O., 2010. Mplus User's Guide, 6th ed. ed. Muthén & Muthén, Los Angeles, CA.

Oliveira MAB, Epifano K, Mathur S, Carvalho FG, Scop M, Carissimi A, Francisco AP, Garay LLS, Adan A, Hidalgo MP, Frey BN, 2020. Validation of the English Version of the Mood Rhythm Instrument. BMC Psychology.

Pereira-Morales, A.J., Casiraghi, L.P., Adan, A., Camargo, A., 2019. Mood rhythmicity is associated with depressive symptoms and caffeinated drinks consumption in South American young adults. Chronobiol. Int. 36, 225–236. https://doi.org/10.1080/07420528.2018.1530257

Pilz, L.K., Carissimi, A., Francisco, A.P., Oliveira, M.A.B., Slyepchenko, A., Epifano, K., Garay, L.L.S., Fabris, R.C., Scop, M., Streiner, D.L., Hidalgo, M.P., Frey, B.N., 2018a. Prospective Assessment of Daily Patterns of Mood-Related Symptoms. Front Psychiatry 9, 370. https://doi.org/10.3389/fpsyt.2018.00370

Pilz, L.K., Carissimi, A., Oliveira, M.A.B., Francisco, A.P., Fabris, R.C., Medeiros, M.S., Scop, M., Frey, B.N., Adan, A., Hidalgo, M.P., 2018b. Rhythmicity of Mood Symptoms in Individuals at Risk for

Psychiatric Disorders. Scientific Reports 8, 11402. https://doi.org/10.1038/s41598-018-29348-z

Revelle, W., 2019. psych: Procedures for Psychological, Psychometric, and Personality Research.

Schmittmann, V.D., Cramer, A.O.J., Waldorp, L.J., Epskamp, S., Kievit, R.A., Borsboom, D., 2013. Deconstructing the construct: A network perspective on psychological phenomena. New Ideas in Psychology 31, 43–53. https://doi.org/10.1016/j.newideapsych.2011.02.007

Thorpy, M.J., 2005. Which clinical conditions are responsible for impaired alertness? Sleep Med. 6 Suppl 1, S13-20.

Tonon, A.C., Fuchs, D.F.P., Barbosa Gomes, W., Levandovski, R., Pio de Almeida Fleck, M., Hidalgo, M.P.L., da Silva Alencastro, L., 2017. Nocturnal motor activity and light exposure: Objective actigraphy-based marks of melancholic and non-melancholic depressive disorder. Brief report. Psychiatry Res 258, 587–590. https://doi.org/10.1016/j.psychres.2017.08.025

Upadhyaya, A., Creed, F., Upadhyaya, M., 1990. Psychiatric morbidity among mothers attending well-baby clinic: a cross-cultural comparison. Acta Psychiatr Scand 81, 148–151.

van der Westhuizen, C., Wyatt, G., Williams, J.K., Stein, D.J., Sorsdahl, K., 2016. Validation of the Self Reporting Questionnaire 20-Item (SRQ-20) for Use in a Low- and Middle-Income Country Emergency Centre Setting. Int J Ment Health Addict 14, 37–48. https://doi.org/10.1007/s11469-015-9566-x

Vanzhula, I.A., Calebs, B., Fewell, L., Levinson, C.A., 2019. Illness pathways between eating disorder and post-traumatic stress disorder symptoms: Understanding comorbidity with network analysis. Eur Eat Disord Rev 27, 147–160. https://doi.org/10.1002/erv.2634

Wickham, H., Chang, W., Henry, L., Pedersen, T.L., Takahashi, K., Wilke, C., Woo, K., Yutani, H., Dunnington, D., RStudio, 2020. ggplot2: Create Elegant Data Visualisations Using the Grammar of Graphics.

Wulff, K., Gatti, S., Wettstein, J.G., Foster, R.G., 2010. Sleep and circadian rhythm disruption in psychiatric and neurodegenerative disease. Nat. Rev. Neurosci. 11, 589–599. https://doi.org/10.1038/nrn2868

Yatham, L.N., Kennedy, S.H., Parikh, S.V., Schaffer, A., Bond, D.J., Frey, B.N., Sharma, V., Goldstein, B.I., Rej, S., Beaulieu, S., Alda, M., MacQueen, G., Milev, R.V., Ravindran, A., O'Donovan, C., McIntosh, D., Lam, R.W., Vazquez, G., Kapczinski, F., McIntyre, R.S., Kozicky, J., Kanba, S., Lafer, B., Suppes, T., Calabrese, J.R., Vieta, E., Malhi, G., Post, R.M., Berk, M., 2018. Canadian Network for Mood and Anxiety Treatments (CANMAT) and International Society for Bipolar Disorders (ISBD) 2018 guidelines for the management of patients with bipolar disorder. Bipolar Disord 20, 97–170. https://doi.org/10.1111/bdi.12609

Supplementary Materials

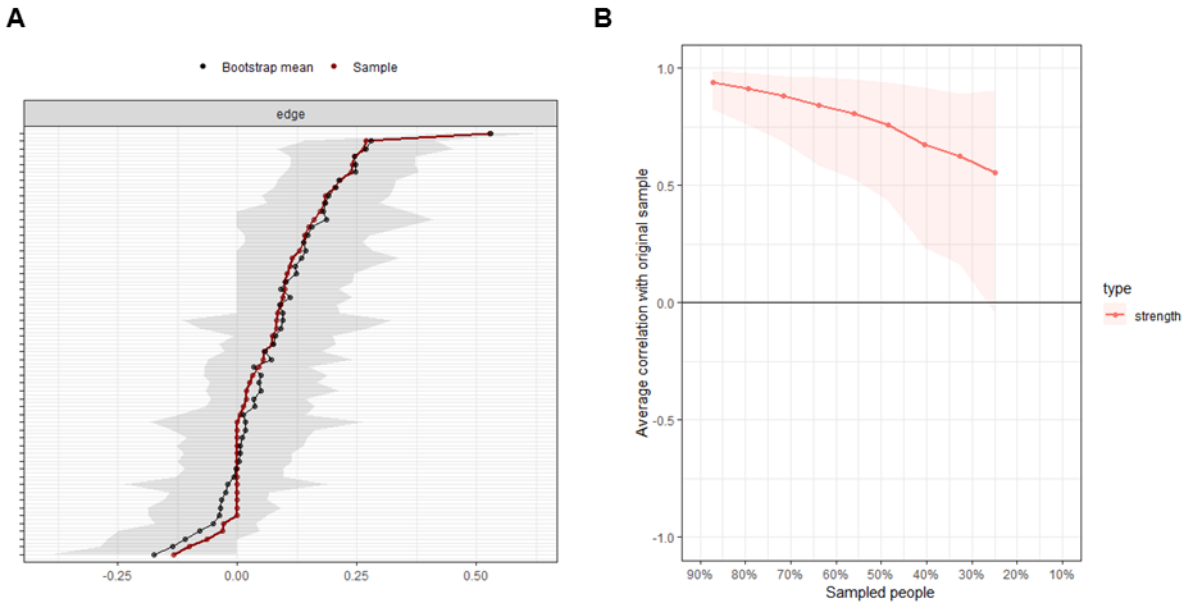


Fig. S1: Network Stability of MRhI-r items (n= 1275). A: Edge-weight accuracy. Bootstrapped 95% confidence intervals (CI) of estimated edge-weights for the MRhI-r network are displayed as gray area. Horizontal lines represent each of the edges of the network, ordered from the edge with the highest to the one with the lowest edge-weight. The smaller the CIs, the higher is the accuracy of network estimation. B: Stability of strength centrality. Applying the *case-dropping subset bootstrap we* verified if centrality estimates remain the same with less cases. To quantify the stability, we used the *CS*-coefficient, which should not be below 0.25 according to Epskamp, Borsboom, & Fried, 2018. When the correlation after dropping a large number of participants remains high, it means that the centrality estimates in the original network can be considered stable. The *CS-coefficient* calculated was 0.28.

Table S1: Eigenvalues for sample correlation matrix.

Factors to retain	Eigenvalue	Proportional explained variance	Cumulative explained variance
1	4.902	32.7%	32.7%
2	1.899	12.7%	45.3%
3	1.193	8.0%	53.3%
4	1.064	7.1%	60.4%
5	0.878	5.9%	66.2%
6	0.759	5.1%	71.3%
7	0.684	4.6%	75.9%
8	0.621	4.1%	80.0%
9	0.582	3.9%	83.9%
10	0.570	3.8%	87.7%
11	0.464	3.1%	90.8%
12	0.445	3.0%	93.7%
13	0.395	2.6%	96.4%
14	0.293	2.0%	98.3%
15	0.251	1.7%	100.0%

Table S2: Factor analysis (four factors).

	F1	F2	F3	F4	*U*
Q2 Sleepiness	0.87 *	0.05	−0.04	−0.08	0.23
Q6 Appetite	0.49 *	−0.02	0.06	0.08	0.72
Q5 Concentration	−0.04	0.83 *	−0.01	−0.00	0.36
Q1 Alertness	0.10	0.62 *	0.08	−0.20 *	0.55
Q15 Energy	0.05	0.58 *	0.15 *	−0.01	0.56
Q3 Problem-solving	0.04	0.58 *	−0.03	0.23 *	0.52
Q12 Memory	−0.09	0.56 *	−0.01	0.32 *	0.55
Q13 Pessimism	0.01	0.01	0.84 *	0.01	0.29
Q10 Sadness	−0.06	−0.01	0.80 *	0.14	0.30
Q9 Anxiety	0.15	0.06	0.63 *	−0.06	0.53
Q8 Irritability	0.31 *	0.01	0.41 *	0.07	0.63
Q11 Physical Exercise	0.25 *	0.26 *	−0.27 *	0.11	0.79
Q4 Self-esteem	0.32	0.04	0.19 *	0.45 *	0.49
Q7 Sexual Arousal	0.37 *	−0.05	0.02	0.38 *	0.69
Q14 Talking to Friends	0.04	0.12	0.13	0.40 *	0.72

*$p < 0.05$.

Table S3: Factor analysis (five factors).

	F1	F2	F3	F4	F5	*U*
Q5 Concentration	0.80 *	0.03	−0.04	−0.00	0.04	0.35
Q1 Alertness	0.60 *	0.13	0.06	−0.02	−0.12	0.58
Q15 Energy	0.56 *	0.10	0.12*	−0.01	0.06	0.55
Q3 Problem-solving	0.56 *	−0.08	−0.01	0.29*	0.02	0.52
Q12 Memory	0.52 *	−0.18	0.02	0.30*	0.03	0.57
Q2 Sleepiness	0.10	0.64 *	−0.03	0.26	−0.05	0.34
Q6 Appetite	−0.04	0.55 *	0.04	0.05	0.20	0.64
Q13 Pessimism	0.02	0.02	0.85 *	0.02	−0.06	0.28
Q10 Sadness	−0.01	−0.08	0.82 *	0.10	−0.02	0.30
Q9 Anxiety	0.07	0.18*	0.62 *	−0.05	0.01	0.53
Q8 Irritability	0.01	0.33*	0.41 *	0.01	0.15	0.61
Q4 Self-esteem	0.02	0.04	0.25 *	0.57 *	0.06	0.46
Q7 Sexual Arousal	−0.06	0.12	0.07	0.49 *	0.05	0.68
Q11 Physical Exercise	0.25*	0.09	−0.24 *	0.31 *	−0.10	0.76
Q14 Talking to Friends	0.04	0.00	−0.00	0.02	0.97 *	0.03

*$p < 0.05$.

Table S4: Bivariate and regularized regressions correlations of the MRhI-r items.

	Problem-solving	Concentration	Memory	Energy	Irritability	Anxiety	Sadness	Pessimism	Sleepiness	Appetite	Sexual Arousal
Problem-solving		0.26	0.21	0.19	0.1	0.05	0.02	−0.07	0.16	−0.17	0.15
Concentration	0.51		0.28	0.25	0.00	−0.15	0.01	0.11	0.10	0.15	−0.06
Memory	0.46	0.49		0.09	−0.01	0.05	0.03	0.01	−0.04	0.01	0.11
Energy	0.45	0.49	0.38		−0.02	0.15	0.07	−0.01	0.09	0.09	−0.07
Irritability	0.27	0.25	0.20	0.26		0.08	0.10	0.20	0.19	0.17	−0.04
Anxiety	0.23	0.16	0.21	0.33	0.40		0.21	0.26	0.15	0.06	−0.06
Sadness	0.19	0.20	0.22	0.27	0.40	0.52		0.53	−0.20	0.07	0.05
Pessimism	0.20	0.24	0.22	0.26	0.46	0.55	0.71		0.02	−0.12	0.14
Sleepiness	0.37	0.35	0.23	0.34	0.36	0.28	0.08	0.18		0.27	0.19
Appetite	0.14	0.28	0.17	0.26	0.32	0.22	0.16	0.15	0.42		0.17
Sexual Arousal	0.28	0.20	0.25	0.16	0.20	0.18	0.21	0.26	0.33	0.28	

Note: Values below the diagonal represent bivariate tetrachoric correlations (white); values above indicate partial correlations based on regularized regressions (shaded).

CAPÍTULO 5 – DISCUSSÃO E CONCLUSÃO

1. CONTRIBUIÇÕES METODOLÓGICAS DESTA TESE

No estudo acerca das recomendações para estudos considerando aspectos básicos envolvendo ritmos biológicos, a tabela contendo as informações mínimas que deveriam ser reportadas representa um produto importante desta tese que visa contribuir com a Cronomedicina. Compreendendo a importância das variações temporais que podem se manifestar e influenciar os achados dos estudos, é preocupante que trabalhos como os inseridos nas áreas de imunologia, farmacologia, endocrinologia e oncologia ainda majoritariamente não levem em consideração, por exemplo, momento do dia de suas intervenções e observações (109). Esperamos que este trabalho seja um guia útil para instruir estudos que possam servir de base para ações de prevenção, identificação e tratamento de doenças levando ritmos biológicos em consideração.

Durante o mestrado foi desenvolvido modelo para avaliar impactos de diferentes sistemas de iluminação nos ritmos de atividade-respouso de roedores. Nesta tese, o modelo incluiu e estudo de ritmos cerebrais. Portanto, contribuo com um modelo viável para reprodução com os protocolos operacionais descritos, que incluem implantação de iButtons (Apêndice 3), manufatura e implantação de eletrodos corticais em roedores (Apêndice 4 e 5) e registro de EEG concomitante com actimetria.

Em relação ao MRhI, ele tem se mostrado uma ferramenta útil na identificação de padrões diários de sintomas de humor e apresenta potencial de aplicação na prática clínica que envolva especialmente transtornos de humor. Tanto a versão validada para o espanhol, quanto a versão traduzida e validada para o inglês (Anexos 1 e 2) mostraram-se de fácil aplicação e compreensão, o que pode ser útil na identificação de sintomas de humor cujos padrões de manifestação possam estar associados a condições patológicas. O instrumento também já demosntrou não apresentar viés de memória, após estudo com a versão diária do MRhI (MRhi-d; Anexo 6). A análise psicométrica das amostras brasileira, espanhola e canadense (Capítulo 4) reuniu na versão revisada os items que se comportam de maneira semelhante dentro dos domínios cognitivo, somático e afetivo do instrumento independente do aspecto socio-geográfico. Sendo assim, este estudo foi capaz de gerar um instrumento mais aplicável em larga escala em populações saudáveis, que diferente dos atuais instrumentos de avaliação de comportamento e humor, leva em consideração a percepção da variação de sintomas

relacionados ao humor ao longo do dia. No entanto, a utilização do MRhI em amostras clínicas ainda vem sendo estudada no Brasil e no Canadá, e poderá indicar quais sintomas de humor e seus respectivos padrões de manifestação ao longo do dia podem estar alterados em transtornos de humor como depressão maior.

O fluxograma abaixo ilustra o processo de desenvolvimento do MRhI, que, recentemente, também foi adaptado para aplicação em adolescentes (MRhI-Y) e vem sendo utilizado para estudar jovens com depressão (110). Além disso, alguns items do MRhI compuseram uma bateria de perguntas que foram respondidas diariamente por indivíduos em distanciamento social durante a pandemia de COVID-19 e cujos padrões de variabilidade durante o dia e entre os dias esteveram associados a sintomas depressivos e ansiosos. Estes mesmos itens foram selecionados para o aplicativo ReGente, que tem como objetivo promover saúde física e mental após a pandemia. Por fim, o MRhI está sendo validado para o persa e, os dados do MRhI obtidos em uma amostra de estudantes mexicanos de ensino médio também está sendo analisada.

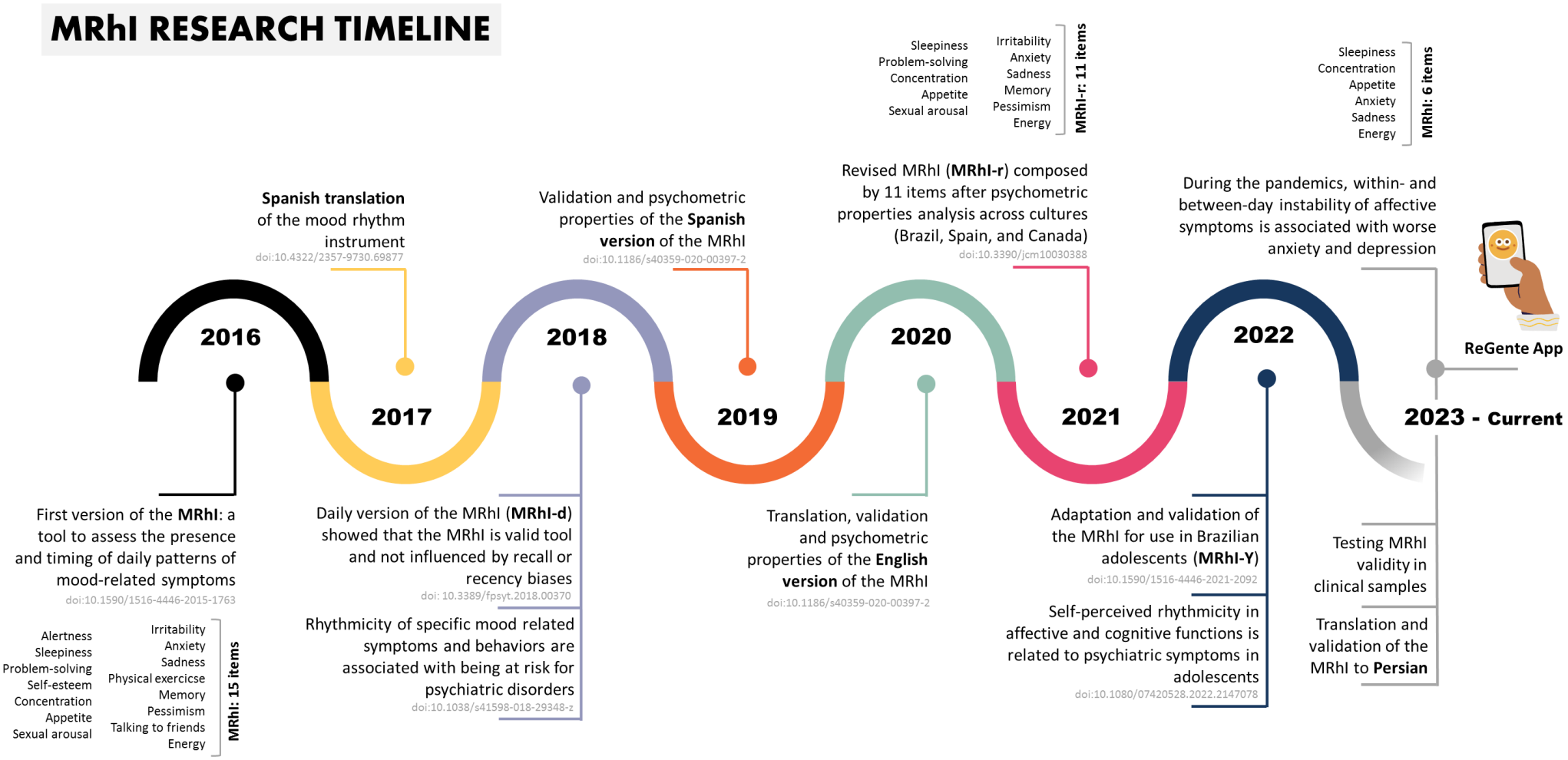


Figura 28. Linha do tempo contendo os estudos com o Instrumento de Ritmo de Humor (MRhI). O MRhI foi criado em 2016 contendo 15 itens. Em 2021, a escala revisada com 11 itens foi desenvolvida e atualmente vem sendo aplicada em amostras clínicas de transtornos de humor.

2. IMPLICAÇÕES DOS PRINCIPAIS ACHADOS DOS ESTUDOS CLÍNICOS E EXPERIMENTAIS

O futuro da medicina circadiana pode ser visto a partir do modelo proposto do Kramer e colaboradores, como um paradigma que integra diferentes abordagens que precisam ser igualmente desenvolvidas, e cujo foco final é tratamento personalizado do indivíduo, como um ser que pertence a um ambiente cíclico e que, portanto, apresenta seus ritmos e particularidades (104). Esta tese contribui em diversas bases deste modelo: ensino e comunicação, pesquisa sobre mecanismos, além de ferramentas para detectar e intervir nos ritmos.

Primeiramente, propusemos orientações para cientistas que buscam realizar estudos à luz dos ritmos biológicos e do impacto que eles exercem sobre a fisiologia e comportamento dos seres vivos. Estudos que respeitem aspectos básicos da cronobiologia têm grande potencial de se tornarem ferramentas importantes para o desenvolvimento de terapêuticas na medicina circadiana (105). Além disso, também encontramos no estudo experimental com roedores, a investigação sobre efeitos da luz em suas diferentes manifestações que nos dão mais evidências sobre mecanismos de regulação deste importante *zeitgeber*. Além do impacto nos ritmos de atividade-repouso já esperados conforme semelhantes modelos de manipulação no comprimento dos fotoperíodos (52,111,112), nosso estudo mostrou que diferentes regimes de luz afetam significativamente as oscilações cerebrais em ratos jovens e adultos, e possivelmente impactam o comportamento sono-vigília. Os próximos passos serão com análise da arquitetura de sono, e para suprir uma importante limitação deste estudo, enfatizamos a importância da avaliação conjunta da exposição aos sistemas de iluminação e o comportamento tipo-depressivo e tipo-ansioso nos roedores.

Em relação as intervenções nos ritmos, encontramos potencial em um produto já disponível na rede de saúde, as máscaras para fototerapia, que fortalecem a pista temporal de escuro durante a noite e produzem impacto fisiológico - melhora geral da condição de saúde para alta hospitalar; social – com tudo o que representa a família poder retornar para o lar; e econômico – com redução dos custos representados por cada dia de internação. O uso de máscaras é modelo a ser replicado em estudos clínicos em outros tipos de internação, e nos faz pensar em protocolos assistenciais que incluam manipulação luz em unidades hospitalares.

Por fim, em relação às ferramentas para detectar ritmos, o MRhI se mostrou um instrumento inovador no estudo de transtornos de humor. Os instrumentos atualmente disponíveis, têm como objetivo medir a intensidade de humor ou presença de transtorno de

humor, enquanto o MRhI leva em consideração as possíveis percepções das oscilações de comportamentos ao longo do dia. Estudos adicionais que avaliem a percepção de mudanças diárias do humor em amostras clínicas podem contribuir para a aplicação deste instrumento no diagnóstico e tratamento de transtornos psiquiátricos.

3. CONCLUSÃO

Apesar do corpo sólido de evidências de que grande parte dos processos fisiológicos e comportamentais nos organismos vivos exibem ritmicidade e são regulados dentro de um ciclo de 24 horas, ainda existe a necessidade de incorporar em protocolos de amostragens bem como de intervenções, a variável "tempo" como importante moduladora de desfechos em saúde.

Os resultados e produtos desta tese demonstram a aplicabilidade das ferramentas de medida e interveção em ritmos biológicos e do potencial para contribuir com o futuro da Cronomedicina. Desde de estudos de revisão metodológica, passando por estudo experimental, clínico até epidemiológico, demonstrou-se a capacidade e potencial de translação dos conhecimentos básicos de cronobiologia para a prática clínica.

4. PERSPECTIVAS

Nos próximos anos, pretendo seguir contribuindo com linhas de pesquisa do Laboratório de Cronobiologia e Sono HCPA/UFRGS e do Laboratório de Neurofisiologia e Neuroquímica da Excitabilidade Neuronal e Plasticidade Sináptica (NNNESP Lab) como pesquisadora colaboradora, além de dar início ao meu pós-doutoramento junto ao programa NeuroInsight em Dublin, onde trabalharei com pesquisa na interface entre Cronobiologia e Epilepsia.

Primeiramente, pretendo submeter para publicação o segundo artigo descrito nesta tese, onde avaliamos os efeitos de diferentes sistemas de iluminação nos ritmos cerebrais de roedores. Além disso, durante o período de doutoramento, fui capaz de coletar mais dados de EEG juntamente com dados de eletromiografia, que possibilitarão o estagiamento dos períodos de sono dos roedores, o que nos auxiliará no entendimento do impacto dos sistemas de iluminação no ritmo de sono-vigília destes animais.

Ainda em relação ao estudo dos ritmos cerebrais em roedores, estamos testando a utilização de um aparato para EEG portátil que facilitará imensamente os registros

eletrofisiológicos que forem realizados na Unidade de Experimentação Animal no HCPA. Pelas característacas deste novo equipamento, será possível reproduzir, por exemplo, as mesmas condições ambientais do estudo também durante a realização dos registros de EEG, sem a necessidade de deslocamento até o Instituto de Bioquímica.

Em relação ao projeto desenvolvido com neonatos na UTI, a hipótese de que a associação entre o uso das máscaras durante a noite e a utilização de iluminação dinâmica durante o dia produzirá ainda melhores resultados em relação à recuperação e alta hospitalar dos neonatos, nos incentiva a buscar colaboração para realização da segunda fase do estudo em unidades hospitalares com pouca variação de claro e escuro.

Com a validação do MRhI para as línguas espanhola e inglesa, esse instrumento tem se tornado cada vez mais reconhecido e aplicado internacionalmente. Atualmente, integro duas colaborações externas, uma para validação do MRhI em amostra iraniana, e outra que busca compreender a associação entre o turno escolar e a percepção de ritmos de humor em jovens mexicanos. Ainda, além de o MRhI estar sendo aplicado em amostras clínicas de transtornos de humor, vem sendo incorporado ao desenvolvimento de um aplicativo para smartphones que busca promover saúde física e mental após a pandemia de COVID-19.

REFERÊNCIAS

1. Castellanos MÁ, Escobar C. De la cronobiología a la cronomedicina.

2. Roenneberg T, Merrow M. The Circadian Clock and Human Health. Curr Biol CB. 2016 23;26(10):R432-443.

3. Fishbein AB, Knutson KL, Zee PC. Circadian disruption and human health. J Clin Invest. 2021 Oct 1;131(19):e148286.

4. Cortelli P. Chronomedicine: a necessary concept to manage human diseases. Sleep Med Rev. 2015 Jun;21:1–2.

5. Roenneberg T, Merrow M. Circadian systems: different levels of complexity. Philos Trans R Soc Lond B Biol Sci. 2001 Nov 29;356(1415):1687–96.

6. Moore RY. The suprachiasmatic nucleus and the circadian timing system. Prog Mol Biol Transl Sci. 2013;119:1–28.

7. Hastings MH, Maywood ES, Brancaccio M. Generation of circadian rhythms in the suprachiasmatic nucleus. Nat Rev Neurosci. 2018 Aug;19(8):453–69.

8. Hardin PE, Hall JC, Rosbash M. Feedback of the Drosophila period gene product on circadian cycling of its messenger RNA levels. Nature. 1990 Feb;343(6258):536–40.

9. Mohawk JA, Green CB, Takahashi JS. Central and Peripheral Circadian Clocks in Mammals. Annu Rev Neurosci. 2012;35(1):445–62.

10. Bedrosian TA, Nelson RJ. Timing of light exposure affects mood and brain circuits. Transl Psychiatry. 2017 31;7(1):e1017.

11. Vetter C. Circadian disruption: What do we actually mean? Eur J Neurosci. 2020;51(1):531–50.

12. Antoni Díez-Noguera, Trinitat Cambras Riu, Jordi Vilaplana Hortensi, Nuria Casamitjana Cucurella. Cronobiologia. Editora Livre; 2007. 160 p.

13. Daan S, Aschoff J. The Entrainment of Circadian Systems. In: Takahashi JS, Turek FW, Moore RY, editors. Circadian Clocks [Internet]. Boston, MA: Springer US; 2001 [cited 2023 Jan 18]. p. 7–43. (Handbook of Behavioral Neurobiology). Available from: https://doi.org/10.1007/978-1-4615-1201-1_2

14. Roenneberg T, Merrow M. Circadian clocks — the fall and rise of physiology. Nat Rev Mol Cell Biol. 2005 Dec;6(12):965–71.

15. Emery P, Klarsfeld A, Stanewsky R, Shafer OT. Sensitive Timing: A Reappraisal of Chronobiology's Foundational Texts. J Biol Rhythms. 2023 Jun 1;38(3):245–58.

16. Bell-Pedersen D, Cassone VM, Earnest DJ, Golden SS, Hardin PE, Thomas TL, et al. Circadian rhythms from multiple oscillators: lessons from diverse organisms. Nat Rev Genet. 2005 Jul;6(7):544–56.

17. Weaver DR. The suprachiasmatic nucleus: a 25-year retrospective. J Biol Rhythms. 1998 Apr;13(2):100–12.

18. Stephan FK, Zucker I. Circadian rhythms in drinking behavior and locomotor activity of rats are eliminated by hypothalamic lesions. Proc Natl Acad Sci U S A. 1972 Jun;69(6):1583–6.

19. Moore RY, Eichler VB. Loss of a circadian adrenal corticosterone rhythm following suprachiasmatic lesions in the rat. Brain Res. 1972 Jul 13;42(1):201–6.

20. Patton AP, Hastings MH. The suprachiasmatic nucleus. Curr Biol. 2018 Aug 6;28(15):R816–22.

21. Reppert SM, Weaver DR. Coordination of circadian timing in mammals. Nature. 2002 Aug;418(6901):935–41.

22. Mure LS, Le HD, Benegiamo G, Chang MW, Rios L, Jillani N, et al. Diurnal transcriptome atlas of a primate across major neural and peripheral tissues. Science [Internet]. 2018 Mar 16 [cited 2020 Oct 8];359(6381). Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5924732/

23. Takahashi JS. Transcriptional architecture of the mammalian circadian clock. Nat Rev Genet. 2017 Mar;18(3):164–79.

24. Aschoff J, Hoffmann K, Pohl H, Wever R. Re-entrainment of circadian rhythms after phase-shifts of the Zeitgeber. Chronobiologia. 1975;2(1):23–78.

25. Mistlberger RE, Skene DJ. Nonphotic entrainment in humans? J Biol Rhythms. 2005 Aug;20(4):339–52.

26. Logan RW, McClung CA. Rhythms of life: circadian disruption and brain disorders across the lifespan. Nat Rev Neurosci. 2019 Jan;20(1):49–65.

27. Halberg F, Engeli M, Hamburger C, Hillman D. Spectral Resolution of Low-Frequency, Small-Amplitude Rhythms in Excreted 17-Ketosteroids; Probable Androgen-Induced Circaseptan Desynchronization. Acta Endocrinol Nor. 1965 Dec 1;50(4_Supplement):S5–54.

28. Gyorgy Buzsaki. Rhythms of the brain. Oxford University Press; 2006.

29. Proekt A. Brief Introduction to Electroencephalography. Methods Enzymol. 2018;603:257–77.

30. Buzsáki G, Anastassiou CA, Koch C. The origin of extracellular fields and currents — EEG, ECoG, LFP and spikes. Nat Rev Neurosci. 2012 Jun;13(6):407–20.

31. Luo W, Guan JS. Do Brain Oscillations Orchestrate Memory? Brain Sci Adv. 2018 Sep 1;4(1):16–33.

32. Andrew S. Blum, Seward B. Rutkove. The Clinical Neurophysiology Primer. Humana Press; 2007.

33. Hasan S, Tam SKE, Foster RG, Vyazovskiy VV, Bannerman DM, Peirson SN. Modulation of recognition memory performance by light and its relationship with cortical EEG theta and gamma activities. Biochem Pharmacol. 2021 Sep;191:114404.

34. Mondino A, Cavelli M, González J, Osorio L, Castro-Zaballa S, Costa A, et al. Power and Coherence in the EEG of the Rat: Impact of Behavioral States, Cortical Area, Lateralization and Light/Dark Phases. Clocks Sleep. 2020 Dec 9;2(4):536–56.

35. Alföldi P, Franken P, Tobler I, Borbély AA. Short light-dark cycles influence sleep stages and EEG power spectra in the rat. Behav Brain Res. 1991 May 15;43(2):125–31.

36. Yasuda T, Yasuda K, Brown RA, Krueger JM. State-dependent effects of light-dark cycle on somatosensory and visual cortex EEG in rats. Am J Physiol Regul Integr Comp Physiol. 2005 Oct;289(4):R1083-1089.

37. Hu WP, Li JD, Zhang C, Boehmer L, Siegel JM, Zhou QY. Altered circadian and homeostatic sleep regulation in prokineticin 2-deficient mice. Sleep. 2007 Mar;30(3):247–56.

38. Hattar S, Liao HW, Takao M, Berson DM, Yau KW. Melanopsin-containing retinal ganglion cells: architecture, projections, and intrinsic photosensitivity. Science. 2002 Feb 8;295(5557):1065–70.

39. LeGates TA, Fernandez DC, Hattar S. Light as a central modulator of circadian rhythms, sleep and affect. Nat Rev Neurosci. 2014 Jul;15(7):443–54.

40. Copenhaver AE, Roberts RC, LeGates TA. Light-dependent effects on mood: Mechanistic insights from animal models. Prog Brain Res. 2022;273(1):71–95.

41. Fernandez DC, Fogerson PM, Lazzerini Ospri L, Thomsen MB, Layne RM, Severin D, et al. Light Affects Mood and Learning through Distinct Retina-Brain Pathways. Cell. 2018 Sep 20;175(1):71-84.e18.

42. Wirz-Justice A. Seasonality in affective disorders. Gen Comp Endocrinol. 2018 01;258:244–9.

43. Bedrosian TA, Nelson RJ. Influence of the modern light environment on mood. Mol Psychiatry. 2013 Jul;18(7):751–7.

44. Brown TM, Brainard GC, Cajochen C, Czeisler CA, Hanifin JP, Lockley SW, et al. Recommendations for daytime, evening, and nighttime indoor light exposure to best support physiology, sleep, and wakefulness in healthy adults. PLoS Biol. 2022 Mar;20(3):e3001571.

45. Wirz-Justice A, Terman AM. CME: Light Therapy: Why, What, for Whom, How, and When (And a Postscript about Darkness). Praxis. 2022 Feb;110(2):56–62.

46. Garbazza C, Cirignotta F, D'Agostino A, Cicolin A, Hackethal S, Wirz-Justice A, et al. Sustained remission from perinatal depression after bright light therapy: A pilot randomised, placebo-controlled trial. Acta Psychiatr Scand. 2022 Oct;146(4):350–6.

47. Harb F, Hidalgo MP, Martau B. Lack of exposure to natural light in the workspace is associated with physiological, sleep and depressive symptoms. Chronobiol Int. 2015 Mar 16;32(3):368–75.

48. Carvalho FG, Hidalgo MP, Levandovski R. Differences in circadian patterns between rural and urban populations: an epidemiological study in countryside. Chronobiol Int. 2014 Apr;31(3):442–9.

49. Pilz LK, Levandovski R, Oliveira MAB, Hidalgo MP, Roenneberg T. Sleep and light exposure across different levels of urbanisation in Brazilian communities. Sci Rep. 2018 Jul 30;8(1):11389.

50. Pilz LK, Xavier NB, Levandovski R, Oliveira MAB, Tonon AC, Constantino DB, et al. Circadian Strain, Light Exposure, and Depressive Symptoms in Rural Communities of Southern Brazil. Front Netw Physiol. 2021;1:779136.

51. Tonon AC, Constantino DB, Amando GR, Abreu AC, Francisco AP, de Oliveira MAB, et al. Sleep disturbances, circadian activity, and nocturnal light exposure characterize high risk for and current depression in adolescence. Sleep. 2022 Jul 11;45(7):zsac104.

52. Quiles CL, de Oliveira MAB, Tonon AC, Hidalgo MPL. Biological adaptability under seasonal variation of light/dark cycles. Chronobiol Int. 2016;33(8):964–71.

53. Pilz LK, Quiles CL, Dallegrave E, Levandovski R, Hidalgo MPL, Elisabetsky E. Differential susceptibility of BALB/c, C57BL/6N, and CF1 mice to photoperiod changes. Braz J Psychiatry. 2015 Sep;37(3):185–90.

54. Wirz-Justice A, Skene DJ, Münch M. The relevance of daylight for humans. Biochem Pharmacol. 2021 Sep 1;191:114304.

55. Walmsley L, Hanna L, Mouland J, Martial F, West A, Smedley AR, et al. Colour as a signal for entraining the mammalian circadian clock. PLoS Biol. 2015 Apr;13(4):e1002127.

56. Bonmati-Carrion MA, Baño-Otalora B, Madrid JA, Rol MA. Light color importance for circadian entrainment in a diurnal (Octodon degus) and a nocturnal (Rattus norvegicus) rodent. Sci Rep. 2017 Aug 18;7(1):8846.

57. Oliveira MAB de, Scop M, Abreu ACO, Sanches PRS, Rossi AC, Díez-Noguera A, et al. Entraining effects of variations in light spectral composition on the rest-activity rhythm of a nocturnal rodent. Chronobiol Int. 2019 May 6;0(0):1–11.

58. Sulli G, Manoogian ENC, Taub PR, Panda S. Training the Circadian Clock, Clocking the Drugs, and Drugging the Clock to Prevent, Manage, and Treat Chronic Diseases. Trends Pharmacol Sci. 2018 Sep 1;39(9):812–27.

59. Wulff K, Gatti S, Wettstein JG, Foster RG. Sleep and circadian rhythm disruption in psychiatric and neurodegenerative disease. Nat Rev Neurosci. 2010 Aug;11(8):589–99.

60. Haynes PL, Gengler D, Kelly M. Social Rhythm Therapies for Mood Disorders: an Update. Curr Psychiatry Rep [Internet]. 2016 [cited 2019 May 7];18. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4919368/

61. Murray G, Gottlieb J, Hidalgo MP, Etain B, Ritter P, Skene DJ, et al. Measuring circadian function in bipolar disorders: Empirical and conceptual review of physiological, actigraphic, and self-report approaches. Bipolar Disord. 2020 Nov;22(7):693–710.

62. Liguori C, Mombelli S, Fernandes M, Zucconi M, Plazzi G, Ferini-Strambi L, et al. The evolving role of quantitative actigraphy in clinical sleep medicine. Sleep Med Rev. 2023 Apr;68:101762.

63. Pandi-Perumal SR, Smits M, Spence W, Srinivasan V, Cardinali DP, Lowe AD, et al. Dim light melatonin onset (DLMO): a tool for the analysis of circadian phase in human sleep and chronobiological disorders. Prog Neuropsychopharmacol Biol Psychiatry. 2007 Jan 30;31(1):1–11.

64. Bojkowski CJ, Aldhous ME, English J, Franey C, Poulton AL, Skene DJ, et al. Suppression of nocturnal plasma melatonin and 6-sulphatoxymelatonin by bright and dim light in man. Horm Metab Res Horm Stoffwechselforschung Horm Metab. 1987 Sep;19(9):437–40.

65. Landgraf D, McCarthy MJ, Welsh DK. The role of the circadian clock in animal models of mood disorders. Behav Neurosci. 2014 Jun;128(3):344–59.

66. Ávila Moraes C, Cambras T, Diez-Noguera A, Schimitt R, Dantas G, Levandovski R, et al. A new chronobiological approach to discriminate between acute and chronic depression using peripheral temperature, rest-activity, and light exposure parameters. BMC Psychiatry. 2013 Mar 9;13:77.

67. Lyall LM, Wyse CA, Graham N, Ferguson A, Lyall DM, Cullen B, et al. Association of disrupted circadian rhythmicity with mood disorders, subjective wellbeing, and cognitive function: a cross-sectional study of 91 105 participants from the UK Biobank. Lancet Psychiatry. 2018 Jun;5(6):507–14.

68. Tonon AC, Fuchs DFP, Barbosa Gomes W, Levandovski R, Pio de Almeida Fleck M, Hidalgo MPL, et al. Nocturnal motor activity and light exposure: Objective actigraphy-based marks of melancholic and non-melancholic depressive disorder. Brief report. Psychiatry Res. 2017;258:587–90.

69. Logan RW, Edgar N, Gillman AG, Hoffman D, Zhu X, McClung CA. Chronic Stress Induces Brain Region-Specific Alterations of Molecular Rhythms that Correlate with Depression-like Behavior in Mice. Biol Psychiatry. 2015 Aug 15;78(4):249–58.

70. Adan A, Archer SN, Hidalgo MP, Di Milia L, Natale V, Randler C. Circadian typology: a comprehensive review. Chronobiol Int. 2012 Nov;29(9):1153–75.

71. Jury Freitas J, Bertuol Xavier N, Comiran Tonon A, Carissimi A, Timm Pizutti L, Vieira Ilgenfritz CA, et al. 6-Sulfatoxymelatonin predicts treatment response to fluoxetine in major depressive disorder. Ther Adv Psychopharmacol. 2019 Dec 27;9:2045125319881927.

72. Hidalgo MPL, Caumo W, Dantas G, Franco DG, Torres IL da S, Pezzi J, et al. 6-Sulfatoxymelatonin as a predictor of clinical outcome in depressive patients. Hum Psychopharmacol. 2011 Apr;26(3):252–7.

73. Markus R, Franco D, Carvalho L, Gentil V, Gorenstein C. Acute increase in urinary 6-sulfatoximelatonin after clomipramine, as a predictive measure for emotional improvement. J Psychopharmacol (Oxf). 2010 Jun 1;24(6):855–60.

74. Byrne JEM, Bullock B, Murray G. Development of a Measure of Sleep, Circadian Rhythms, and Mood: The SCRAM Questionnaire. Front Psychol [Internet]. 2017 Dec 1 [cited 2018 Oct 16];8. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5717824/

75. Daviss WB, Birmaher B, Melhem NA, Axelson DA, Michaels SM, Brent DA. Criterion validity of the Mood and Feelings Questionnaire for depressive episodes in clinic and non-clinic subjects. J Child Psychol Psychiatry. 2006 Sep;47(9):927–34.

76. McNair DM, Lorr M, Droppleman LF. Profile of Mood States (POMS)–revised manual. 1992;

77. Watson D, Clark LA, Tellegen A. Development and validation of brief measures of positive and negative affect: the PANAS scales. J Pers Soc Psychol. 1988 Jun;54(6):1063–70.

78. Giglio LMF, Magalhães PV da S, Andreazza AC, Walz JC, Jakobson L, Rucci P, et al. Development and use of a biological rhythm interview. J Affect Disord. 2009 Nov;118(1–3):161–5.

79. Wirz-Justice A. Diurnal variation of depressive symptoms. Dialogues Clin Neurosci. 2008;10(3):337–43.

80. de Souza CM, Carissimi A, Costa D, Francisco AP, Medeiros MS, Ilgenfritz CA, et al. The Mood Rhythm Instrument: development and preliminary report. Braz J Psychiatry. 2016 Jun;38(2):148–53.

81. Pilz LK, Carissimi A, Francisco AP, Oliveira MAB, Slyepchenko A, Epifano K, et al. Prospective Assessment of Daily Patterns of Mood-Related Symptoms. Front Psychiatry. 2018;9:370.

82. Pilz LK, Carissimi A, Oliveira MAB, Francisco AP, Fabris RC, Medeiros MS, et al. Rhythmicity of Mood Symptoms in Individuals at Risk for Psychiatric Disorders. Sci Rep. 2018 Jul 30;8(1):11402.

83. Edward Batschelet. Introdução à Matemática para Biocientistas. Editora Interciência; Ed da Universidade de São Paulo; 1978. 618 p.

84. REFINETTI R, LISSEN GC, HALBERG F. Procedures for numerical analysis of circadian rhythms. Biol Rhythm Res. 2007;38(4):275–325.

85. Enright JT. The search for rhythmicity in biological time-series. J Theor Biol. 1965 May 1;8(3):426–68.

86. Sokolove PG, Bushell WN. The chi square periodogram: Its utility for analysis of circadian rhythms. J Theor Biol. 1978 May 8;72(1):131–60.

87. Lomb NR. Least-squares frequency analysis of unequally spaced data. Astrophys Space Sci. 1976 Feb 1;39(2):447–62.

88. Díez-Noguera A. Methods for serial analysis of long time series in the study of biological rhythms. J Circadian Rhythms. 2013 Jul 18;11:7.

89. Lepage WR. Symbolic nomenclature for sinusoids. Electr Eng. 1949 Jul;68(7):561–5.

90. Halberg F, Tong YL, Johnson EA. Circadian System Phase — An Aspect of Temporal Morphology; Procedures and Illustrative Examples. In: von Mayersbach H, editor. The Cellular Aspects of Biorhythms: Symposium on Rhythmic Research Sponsored by the VIIIth International Congress of Anatomy Wiesbaden 8–14 August 1965 [Internet]. Berlin, Heidelberg: Springer; 1967 [cited 2020 Nov 30]. p. 20–48. Available from: https://doi.org/10.1007/978-3-642-88394-1_2

91. Cornelissen G. Cosinor-based rhythmometry. Theor Biol Med Model. 2014 Apr 11;11(1):16.

92. Witting W, Kwa IH, Eikelenboom P, Mirmiran M, Swaab DF. Alterations in the circadian rest-activity rhythm in aging and Alzheimer's disease. Biol Psychiatry. 1990 Mar 15;27(6):563–72.

93. Gonçalves BSB, Adamowicz T, Louzada FM, Moreno CR, Araujo JF. A fresh look at the use of nonparametric analysis in actimetry. Sleep Med Rev. 2015 Apr;20:84–91.

94. Suibkitwanchai K, Sykulski AM, Algorta GP, Waller D, Walshe C. Nonparametric time series summary statistics for high-frequency accelerometry data from individuals with advanced dementia. PLOS ONE. 2020 Sep 25;15(9):e0239368.

95. Jones SH, Hare DJ, Evershed K. Actigraphic assessment of circadian activity and sleep patterns in bipolar disorder. Bipolar Disord. 2005 Apr;7(2):176–86.

96. Zhao X, Tang Q, Feng Z, Zhào H. Executive Performance Is Associated With Rest-Activity Rhythm in Nurses Working Rotating Shifts. Front Neurosci. 2022;16:805039.

97. Dagan Y, Ayalon L. Case study: psychiatric misdiagnosis of non-24-hours sleep-wake schedule disorder resolved by melatonin. J Am Acad Child Adolesc Psychiatry. 2005 Dec;44(12):1271–5.

98. N. I. Fisher. Statistical analysis of circular data. 1st ed. Cambridge University Press; 1993.

99. Edward Batschelet. Circular Statistics in Biology. Londres: Robin Sibson, Joel E. Cohen. Academic Press; 1981. 371 p.

100. Kanti V. Mardia, Peter E. Jupp. Directional Statistics. John Wiley & Sons, Inc.; 2000.

101. Comiran Tonon A, Pilz LK, Amando GR, Constantino DB, Boff Borges R, Caye A, et al. Handling missing data in rest-activity time series measured by actimetry. Chronobiol Int. 2022 Jul;39(7):964–75.

102. Erren TC, Koch MS, Groß JV, Kämmerer-Cruchon S, Fuchs A, Pinger A, et al. Chronomedicine: an old concept's fledging? A selective literature search. Neuro Endocrinol Lett. 2012;33(4):357–60.

103. Klerman E, Kramer A, Zee PC. From Bench to Bedside and Back Again: translating circadian science to medicine. J Biol Rhythms. 2023 Apr;38(2):125–30.

104. Kramer A, Lange T, Spies C, Finger AM, Berg D, Oster H. Foundations of circadian medicine. PLoS Biol. 2022 Mar;20(3):e3001567.

105. Klerman EB, Brager A, Carskadon MA, Depner CM, Foster R, Goel N, et al. Keeping an eye on circadian time in clinical research and medicine. Clin Transl Med. 2022 Dec;12(12):e1131.

106. Awad K, Banach M. The optimal time of day for statin administration: a review of current evidence. Curr Opin Lipidol. 2018 Aug;29(4):340–5.

107. Smolensky MH, Lemmer B, Reinberg AE. Chronobiology and chronotherapy of allergic rhinitis and bronchial asthma. Adv Drug Deliv Rev. 2007 Aug 31;59(9–10):852–82.

108. Münch M, Kramer A. Timing matters: New tools for personalized chronomedicine and circadian health. Acta Physiol Oxf Engl. 2019 Oct;227(2):e13300.

109. Nelson RJ, Bumgarner JR, Liu JA, Love JA, Meléndez-Fernández OH, Becker-Krail DD, et al. Time of day as a critical variable in biology. BMC Biol. 2022 Jun 15;20(1):142.

110. Francisco AP, Cunha AMD, Tonon AC, Scop M, Mathur S, Caropreso L, et al. Adaptation and validation of the Mood Rhythm Instrument for use in Brazilian adolescents. Rev Bras Psiquiatr Sao Paulo Braz 1999. 2022;44(3):264–70.

111. de Oliveira MAB, Scop M, Abreu ACO, Sanches PRS, Rossi AC, Díez-Noguera A, et al. Entraining effects of variations in light spectral composition on the rest-activity rhythm of a nocturnal rodent. Chronobiol Int. 2019;36(7):934–44.

112. Chiesa JJ, Cambras T, Carpentieri AR, Díez-Noguera A. Arrhythmic rats after SCN lesions and constant light differ in short time scale regulation of locomotor activity. J Biol Rhythms. 2010 Feb;25(1):37–46.

APÊNDICE 1 – OUTRAS PRODUÇÕES E PARTICIPAÇÕES

Outros Artigos Publicados Durante o Período de Doutoramento

1. Pilz LK, de Oliveira MAB, Steibel EG, Policarpo LM, Carissimi A, Carvalho FG, Constantino DB, Tonon AC, Xavier NB, da Rosa Righi R, Hidalgo MP. *Development and testing of methods for detecting off-wrist in actimetry recordings*. 2022.
   DOI: 10.1093/sleep/zsac118.
   Revista: Sleep

2. Tonon AC, Constantino DB, Amando GR, Abreu AC, Francisco AP, de Oliveira MAB, Pilz LK, Xavier NB, Rohrsetzer F, Souza L, Piccin J, Caye A, Petresco S, Manfro PH, Pereira R, Martini T, Kohrt BA, Fisher HL, Mondelli V, Kieling C, Hidalgo MPL. *Sleep disturbances, circadian activity, and nocturnal light exposure characterize high risk for and current depression in adolescence*. 2022.
   DOI: 10.1093/sleep/zsac104.
   Revista: Sleep

3. Constantino DB, Tonon AC, de Oliveira MAB, Amando GR, Freitas JJ, Xavier NB, Ribeiro RJ, Idiart M, Hidalgo MPL. *Effects of lighting patterns in pubertal development and metabolism of female wistar rats*. 2022.
   DOI: 10.1016/j.physbeh.2021.113641.
   Revista: Physiology & Behavior

4. Pilz LK, Couto Pereira NS, Francisco AP, Carissimi A, Constantino DB, Caus LB, Abreu ACO, Amando GR, Bonatto FS, Carvalho PVV, Cipolla-Neto J, Harb A, Lazzarotto G, Marafiga JR, Minuzzi L, Montagner F, Nishino FA, Oliveira MAB, Dos Santos BGT, Steibel EG, Tavares PS, Tonon AC, Xavier NB, Zanona QK, Amaral FG, Calcagnotto ME, Frey BN, Hidalgo MP, Idiart M, Russomano T. *Effective recommendations towards healthy routines to preserve mental health during the COVID-19 pandemic*. 2022.
   DOI: 10.1590/1516-4446-2021-2109.
   Revista: Brazilian Journal of Psychiatry

5. Pilz, Luísa K.; Xavier, Nicóli B.; Levandovski, Rosa; Oliveira, Melissa A. B.; Tonon, André C.; Constantino, Débora B.; Machado, Valdomiro; Roenneberg, Till; Hidalgo, Maria Paz. *Circadian Strain, Light Exposure, and Depressive Symptoms in Rural Communities of Southern Brazil*. 2022.
   DOI: 10.3389/fnetp.2021.779136.
   Revista: Frontiers in Network Physiology

6. Rosa GSD, Andrades GS, Caye A, Hidalgo MP, Oliveira MAB, Pilz LK. *Thirteen Reasons Why: The impact of suicide portrayal on adolescents' mental health*. 2019.
   DOI: 10.1016/j.jpsychires.2018.10.018.
   Revista: Journal of Psychiatric Research

Artigos submetidos

1. Adile Nexhaa, Luisa K. Pilz, Melissa A. B. Oliveira, Nicoli B. Xavier, Rogério Boff Borges, Benicio N. Frey, Maria Paz L. Hidalgo. *Greater within- and between-day instability is associated with worse anxiety and depression symptoms*. 2023.
   Revista: Journal of Affective Disorders

Resumos Publicados em Anais de Eventos Científicos

1. Amando GR, Constantino DB, Tonon AC, Oliveira MAB, Freitas JJ, Hidalgo MP. *Avaliação do efeito de diferentes padrões de iluminação no desenvolvimento puberal de ratas wistar.*
   Evento: 39ª Semana Científica do Hospital de Clínicas de Porto Alegre - 2019
   URL: http://hdl.handle.net/10183/211895
2. Rossi AC, Silva Junior DP, Sanches PRS, Medeiros MS, Oliveira MAB, Tonon AC, Hidalgo MP, Schmid M, Magalhães PV, Muller AF. *Equipamento para caracterização de iluminação.*
   Evento: 39ª Semana Científica do Hospital de Clínicas de Porto Alegre - 2019
   URL: http://hdl.handle.net/10183/211833

3. Silva MM; Garay LLS; Serafim PHM; Amando GR; Greco TM; Oliveira MAB; Medeiros MS; Frey BN; Carissimi A; Hidalgo MP. *A influência do humor deprimido nos aspectos neuropsicológicos e falsas memórias*.
   Evento: 39ª Semana Científica do Hospital de Clínicas de Porto Alegre - 2019
   URL: https://hdl.handle.net/10183/213284

4. Serafim PHM; Garay LLS; Amando GR; Silva MM; Greco TM; Oliveira MAB; Thiago Maia Greco; Medeiros MS; Frey BN; Carissimi A; Hidalgo MP. *Avaliação da ritmicidade de humor e sintomas depressivos através do instrumento de ritmo de humor (MRI).*
   Evento: 39ª Semana Científica do Hospital de Clínicas de Porto Alegre - 2019
   URL: https://hdl.handle.net/10183/213285

5. Silva MM; Tonon AC; Oliveira MAB; Constantino DB; Zanona GK; Amando GR; Aniola LL; Enriconi MAA; Calcagnotto MA; Hidalgo MP. *O Impacto do Ensino Remoto Sobre Ciência para Adolescentes durante da Pandemia da Covid-19*
   Evento: 40ª Semana Científica do Hospital de Clínicas de Porto Alegre – 2020
   URL:https://hdl.handle.net/10183/221567

6. Abreu ACOV; Oliveira MAB; Zanona GK; Calcagnotto MA; Hidalgo MP. *Análise Preliminar dos Padrões de Oscilações Cerebrais de Roedores Expostos a Diferentes Sistemas de Iluminação*
   Evento: 40ª Semana Científica do Hospital de Clínicas de Porto Alegre – 2020
   URL:https://hdl.handle.net/10183/233616

7. Abreu ACOV; Oliveira MAB; Alquati T; Tonon AC; Reis MN; Rossi AC; Bonatto FS, Hidalgo MP. *Uso de equipamento de proteção contra luz à noite reduz o tempo até a alta de Unidade de Terapia Intensiva Neonatal*.
   Evento: 42ª Semana Científica do Hospital de Clínicas de Porto Alegre – 2022

8. Moschetta R; Pietra Borges PRC; Tonon AC; Amando GR; Oliveira MAB; Bonatto FS; Xavier NB; Hidalgo MP. *Fatores Preditores De Sonolência Excessiva Diurna Nos Trabalhadores Do Hospital De Clínicas De Porto Alegre.*

Evento: 42ª Semana Científica do Hospital de Clínicas de Porto Alegre – 2022

9. Pietra Borges PRC; Moschetta R; Tonon AC; Amando GR; Oliveira MAB; Bonatto FS; Xavier NB; Hidalgo MP. *A Exposição à Luz Natural por Meio de Janelas e sua Relação com Estresse Percebido e Sintomas Depressivos em Trabalhadores do Hospital de Clínicas de Porto Alegre.*
   Evento: 42ª Semana Científica do Hospital de Clínicas de Porto Alegre – 2022

10. Nexha A,Pilz LK, Oliveira MAB, Xavier NB, Frey BN, Hidalgo MP. *Within- and between-day variability of affective, somatic, and cognitive symptoms against validated scores of anxiety and depression.*
    Evento: 42ª Semana Científica do Hospital de Clínicas de Porto Alegre – 2022

Outras Apresentações de Trabalhos em Eventos Científicos

1. International Society for Bipolar Disorders, Virtual Conference 2020 - Pôster "THE MOOD RHYTHM INSTRUMENT: A CHRONOBIOLOGICAL APPROACH TO EVALUATING MOOD IN CLINICAL SETTINGS.
2. XXXIV FeSBE Annual Meeting. Pôster "VARIATIONS IN SPECTRAL LIGHT COMPOSITION AND ITS EFFECT ON THE REST-ACTIVITY RHYTHM OF WISTAR RATS". 09 a 13 de setembro de 2019, Campos do Jordão Convention Center em Campos do Jordão/SP.

Palestras, aulas e cursos

- Curso na XXXVI Reunião Anual da FESBE. "Ritmos Biológicos: mais perto da sua pesquisa do que você imagina. 2022

- Curso na XLIII SBNeC Annual Meeting. "Biological Rhythms: closer to your research than you ever imagine". 2020

- Curso na XXXIV FeSBE Annual Meeting. "Biological Rhythms: closer to your research than you ever imagine". 2019

- Aula sobre confecção de eletrodos corticais para disciplina de graduação da UFRGS "Eletrofisiologia do Sistema Nervoso Central" coordenada pela professora Maria Elisa Calcagnotto. 2022, 2023.

- Monitoria na disciplina do PPG Psiquiatria e Ciências do Comportamento "Análises de Séries Temporais – Teoria e Prática". 2018.

Extensão Universitária

Participei como tutora no programa de iniciação científica júnior ("IC Jr.", CEP-HCPA 2017-0441) coordenado pelas professoras Maria Paz Hidalgo e Maria Elisa Calcagnotto, cujo objetivo é a introdução de estudantes do ensino médio ao pensamento científico, mas que também possibilita que estes alunos desenvolvam seu próprio projeto e analisem seus resultados.

Durante o doutoramento, orientei os alunos e alguns resultados dos projetos foram apresentados em eventos científicos (Semana Científica HCPA, Salão UFRGS Jovem e MOSTRATEC).

Premiações

- Mérito científico e menção honrosa ao trabalho "O IMPACTO DO ENSINO EM CIÊNCIA A ADOLESCENTES DURANTE A PANDEMIA DA COVID-19", ENDESE ENCONTRO NACIONAL DE DESENVOLVIMENTO EDUCACIONAL (2021).
- Prêmio Jovem Pesquisador (Apresentação: Marina Scop Medeiros. Entraining Effects of Variations in Light Spectral Composition on the Rest-activity Rhythm of a Nocturnal Rodent), Congresso Brain, Behavior and Emotions.

Organização eventos

- CALCAGNOTTO, M. E.; HIDALGO, M. P.; PILZ, L. K.; OLIVEIRA, M. A. B.; TONON, A. C.; CONSTANTINO, D. B.; XAVIER, N. B. Care Conference A Carbon Reduced Conference: The Circadian Clock and its pervasive impact on metabolism from behavior to mechanism. 2019.

Participação em bancas de comissões julgadoras

- OLIVEIRA, M. A. B. XV Salão de Ensino (Sessão Experiências de Ensino na Graduação). 2019. Universidade Federal do Rio Grande do Sul.

Orientações e supervisões

1. Trabalho de conclusão de curso de graduação

Ana Carolina Odebrecht Vergne de Abreu. Estudo randomizado sobre o uso de equipamentos de proteção contra a luz artificial na recuperação de neonatos prematuros. 2021.
Trabalho de Conclusão de Curso (Graduação em Biomedicina) - Universidade Federal do Rio Grande do Sul. Orientadora: Maria Paz Hidalgo. Co-orientadora: Melissa Alves Braga de Oliveira.

2. Iniciação científica

Co-orientação de alunos de graduação e de iniciação científica no Laboratório de Cronobiologia e Sono HCPA/UFRGS (concluídas: 16).

3. Iniciação científica júnior

Co-orientação de alunos de ensino médio e de iniciação científica júnior no Laboratório de Cronobiologia e Sono HCPA/UFRGS (concluídas: 12).

APÊNDICE 2 – ORIENTAÇÕES SOBRE USO DO TEMPLATE DE ESTATÍSTICA CIRCULAR

A imagem abaixo é de um template originalmente criado e compartilhado pelo professor Antoni Diez-Noguera com o Laboratório de Cronobiologia e Sono HCPA/UFRGS. Trata-se de um arquivo de excel contendo as fórmulas e cálculos para alguns dos principais e mais usuais teste de estatística circular, como teste para verificação da uniformidade de distribuição dos dados (Rayleigh), teste paramétrico para comparação entre dois ou mais grupos (Watson-Williams) e teste não paramétrico para comparação de dois (Wheeler-Watson) ou mais grupos (Wheeler-Watson-Mardia).

O template é de fácil uso e permite a realização de análises estatísticas que, nem sempre, estão disponíveis de forma gratuita em softwares mais usuais de estatística. No entanto, deve-se considerar as seguintes orientações:

- Verificar a quantidade padrão para entrada de grupos e número dados para as análises. O template permite edição das fórmulas para abranger mais dados e mais grupos se necessário;
- Os dados inseridos no arquivo devem estar na unidade radianos para que os cálculos sejam aplicados adequadamente;
- Para cada dado circular, calcula-se o seno e o cosseno do respectivo ângulo. A partir da soma dos senos (S) e cossenos (C) dos dados de um determinado grupo, calcula-se o comprimento do vetor resultante (R) e também o ângulo médio do grupo (ϕ). As fórmulas para os diferentes testes vão variar, mas levam em consideração estas variáveis inicialmente calculadas.

índice dos grupos | índice dos grupos a comparar. 0=>não se usa | XX = não paramétrico

grupo	dados
1	2,10
1	2,20
1	2,75
1	2,00
1	3,00
1	2,35
1	2,25
1	2,15
1	2,60
1	2,65
2	4,20
2	4,45
2	4,00
2	4,25
2	4,45
2	4,15
2	4,71
2	4,30
2	4,25
2	4,10
3	6,15
3	2,40
3	5,70
3	5,30
3	2,15
3	3,25
3	6,00
3	5,90
3	6,10
3	1,20

			Rayleigh
1	n·1	10	
	C·1	-7,0401	
	S·1	6,41878	
	R·1	9,52699	
	c·1	-0,704	
	s·1	0,64188	
	sd·1	0,30757	
	ϕ·1	2,40232	
	r·1	**0,9527**	
	p(r)·1	**5,2E-06**	
2	n·2	10	
	C·2	-4,0625	
	S·2	-8,9306	
	R·2	9,81125	
	c·2	-0,4063	
	s·2	-0,8931	
	sd·2	0,1943	
	ϕ·2	-1,9977	
	r·2	**0,98112**	
	p(r)·2	**1,3E-06**	
3	n·3	10	
	C·3	3,33462	
	S·3	-0,015	
	R·3	3,33466	
	c·3	0,33346	
	s·3	-0,0015	
	sd·3	1,15459	
	ϕ·3	-0,0045	
	r·3	**0,33347**	
	p(r)·3	**0,33716**	

Wheeler-Watson

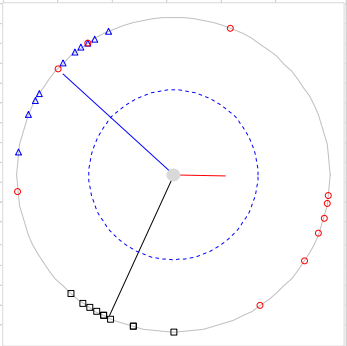
1	n·1	10
	C·1	1
	S·1	-6,31375
	R·1	6,39245
	W·1	8,17269
2	n·2	10
	C·2	-0,98644
	S·2	6,35548
	R·2	6,43158
	W·2	8,27305
	W	**16,4457**
	χ^2	5,9915
	p(W)	**0,00027**

Watson-Williams

	k	3
	n	30
	C	-7,76801
	S	-2,52685
	R	8,16865
	r-	0,75576
	n-	10
	( )	2,4111
	κ	2,32815
	g	**1,1611**
	F	**31,0281**
	F(2,27)	3,3541
	p(F)	**0,00142**

Wheeler-Watson-Mardia

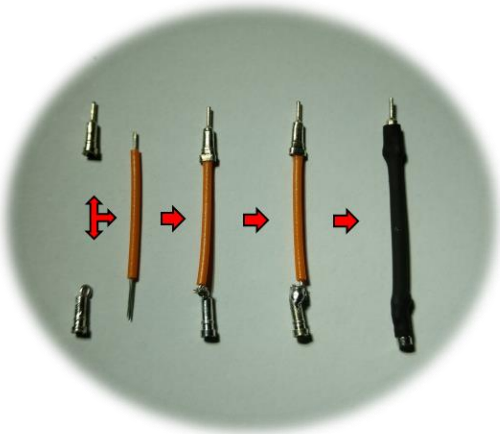
1	n·1	10
	C·1	1,26122
	S·1	-7,2434
	R·1	7,35238
	W·1	10,8115
2	n·2	10
	C·2	-6,1674
	S·2	5,56184
	R·2	8,30489
	W·2	13,7942
3	n·3	10
	C·3	4,90393
	S·3	1,69227
	R·3	5,18771
	W·3	5,38246
	W	**29,9882**
	χ^2	9,4877
	p(W)	**4,9E-06**



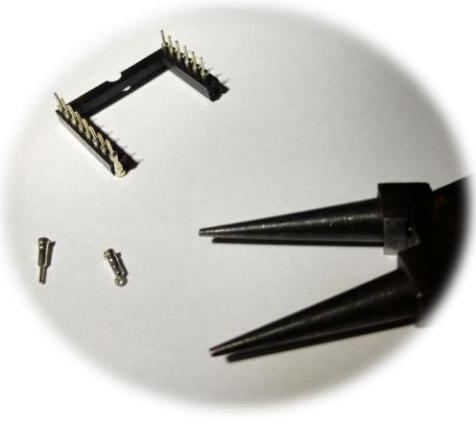
APÊNDICE 3 – PROTOCOLO DE CONFECÇÃO DE ELETRODOS CORTICAIS E MUSCULARES PARA EEG

PASSO A PASSO (obsvervar os números das imagens)

1. Retirar os pinos torneados do soquete com um alicate de ponta fina e outro de ponta chata, fazer um anel em metade dos pinos retirados. Para cada eletrodo, se usa 1 pino reto e 1 com a ponta em anel;
2. Cortar um pedaço de fio, desencapar as extremidades (pode ser com alicate de desencapar ou com uma lâmina de bisturi) cuidando para que, em uma das extremidades, sobre mais de fio desencapado do que na outra;
3. Na extremidade mais curta, encaixar o pino reto e apertar com alicate bem no local de sua junção com o fio. Não apertar muito forte para não quebrar os fios. Na extremidade mais longa, passar os fios de metal desencapados pelo anel formado na ponta do pino e enrolar no próprio anel;
4. Aplicar solda na junção dos fios desencapados com o pino com ponta em anel;
5. Cortar tubo termo retrátil suficiente para cobrir praticamente todo o eletrodo e, com um isqueiro, esquentar o tubo para que ele colabe e sele o eletrodo;



Evolução da construção



Pinos em detalhe

APÊNDICE 3 – PROTOCOLO PARA IMPLANTE DE ELETRODOS CORTICAIS

O protocolo foi desenvolvido juntamente com a equipe da Unidade de Experimentação Animal (UEA) do Hospital de Clínicas de Porto Alegre (HCPA).

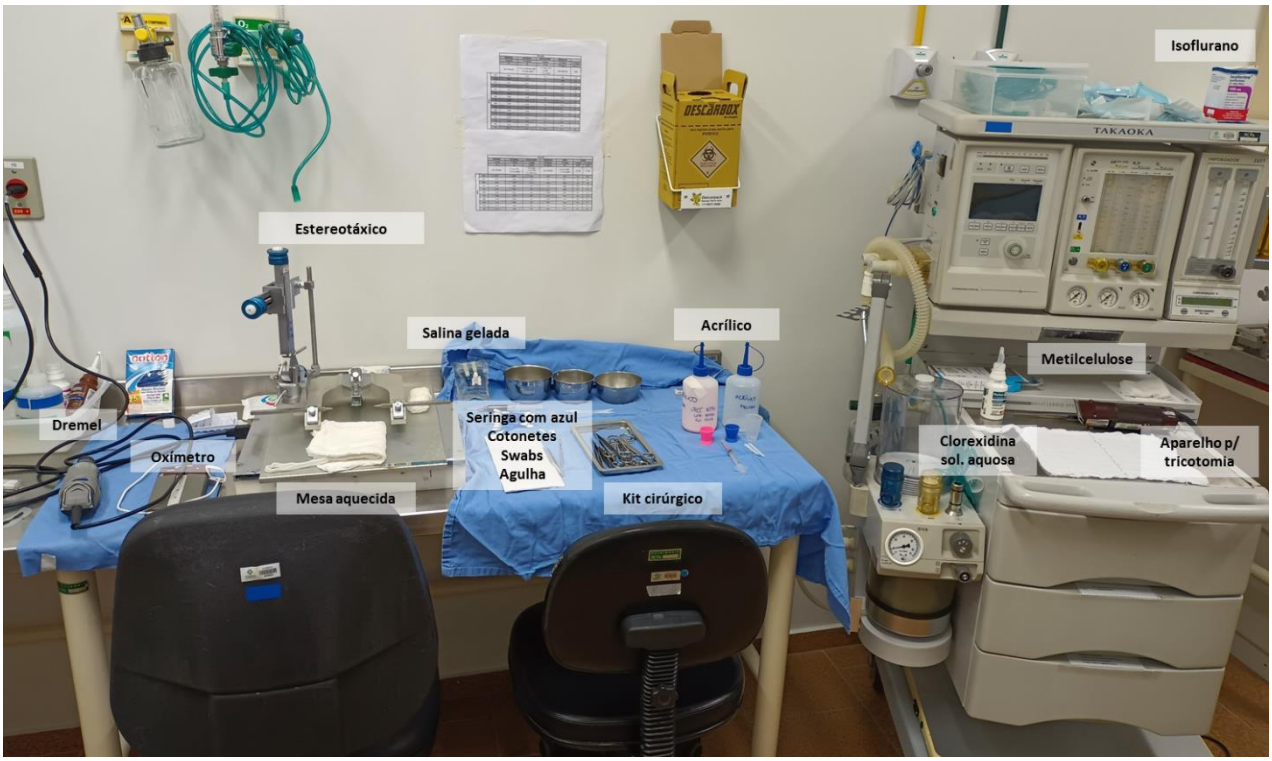
Local de execução: Bancada de experimentação para ratos (UEA)
Resultados esperados: Implantação de 5 eletrodos corticais na calota craniana e eletrodo muscular para monitoramento da atividade eletroencefalográfica (EEG) e muscular.

MATERIAIS

EPIs específicos da área de experimentação (roupa, touca, luvas, máscara)
Aparelho de anestesia/vaporizador
Mesa de estereotáxica
Mesa aquecida
Compressa para a elevação do corpo do animal (posicionamento na mesa de estereotáxica)
Kit de anestesia para roedores (tubo e bocais)
Oxímetro NONIN + adesivos próprios para fixação do sensor
Tricotomizador
Furadeira DREMEL
Broca cortante 43R AESCULAP
Parafusos de relojoeiro para ancoragem do capacete no crânio
Chave para rosquear os parafusos
Extensão elétrica
Incubadora
Bandeja de instrumental para microcirurgia (tesoura pequena, pinças)
Descolador e sindesmótomo
Cubas – para salina resfriada e para descarte das gazes e cotonetes com sangue
Eletrodos prontos e embebidos no álcool 70
Gazes, cotonetes e torundas esterilizadas
Luvas cirúrgicas estéreis
Acrílico auto-polimerizante (pó e líquido)
Copo de silicone (dappen) para o preparo do acrílico
1 Soro fisiológico 100ml – colocar na geladeira para resfriar
Seringas 3 ml e agulhas 25x12 – preencher 1 seringa com solução salina e outra será utilizada para aplicação do acrílico
Metilcelulose para proteção ocular
Esponja Hemostática Cutanplast
Campo cirúrgico estéril
Ampola de adrenalina
Frasco de bupivacaína para infiltração sob a pele da cabeça (misturar 0,1 ml de adrenalina no frasco de 20 mL de bupivacaína 0,5% - o restante da adrenalina colocar em seringa de 1 mL IDENTIFICADA)
Isoflurano
Ampolas de morfina (2,5 mg/kg – 0,025 mL/100 g), dipirona (500 mg/kg – 0,1 mL/100 g), tramadol (20 mg/kg – 0,04 mL/100 g)
Micropore ou fita adesiva

PROCEDIMENTOS

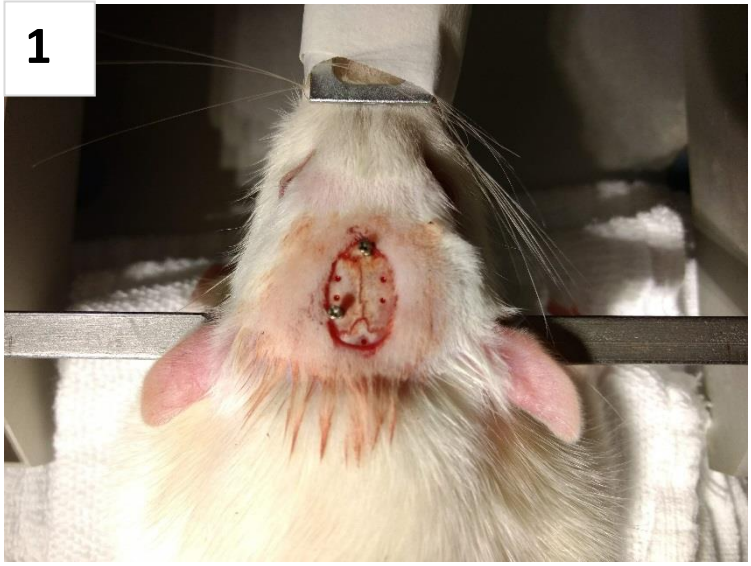
De preferência, deixar a bancada pronta com antecedência, apenas deixando para abrir os itens esterilizados na hora do procedimento. Abaixo, uma foto ilustrativa.



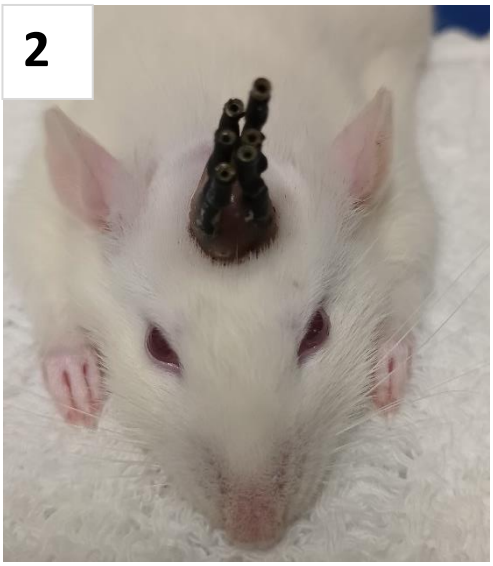
1. Na sala com bancada de experimentação para ratos, ligar a incubadora (30 graus; fluxo 2 L/min) e a mesa aquecida, que deve estar posicionada sob a mesa de estereotaxia;
2. Pesar os animais, calcular as medicações e preencher as fichas anestésicas (modelo ao final do protocolo);
3. Sobre a bancada do aparelho de anestesia inalatória, anestesiar o animais com isoflurano via máscara (500 mL/min de O2 + isoflurano 5% ou 100 mL/min). Após perda do tonus postural, reduzir o isoflurano para 2% ou 30 mL/min. Anotar o tempo de indução;
4. Aplicar a morfina (2,5mg/kg) e anotar o tempo da aplicação;
5. Avaliar a perda do reflexo digital e ajustar o plano para que ele se mantenha ausente;
6. Realizar a tricotomia da área cirúrgica;
7. Aplicar metilcelulose nos olhos;
8. Realizar a antissepsia da área cirúrgica com clorexidine aquoso 2%;
9. Realizar a anestesia local com infiltração de bupivacaina/adrenalina (4mg/kg), a qual deve ser dispersa por toda a calota (não somente na linha de incisão);
10. Posicionar o animal em decúbito esternal sobre o estereotáxico, movendo o tubo com anestésico inalatório juntamente com o animal. Posicionar a boca no animal no aparelho, ajustar na área do focinho, retirar máscara do tubo e usar fita adesiva para criar “cabana” que concentre o anestésico inalatório próximo às narinas e impeça que o animal respire pela boca (foto 1);
11. Posicionar barras auriculares com cuidado para não romper tímpano nem pressionar nervos. Se o animal piscar, ajustar posição das barras;
12. Posicionar o sensor do oxímetro no membro pélvico com o auxílio do adesivo próprio;

13. Disponibilizar sobre o campo de tecido o instrumental e demais materiais necessários ao procedimento e posicionar broca na Dremel;
14. Calçar luvas cirúrgicas;
15. Realizar nova antissepsia do sítio de incisão com clorexidine aquoso 2%;
16. Incisar pele e divulsionar o tecido subjacente (subcutâneo + periósteo);
17. Anotar o horário do início do procedimento cirúrgico;
18. Utilizar solução salina resfriada para limpar a área e auxiliar na hemostasia;
19. Com base nas suturas cranianas, realizar as medições dos locais para perfuração;
20. Embeber a ponta da agulha guia no estereotáxico com azul de tolueno e marcar na calota craniana os locais para os furos;
21. Iniciar a perfuração com a broca. Fazer todos os orifícios correspondentes aos 2 parafusos de ancoragem e aos 5 eletrodos de registro (foto 1);
22. Em caso de sangramento, manter a esponja hemostática por 5 minutos para a realização da hemostasia e, em caso de insucesso, pingar uma gota de adrenalina;
23. Posicionar e rosquear os parafusos de ancoragem até que atravessem o crânio, mas não lesionem o córtex. Eles precisam estar muito firmes e fixos para correta ancoragem do capacete;
24. Posicionar todos os eletrodos nos furos (menos o muscular), segurá-los com firmeza, mas sem pressioná-los, limpar com cotonete e swab qualquer sangue que saia dos furos nesta hora para que o crânio fique seco e, ao mesmo tempo, preparar a resina acrílica (um pouco mais líquida nesta fase);
25. Colocar o acrílico em uma seringa de 3ml e com uma agulha 25x12 (grossa), aplicar a resina entre os eletrodos, cuidando para ela permanecer apenas no local desejado e não escorrer para a pele. Esperar secar um pouco e posicionar o eletrodo muscular;
26. Aplicar resina mais firme em toda a região juntando o eletrodo muscular com o resto do capacete até cobrir completamente os parafusos;
27. ATENÇÃO: a resina acrílica não pode entrar em contato com o fio do eletrodo muscular, apenas com o resto do eletrodo onde há cobertura com tubo preto termoretrátil;
28. Antes da resina secar e endurecer por completo, desgrudar o capacete aderido à pele para diminuir o desconforto do animal, a vontade de coçar e o risco de remover o capacete (resultado final na foto 2);
29. Com o capecete seco, desligar o isoflurano e deixar apenas oxigênio;
30. Esperar cerca de 1 minuto para retirar o animal do aparelho, deixá-lo sobre a mesa aquecida e aproveitar que o animal ainda não acordou para cobrir as pontas dos eletrodos com micropore ou fita adesiva;
31. Aplicar dipirona IP (500mg/kg) com fluído até completar a seringa;
32. Esperar que o animal esteja bem ativo para aplicar o meloxicam (1mg/kg);
33. Colocá-lo em sua caixa moradia limpa, forrada com papel, com comida e água, dentro da incubadora aquecida e oxigenada. Lembrar de deixar a grade invertida;
34. No final do dia, iniciar protocolo com tramadol IP (20mg/kg) e meloxicam IP (1mg/kg) a cada 12h durante 3 dias. Levar o animal de volta para a estante de fotoperíodo em sua caixa moradia, desta vez com maravalha, comida dentro da caixa e água sobre a grade invertida (foto 3).

1



2



3

FICHA DE REGISTRO ANESTÉSICO

Data: ____/____/______ **Animal n°: ________________________** **Peso:_________________**

Med. pré-anestésica:_____________________________________ Hora: _________ Via: ____________

HORA														
FC														
$Sp0_2$														
FR														
ISSO/0_2														
FLUIDO														
TEMP														

HORA INÍCIO CIRURGIA: ____________ HORA FIM CIRURGIA: ______________

Observações:____
____

Data: ____/____/______ **Animal n°: ________________________** **Peso:_________________**

Med. pré-anestésica:_____________________________________ Hora: _________ Via: ____________

HORA														
FC														
$Sp0_2$														
FR														
ISSO/0_2														
FLUIDO														
TEMP														

HORA INÍCIO CIRURGIA: ____________ HORA FIM CIRURGIA: ______________

Observações:____
____

Data: ____/____/______ **Animal n°: ________________________** **Peso:_________________**

Med. pré-anestésica:_____________________________________ Hora: _________ Via: ____________

HORA														
FC														
$Sp0_2$														
FR														
ISSO/0_2														
FLUIDO														
TEMP														

HORA INÍCIO CIRURGIA: ____________ HORA FIM CIRURGIA: ______________

Observações:____
____

APÊNDICE 4 - PROTOCOLO DE CIRURGIA DE IMPLANTAÇÃO DE IBUTTON

iButton: aparelho utilizado para registro contínuo de temperatura corporal.

Materiais

- IButton (limpar com álcool previamente)
- Isoflurano
- Kit de anestesia para roedores (tubo e bocais)
- Tramadol
- Seringa de insulina
- Mesa aquecida
- Tricotomizador
- Álcool iodado (preferência para o hidroalcoólico)
- Lâmina de bisturi nº 15
- Bisturi
- Fio monolyn 4.0
- 2 pinças (uma pequena e a outra de preferência com garra)
- Porta agulha
- Tesoura pequena
- Gaze
- Campo estéril
- Luva cirúrgica
- Fita adesiva/esparadrapo

Preparação

1. Cobrir mesa do bloco cirúrgico com campo azul, sobre ela colocar a mesa aquecida, e sobre a mesa aquecida outro campo estéril menor. Ligar a mesa aquecida.
2. Preparar seringa com Tramadol. Utiliza-se dose de 10% o peso do animal em microlitro (µl). Considerando que 1 µl = 0,001ml, um animal de aproximadamente 400g receberá 0,04ml.
3. Ajustar o kit anestésico ao equipamento de anestesia disponível (bomba ou vaporizador).
4. Encher compartimento do isoflurano na máquina, lembrando que para cirurgia de dois ratos são usados, com folga, 10ml de isoflurano. Ajustar 500 mL/min de O2. +.
5. Anestesiar o animal. Para indução usar isoflurano 5% ou 100 mL/min. Após perda do tonus postural, reduzir o isoflurano para 2% ou 30 mL/min.
6. Quando o animal estiver sem reflexo doloroso, posicioná-lo em decúbito dorsal sobre a mesa aquecida, ajustando e fixando o tubo da anestesia inalatória.

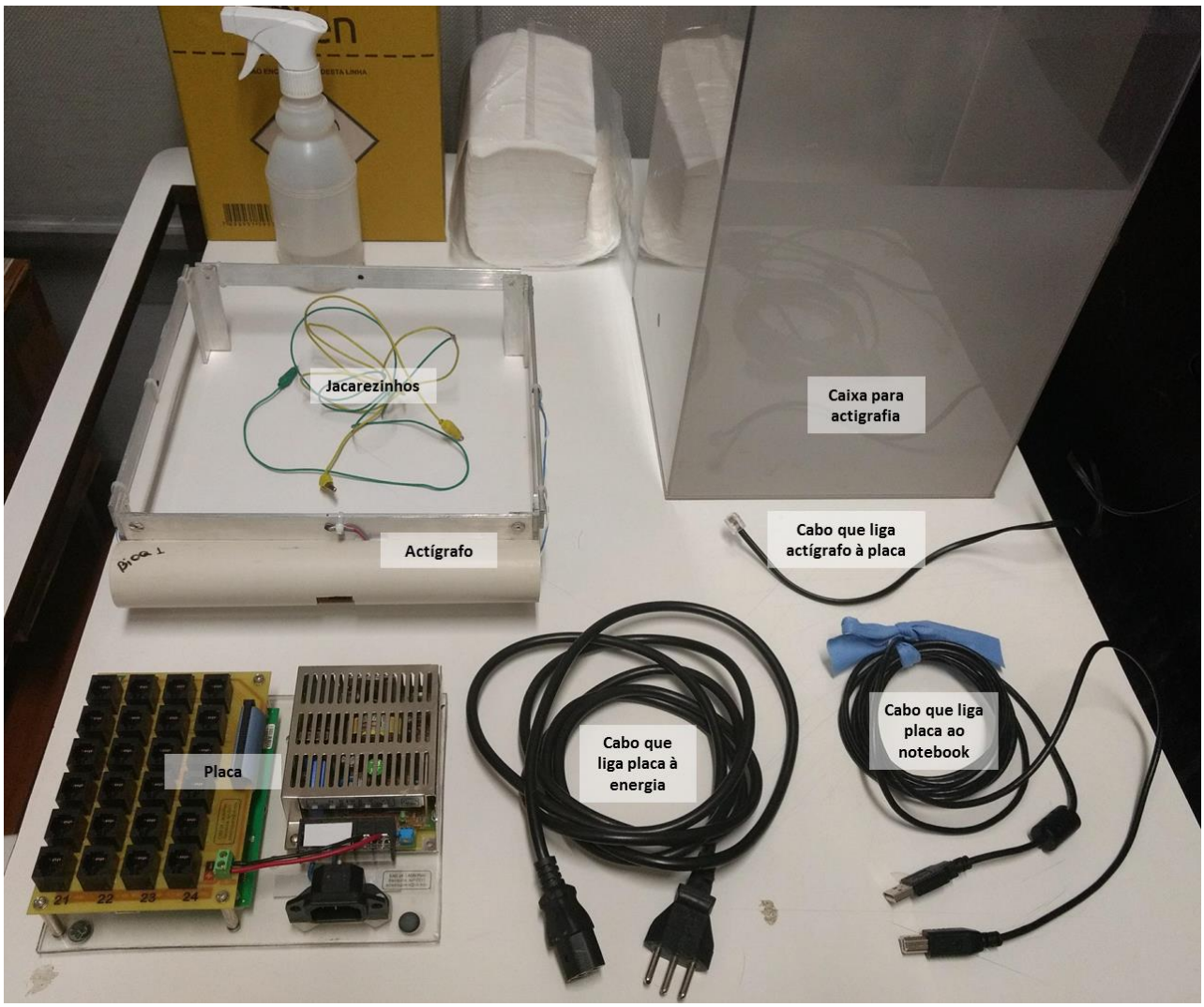
Cirurgia

1. Realizar tricotomia no abdômen.
2. Realizar a antissepsia da área cirúrgica com clorexidine aquoso 2%;
3. Aplicar Tramadol via intraperitoneal.
4. Colocar lâmina no bisturi.
5. Calçar lúvas cirúrgicas.
6. Com o bisturi, realizar incisão longitudinal na pele do abdômen de cerca de 2,5cm.
7. Pinçar e cortar o músculo abdominal com tesoura até o mesmo tamanho de abertura da pele, seguindo a linha alba (mais esbranquiçada). Se enxergar o intestino, estará no lugar certo, ou seja, cavidade abdominal, e não entre pele/músculo/fáscia.
8. Inserir o iButton pela abertura em direção às patas direitas do animal, atentando para que a parte de maior superfície (parte plano do iButton) fique em contato com os órgãos e não com a pele.
9. Com o tamanho de abertura sugerida, normalmente são feitos de 2 a 3 pontos, deixando distância de aproximadamente 1-2 mm entre eles.
10. Após fechar o tecido muscular, fechar a pele seguindo o mesmo procedimento. Contudo, deixar ± 1cm de fio sobrando nos dois lados do ponto.
11. Desligar anestesia e manter oxigênio.
12. Passar gaze com álcool iodado na cicatriz cirúrgica.
13. Esperar animal acordar e coloca-lo em caixa limpa posicionado de lado.

APÊNDICE 5 - PROTOCOLO DE VÍDEO EEG COM ACTIGRAFIA

Na sala de eletrofisiologia *in vivo*, começamos ajustando os equipamentos para **actigrafia**:

1. Actígrafo deve estar posicionado ao redor da caixa moradia, e ambos dentro da caixa preta de registro;
2. Cabo que liga o actígrafo à placa deve estar conectado. Ele sai do actígrafo, sobre por onde tem a câmera e os fios do equipamento de EEG, passa por cima da gaiola até chegar na placa que está sobre a mesa na frente da porta;
3. Placa deve estar ligada à energia através de cabo preto;
4. Placa deve estar conectada ao notebook através de outro cabo;
5. Computador deve estar equipado com o software DAS192USB;
6. Conferir se luz vermelha do actígrafo acende cada vez que os feixes infravermelhos são cruzados (simular animal andando dentro da caixa);
7. Não se esquecer de aterrar o actígrafo, colocando um dos "jacarezinhos" no aparelho e outro na gaiola;
8. Com todo ok, seguir os passos referentes ao EEG.



Vídeo EEG

1. Ligar o comutador (caixa azul em cima da gaiola);
2. Ligar o PLEXON (botão preto –**o** na caixa de metal sobre o computador);
3. Ligar o computador (tatear até achar o botão na CPU);
4. Na área de trabalho do computador

   4.1 Abrir “Server” e esperar até aparecer “Program loaded”;

   4.2 Abrir “SortClient”

   4.2.1 Selecionar os canais desejados em “Analog channels”

   4.2.2 Apertar “Start”

   4.2.3 Se for necessário mudar de canais, selecionar os desejados e desativar os outros e depois apertar em “Update Server”

   4.2.4 Conectar fios no animal

   4.2.5 Ligar amplificador na primeira chave do PLEXON

   4.2.6 Visualizar traçados em “Activity Display” (ícone preto com oscilações em verde)

   4.3 Abrir “CPX Studio”

   4.3.1 Checar pasta de destino dos arquivos

   4.3.2 Armar câmera no **A**

   4.4 No “SortClient” clicar em “Start” (ícone com disquete e sinal de play >)

   4.4.1 Para registro com tempo máximo, que é nosso caso já que o registro é longo e os arquivos não podem ter mais de 3h, entrar no menu “ Start and Stop”

   4.4.2 Selecionar “Time-based Start and Stop”

   4.4.3 Clicar em “Stop and re-start options”

   4.4.4 Colocar tempo desejado de 3h

   4.4.5 Em “File generation” escolher o nome dos arquivos

   4.5 No “SortClient”, dar zoom na segunda lupa com um + para ver melhor os traçados
5. Para parar o registro, dar “Stop” no “SortClient” and desarmar a câmera no “CPX Studio”

APÊNDICE 6 - OUTROS PROJETOS E COLABORAÇÕES DESENVOLVIDAS

Participação em outros projetos

Análise e discussão dados do projeto que buscou compreender rotinas de sono, ritmo de atividade e repouso, exposição à luz e sintomas depressivos em comunidades quilombolas no sul do Brasil (CEP-HCPA 2011–0502 e 2015–0568)

1. Desenho, coleta, análise e discussão de dados de estudo experimental sobre a influência da exposição a diferentes sistemas de sistemas de iluminação no desenvolvimento puberal e no metabolismo de ratas Wistar (CEP-HCPA 2016-0378)
2. Criação de sensores de ambiente e caracterização da iluminação da Unidade de Internação Psiquiátrica do HCPA (CEP-HCPA 2017-0425)
3. Tutoria de alunos de iniciação científica júnior no projeto de extensão "Construindo Pontes entre a Escola e a Universidade Pública: Iniciação ao Ensino de Ciências, Pesquisa e Inovação" (CEP-HCPA 2017-0441)
4. Desenho do estudo de avaliação do impacto dos ambientes físicos de trabalho na saúde mental e nos ritmos biológicos de trabalhadores do Hospital de Clínicas de Porto Alegre (2020-0272)
5. Desenho, coleta, análise e discussão dos dados do estudo sobre ritmos biológicos, sono e humor em indivíduos em distanciamento social (CEP-HCPA 2020-0128)

Colaborações internacionais durante o período de doutoramento

Desde meu mestrado iniciado em 2016, sigo em colaboração com o professor Antoni Díez-Noguera, professor da Universitat de Barcelona, que possui extensa experiência em estudos experimentais com roedores, actigrafia e análise de dados seriados. Juntos ministramos cursos acerca dos métodos de análise de séries temporais para o Programa de Pós-graduação de Psiquiatria e Ciências do Comportamento e publicamos o artigo fruto do meu mestrado em revista internacional relevante na área de Cronobiologia.

Com o desenvolvimento do instrumento para avaliação do ritmo de humor me 2016, venho contribuindo para a manutenção das colaborações internacionais com a professora Ana Adan da Universitat de Barcelona na Espanha e do professor Benicio Frey da McMaster University no Canadá, através das quais, traduzimos e validamos as versões espanhola e inglesa do MRhI. Além disso, o Dr Euclides Mendonça, atualmente da McGill University, é um importante colaborador com conhecimento importante em psicometria que vem contribuindo nos estudos em andamento no Laboratório de Cronobiologia e Sono do HCPA/UFRGS com aplicação do MRhI em amostras clínicas.

Atualmente, mantenho colaborações ativas para tradução e validação do MRhI para persa juntamente com Mohammad Niroumand Sarvandani da Shahroud University of Medical Sciences no Irã, e para validação no MRhI entre estudantes de ensino médio no México juntamente com Arturo Arrona Palacios da Harvard Medical School nos Estados Unidos.

LISTA DE ANEXOS

ANEXO 1

Supplemental material for **English Version of Mood Rhythm Instrument**

Mood Rhythm Instrument - MRhI

Date: ____/_____/_____ Time: _____ Sex: ( ) F ( ) M Subject ID: _____
Date of last period: ____/____/_____

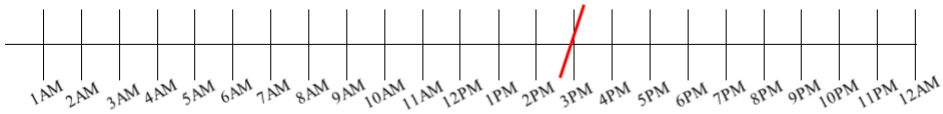
Age: _____ Level of education (years of schooling): _____

Please read carefully the following examples before answering the questions:

Following each question, a 24-hour period is shown. Each number represents an hour of the clock. The line between the numbers represents the minutes.

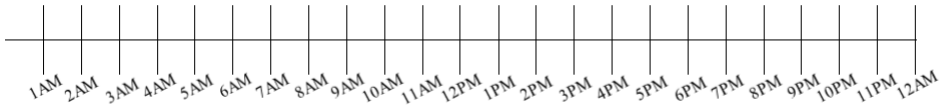
Example (A): if you mark a line between 7AM and 8AM, it will represent 7:30 AM

(B) If you mark a line on 3PM, it will represent 3:00 PM



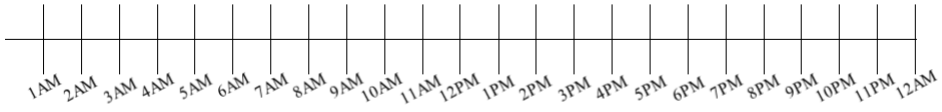
Answer the following questions according to the **last 15 days**, taking into account how you have felt most of the time, on the majority of the days.

1. Is there a specific time of the day when you have felt more alert?
( ) Yes ( ) No
If you answer yes, indicate below the approximate hour:



2. Is there a specific time of the day when you have felt sleepier?
( ) Yes ( ) No
If you answer yes, indicate below the approximate hour:

3. Is there a specific time of the day when you have felt more capable of solving daily problems?
( ) Yes ( ) No
If you answer yes, indicate below the approximate hour:



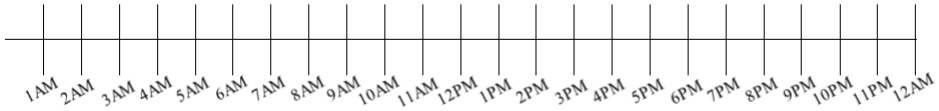
Supplemental material for **English Version of Mood Rhythm Instrument**

4. Is there a specific time of the day when your self-esteem has been higher?
( ) Yes ( ) No
If you answer yes, indicate below the approximate hour:

5. Is there a specific time of the day when you been able to concentrate better?
( ) Yes ( ) No
If you answer yes, indicate below the approximate hour:

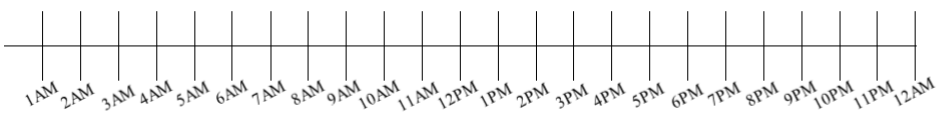
6. Is there a specific time of the day when you have had an increased appetite?
( ) Yes ( ) No
If you answer yes, indicate below the approximate hour:

7. Is there a specific time of the day when your libido (sexual arousal) has been higher?
( ) Yes ( ) No
If you answer yes, indicate below the approximate hour:



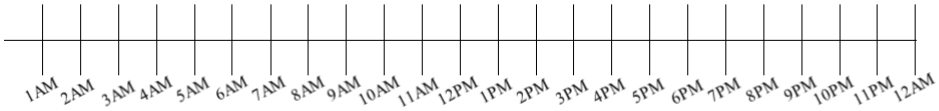
8. Is there a specific time of the day when you have felt more irritable?
( ) Yes ( ) No
If you answer yes, indicate below the approximate hour:

9. Is there a specific time of the day when you have felt more anxious?
( ) Yes ( ) No
If you answer yes, indicate below the approximate hour:



Supplemental material for **English Version of Mood Rhythm Instrument**

10. Is there a specific time of the day when you have felt more sad?
( ) Yes ( ) No
If you answer yes, indicate below the approximate hour:



11. Is there a specific time of the day when you have felt more motivated to exercise?
( ) Yes ( ) No
If you answer yes, indicate below the approximate hour:

12. Is there a specific time of the day when your memory has been better?
( ) Yes ( ) No
If you answer yes, indicate below the approximate hour:

13 Is there a specific time of the day when you have been more pessimistic?
( ) yes ( ) No
If you answer yes, indicate below the approximate hour:

14. Is there a specific time of the day when you have preferred talking to friends face-to-face?
( ) yes ( ) No
If you answer yes, indicate below the approximate hour:

15. Is there a specific time of day when you have had more energy and motivation to do things?
( ) yes ( ) No
If you answer yes, indicate below the approximate hour:

ANEXO 2

INSTRUMENTO DE RITMO DE ESTADO DE HUMOR
(MOOD RHYTHM INSTRUMENT - MRhI)

Fecha: ___/___/___ Horario: _____

Sexo: ( ) Hombre ( ) Mujer Fecha de la última menstruación: ___/___/___

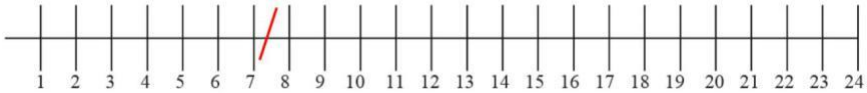
Edad (en años): _____ Fecha de nacimiento: ___/___/___

Enseñanza (Años completos):

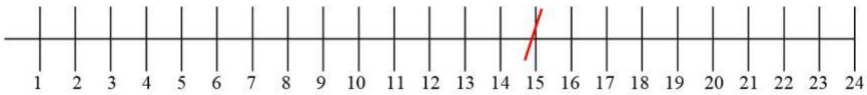
Lea atentamente las siguientes observaciones antes de contestar al cuestionario:

En cada pregunta se muestra un período de 24 horas. Cada número representa la hora del reloj. La línea entre los números representa los minutos.

Por ejemplo, (A) si marca una línea entre 7 y 8 corresponderá a las 7:30h.

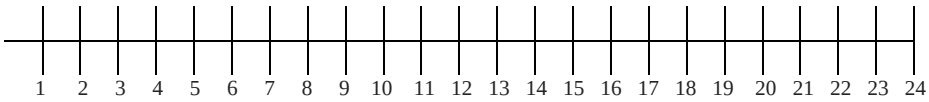


(B) Si marca una línea en el 15 corresponderá a las 15:00h.

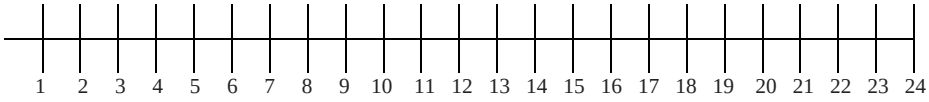


Conteste a las siguientes preguntas **considerando los últimos 15 días**, teniendo en cuenta cómo se siente la mayor parte del tiempo, casi todos los días:

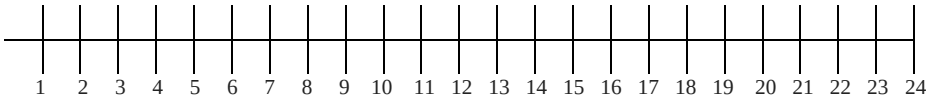
1. ¿Hay alguna hora en la que se sienta más alerta?
( ) Sí ( ) No
En caso afirmativo, indique la hora aproximada:



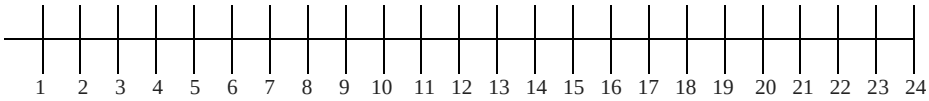
2. ¿Hay alguna hora en la que se sienta con más sueño?
( ) Sí ( ) No
En caso afirmativo, indique la hora aproximada:



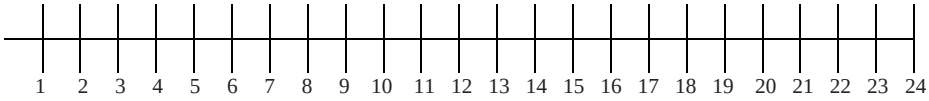
3. ¿Hay alguna hora en la que se sienta con mejor capacidad para solucionar los problemas cotidianos?
( ) Sí ( ) No
En caso afirmativo, indique la hora aproximada:



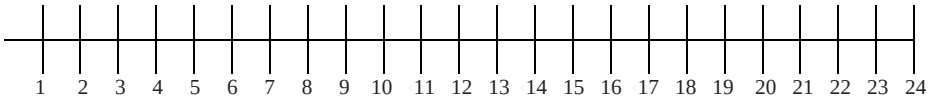
4. ¿Hay alguna hora en la que su autoestima es mejor?
( ) Sí ( ) No
En caso afirmativo, indique la hora aproximada:



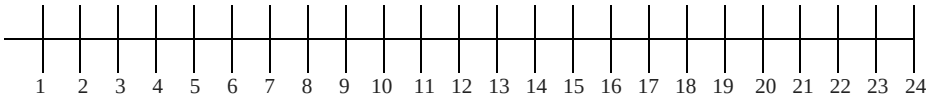
5. ¿Hay alguna hora en la que su concentración es mejor?
( ) Sí ( ) No
En caso afirmativo, indique la hora aproximada:



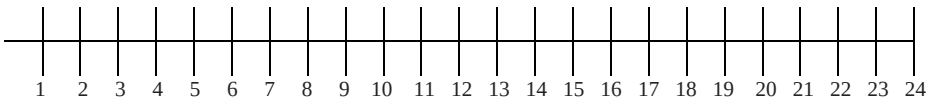
6. ¿Hay alguna hora en la que tiene más apetito?
( ) Sí ( ) No
En caso afirmativo, indique la hora aproximada:



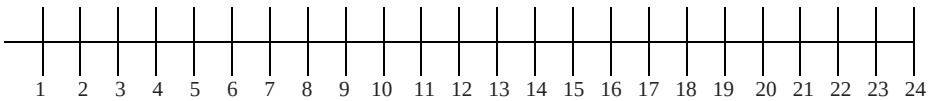
7. ¿Hay alguna hora en la que su libido (deseo sexual) sea mayor?
( ) Sí ( ) No
En caso afirmativo, indique la hora aproximada:



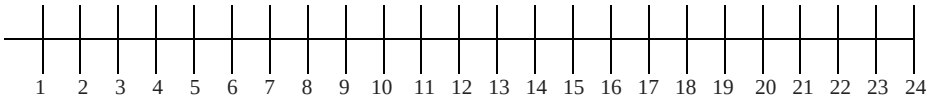
8. ¿Hay alguna hora en la que se sienta más irritable?
( ) Sí ( ) No
En caso afirmativo, indique la hora aproximada:



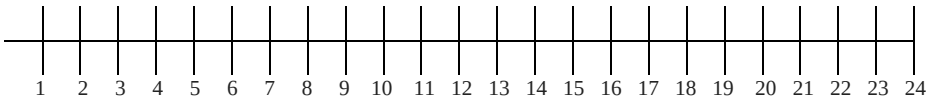
9. ¿Hay alguna hora en la que se sienta más ansioso?
( ) Sí ( ) No
En caso afirmativo, indique la hora aproximada:



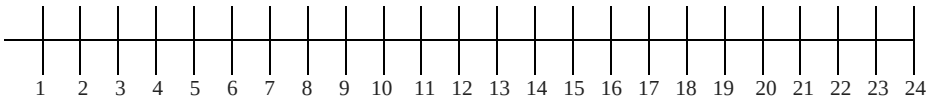
10. ¿Hay alguna hora en la que se sienta más triste?
( ) Sí ( ) No
En caso afirmativo, indique la hora aproximada:



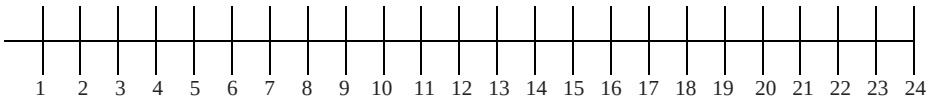
11.¿Hay alguna hora en la que se sienta con más disposición para realizar ejercicio físico?
( ) Sí ( ) No
En caso afirmativo, indique la hora aproximada:



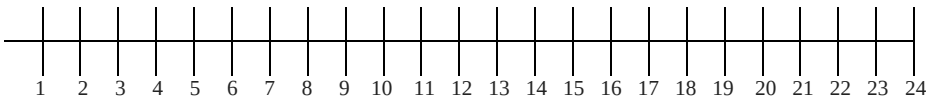
12. ¿Hay alguna hora en la que su memoria es mejor?
( ) Sí ( ) No
En caso afirmativo, indique la hora aproximada:



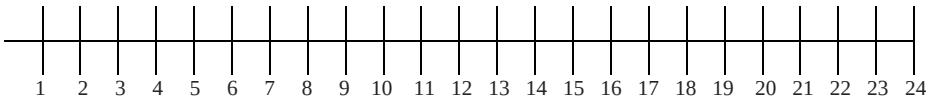
13. ¿Hay alguna hora en la que se sienta más pesimista?
( ) Sí ( ) No
En caso afirmativo, indique la hora aproximada:



14. ¿Hay alguna hora que prefiera para hablar en persona con amigos?
( ) Sí ( ) No
En caso afirmativo, indique la hora aproximada:



15. ¿Hay alguna hora en la que se sienta con mejor disposición?
( ) Sí ( ) No
En caso afirmativo, indique la hora aproximada:



ANEXO 3

INSTRUMENTO DE RITMO DE HUMOR
(MOOD RHYTHM INSTRUMENT - MRhI)

Data: ___/___/_____ Horário:_____ Sexo: ( ) F ( ) M

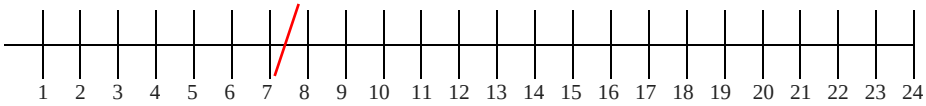
Data da última menstruação: _____

Idade (anos completos): _____ Escolaridade (anos completos): _____

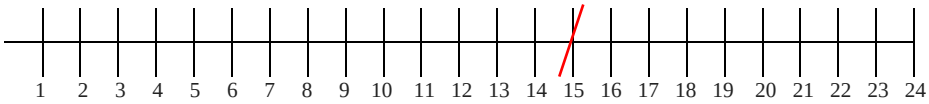
Leia atentamente às observações abaixo antes de responder o questionário.

Abaixo de cada questão está representado um período de 24 horas. Cada número representa a hora do relógio. A linha entre os números representam os minutos.

Por exemplo, (A) se você marcar um traço entre 7 e 8, corresponderá a 7:30h.

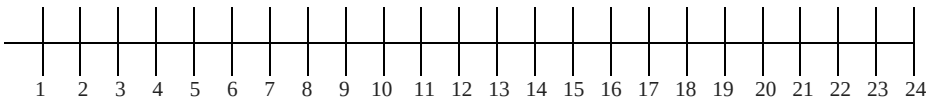


(B) Se você marcar um traço no 15, corresponderá a 15:00h.

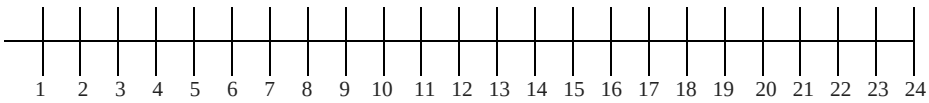


Responda às perguntas abaixo considerando **os últimos 15 dias**, levando em conta como você se sente na maior parte do tempo, na maioria desses dias.

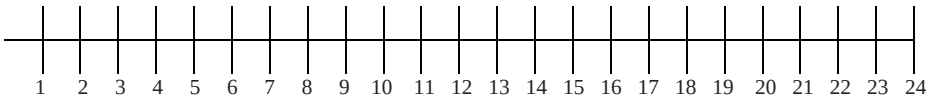
1. Existe algum horário no qual você se sinta com mais sono?
( ) Sim ( ) Não
Se sim, indique abaixo o horário aproximado:



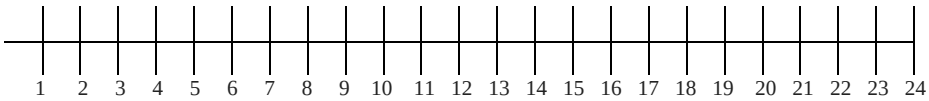
2. Existe algum horário no qual você se sinta com melhor capacidade para resolver problemas do dia-a-dia?
( ) Sim ( ) Não
Se sim, indique abaixo o horário aproximado:



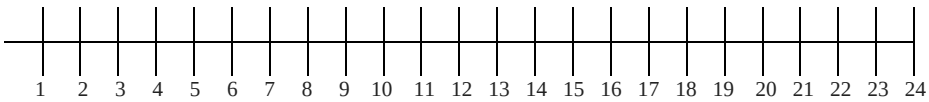
3. Existe algum horário em que sua concentração esteja melhor?
( ) Sim ( ) Não
Se sim, indique abaixo o horário aproximado:



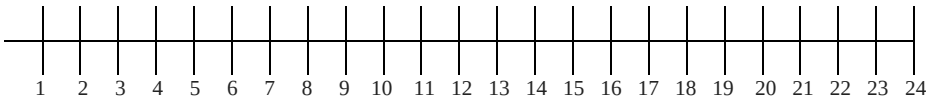
4. Existe algum horário no qual você tenha mais apetite?
( ) Sim ( ) Não
Se sim, indique abaixo o horário aproximado:



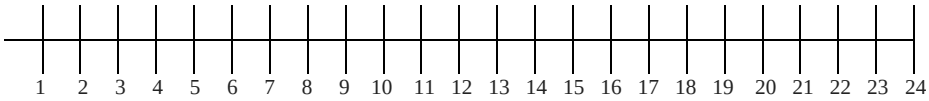
5. Existe algum horário no qual sua libido (desejo sexual) esteja aumentada?
( ) Sim ( ) Não
Se sim, indique abaixo o horário aproximado:



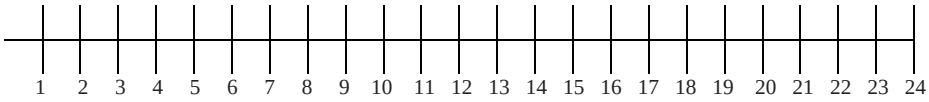
6. Existe algum horário no qual você se sinta mais irritado?
( ) Sim ( ) Não
Se sim, indique abaixo o horário aproximado:



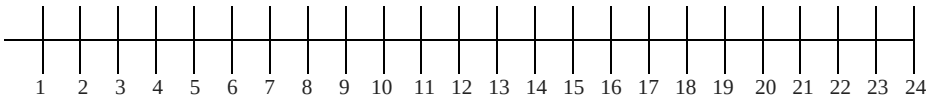
7. Existe algum horário em que você se sinta mais ansioso?
( ) Sim ( ) Não
Se sim, indique abaixo o horário aproximado:



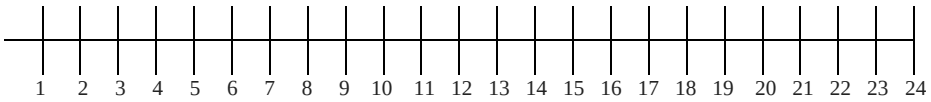
8. Existe algum horário em que você se sinta mais triste?
( ) Sim ( ) Não
Se sim, indique abaixo o horário aproximado:



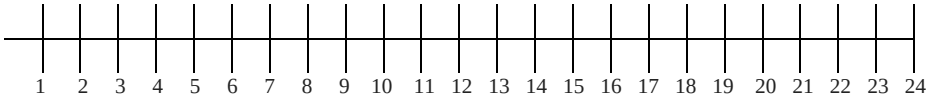
9. Existe algum horário em que sua memória esteja melhor?
( ) Sim ( ) Não
Se sim, indique abaixo o horário aproximado:



10. Existe algum horário em que você esteja mais pessimista?
( ) Sim ( ) Não
Se sim, indique abaixo o horário aproximado:



11. Existe algum horário no qual você se sinta mais disposto?
( ) Sim ( ) Não
Se sim, indique abaixo o horário aproximado:



ANEXO 4

Revised Mood Rhythm Instrument (MRhI-r)

Date: ____/_____/_____ Time (hh:mm): _____ Sex: ( ) F ( ) M Subject ID: _____

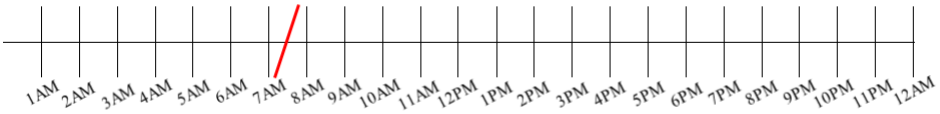
Date of last period: ____/____/_____

Age: _____ Level of education (years of schooling): _____

Please read carefully the following examples before answering the questions:

Following each question, a 24-hour period is shown. Each number represents an hour of the clock. The line between the numbers represents the minutes.

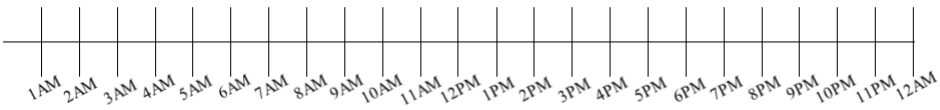
Example (A): if you mark a line between 7AM and 8AM, it will represent 7:30 AM



(B) If you mark a line on 3PM, it will represent 3:00 PM

Answer the following questions according to the **last 15 days**, taking into account how you have felt most of the time, on the majority of the days.

1. Is there a specific time of the day when you have felt sleepier?
( ) Yes ( ) No
If you answer yes, indicate below the approximate hour:



2. Is there a specific time of the day when you have felt more capable of solving daily problems?
( ) Yes ( ) No
If you answer yes, indicate below the approximate hour:

Revised Mood Rhythm Instrument (MRhI-r)

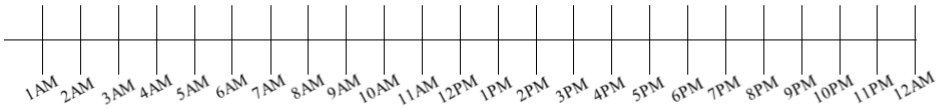
3. Is there a specific time of the day when you been able to concentrate better?
( ) Yes ( ) No
If you answer yes, indicate below the approximate hour:

4. Is there a specific time of the day when you have had an increased appetite?
( ) Yes ( ) No
If you answer yes, indicate below the approximate hour:

5. Is there a specific time of the day when your libido (sexual arousal) has been higher?
( ) Yes ( ) No
If you answer yes, indicate below the approximate hour:

6. Is there a specific time of the day when you have felt more irritable?
( ) Yes ( ) No
If you answer yes, indicate below the approximate hour:

7. Is there a specific time of the day when you have felt more anxious?
( ) Yes ( ) No
If you answer yes, indicate below the approximate hour:



8. Is there a specific time of the day when you have felt more sad?
( ) Yes ( ) No
If you answer yes, indicate below the approximate hour:

Revised Mood Rhythm Instrument (MRhI-r)

9. Is there a specific time of the day when your memory has been better?
( ) Yes ( ) No
If you answer yes, indicate below the approximate hour:

10. Is there a specific time of the day when you have been more pessimistic?
( ) Yes ( ) No
If you answer yes, indicate below the approximate hour:

11. Is there a specific time of day when you have had more energy and motivation to do things?
( ) Yes ( ) No
If you answer yes, indicate below the approximate hour:

ANEXO 5

Instrumento de Ritmo de Humor (MRhI)

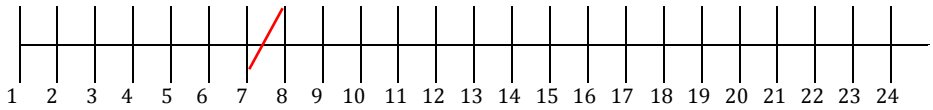
Data e horário de preenchimento: __/__/____ ____:____

Leia atentamente às observações abaixo antes de responder ao questionário.

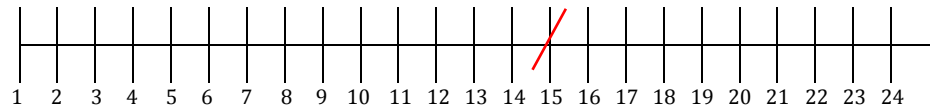
Abaixo de cada questão tem uma linha do tempo com 24 horas. Cada número representa a hora do relógio.

EXEMPLOS:

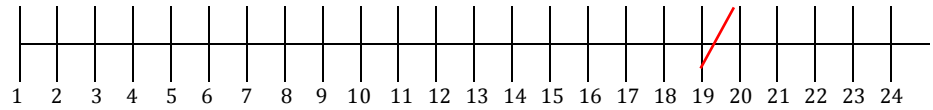
(A) se você marcar um traço entre 7 e 8, corresponderá a 7:30h.



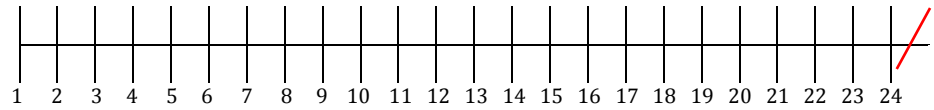
(B) Se você marcar um traço no 15, corresponderá a 15:00h (3h da tarde).



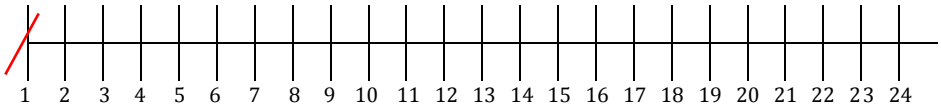
(C) Se você marcar um traço entre 19 e 20, corresponderá a 19:30h (7 e meia da noite).



(D) Se você marcar um traço após 24h, corresponderá a 00:30h (meia noite e meia).



(E) Se você marcar um traço no 1, corresponderá a 1:00h (1h da manhã).

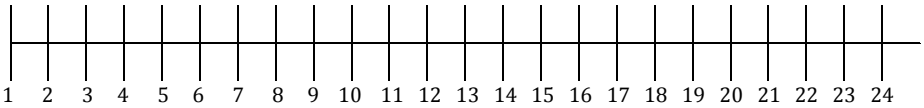


Responda às perguntas abaixo considerando os últimos 15 dias, levando em conta como você se sentiu na maior parte do tempo, nesses 15 dias e na ausência de acontecimentos que tenham lhe causado estresse.

MARQUE <u>SOMENTE</u> EM <u>UM</u> HORÁRIO EM CADA QUESTÃO

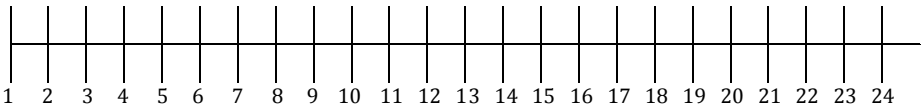
1. Nos últimos 15 dias, na maioria desses dias,
teve um horário no qual você se sentiu mais alerta (mais acordado)?

( ) Sim ( ) Não
Se sim, indique abaixo o horário aproximado: Marque UM traço, somente em UM local.



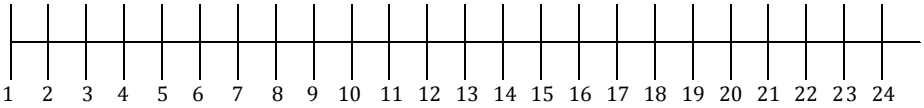
2. Nos últimos 15 dias, na maioria desses dias,
enquanto estava acordado, teve um horário no qual você se sentiu com mais sono?

( ) Sim ( ) Não
Se sim, indique abaixo o horário aproximado: Marque UM traço, somente em UM local.



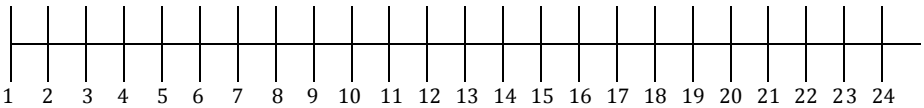
3. Nos últimos 15 dias, na maioria desses dias,
teve um horário no qual você sentiu que estava melhor disposto ou com melhor preparado para resolver problemas do dia-a-dia (por exemplo, fazer temas/lições de casa, trabalhos, arrumar o seu quarto, ajudar em casa, resolver problemas com família e amigos)?

( ) Sim ( ) Não
Se sim, indique abaixo o horário aproximado: Marque UM traço, somente em UM local.



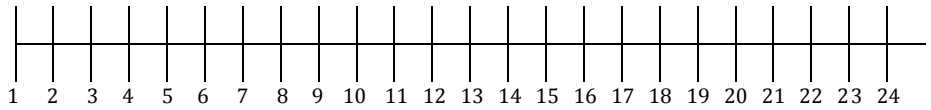
4. Nos últimos 15 dias, na maioria desses dias,
teve um horário no qual a sua autoestima estivesse melhor (ficou mais confiante em você ou estava feliz com quem você é)?

( ) Sim ( ) Não
Se sim, indique abaixo o horário aproximado: Marque UM traço, somente em UM local.



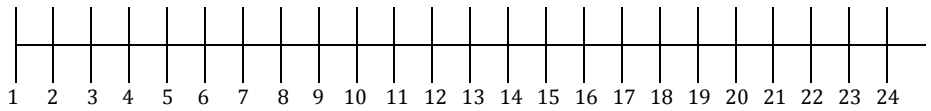
5. Nos últimos 15 dias, na maioria desses dias,
teve um horário em que sua concentração estivesse melhor (conseguiu ter mais foco e atenção para aulas/tarefas no geral/provas)?

( ) Sim ( ) Não
Se sim, indique abaixo o horário aproximado: Marque UM traço, somente em UM local.



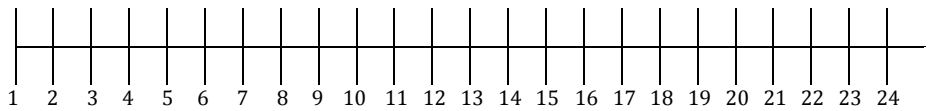
6. Nos últimos 15 dias, na maioria desses dias,
teve um horário no qual você teve mais vontade de comer?

( ) Sim ( ) Não
Se sim, indique abaixo o horário aproximado: Marque UM traço, somente em UM local.



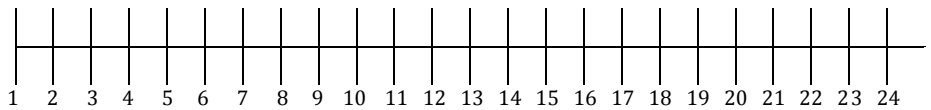
7. Nos últimos 15 dias, na maioria desses dias,
teve um horário no qual você se sentiu mais irritado (zangado, bravo)?

( ) Sim ( ) Não
Se sim, indique abaixo o horário aproximado: Marque UM traço, somente em UM local.



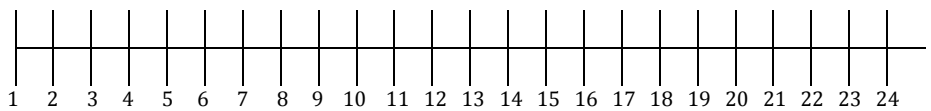
8. Nos últimos 15 dias, na maioria desses dias,
teve um horário em que você se sentiu mais ansioso (preocupado, angustiado)?

( ) Sim ( ) Não
Se sim, indique abaixo o horário aproximado: Marque UM traço, somente em UM local.



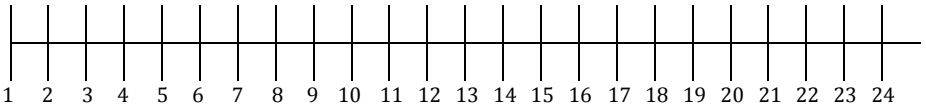
9. Nos últimos 15 dias, na maioria desses dias,
teve um horário do dia em que você se sentiu mais triste?

( ) Sim ( ) Não
Se sim, indique abaixo o horário aproximado: Marque UM traço, somente em UM local.



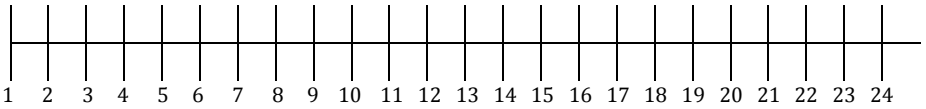
10. Nos últimos 15 dias, na maioria desses dias,
teve um horário em que você sentiu mais vontade de fazer exercício físico ou praticar esportes?

( ) Sim ( ) Não
Se sim, indique abaixo o horário aproximado: Marque UM traço, somente em UM local.



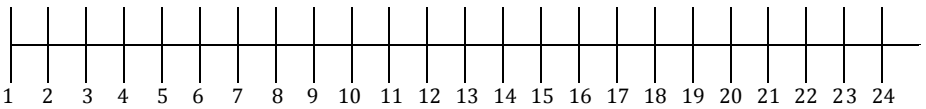
11. Nos últimos 15 dias, na maioria desses dias,
teve um horário em que memorizar coisas foi melhor (foi mais fácil guardar informações que viu ou escutou)?

( ) Sim ( ) Não
Se sim, indique abaixo o horário aproximado: Marque UM traço, somente em UM local.



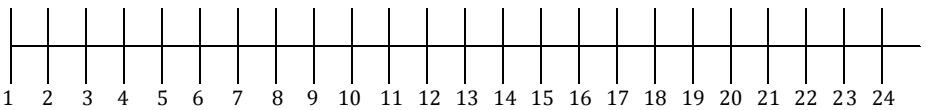
12. Nos últimos 15 dias, na maioria desses dias,
teve um horário em que você estivesse mais pessimista (com o pensamento negativo, achando que as coisas iam dar errado, sem conseguir olhar o lado bom ou positivo das coisas)?

( ) Sim ( ) Não
Se sim, indique abaixo o horário aproximado: Marque UM traço, somente em UM local.



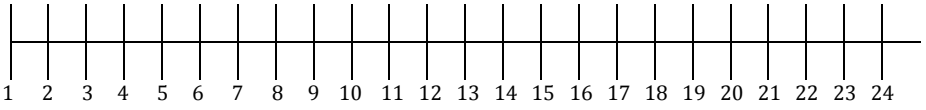
13. Nos últimos 15 dias, na maioria desses dias,
teve um horário em que você teve mais vontade de falar com seus amigos (pessoalmente ou pela internet)?

( ) Sim ( ) Não
Se sim, indique abaixo o horário aproximado: Marque UM traço, somente em UM local.



14. Nos últimos 15 dias, na maioria desses dias,
teve um horário no qual você se sentiu mais disposto (com mais energia)?

( ) Sim ( ) Não
Se sim, indique abaixo o horário aproximado: Marque UM traço, somente em UM local.

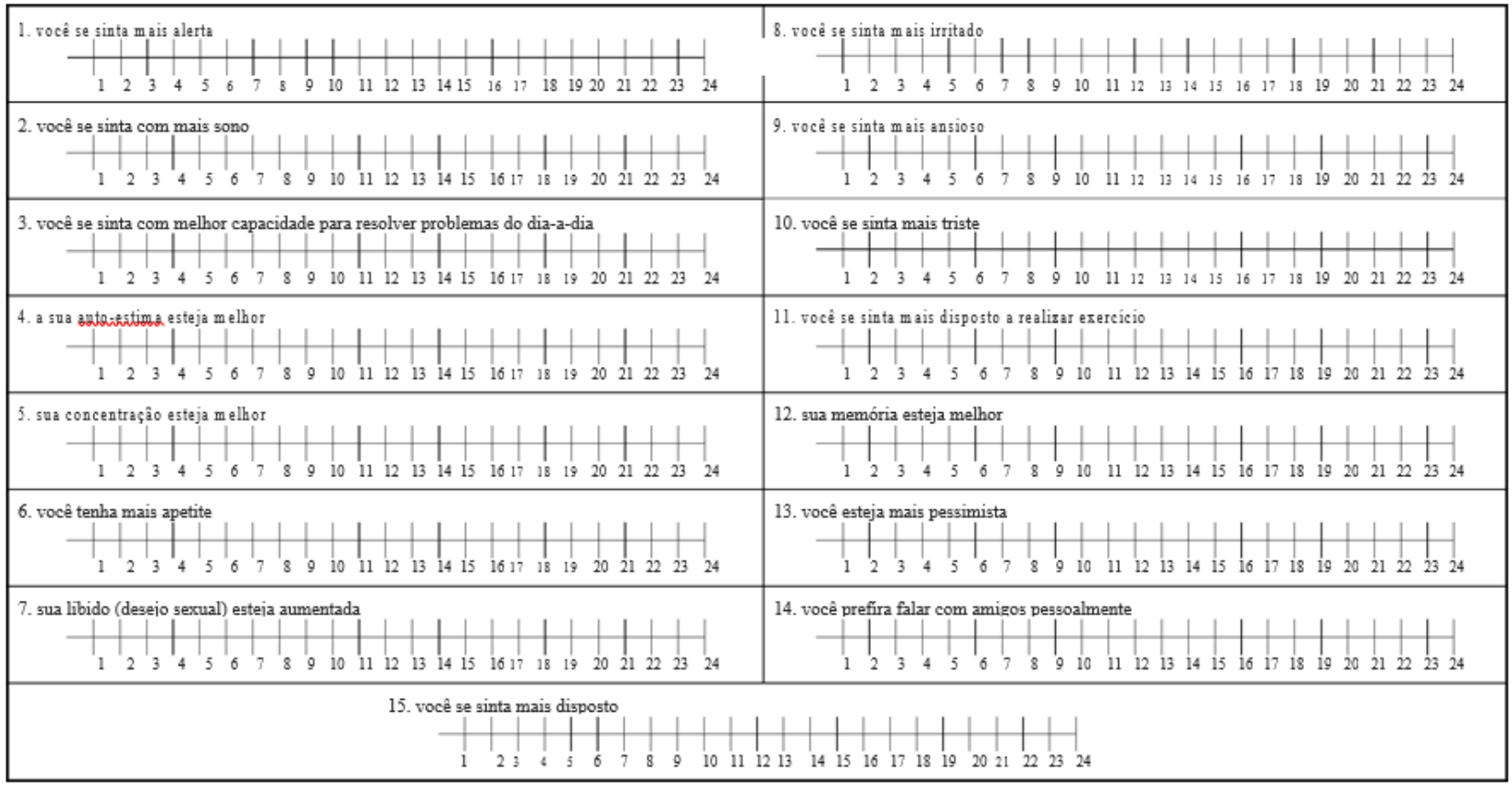


ANEXO 6

INSTRUMENTO DE RITMO DE HUMOR (MOOD RHYTHM INSTRUMENT - MRI)

DATA: ___/___/_____

Leia atentamente às observações abaixo antes de responder o questionário.
Responda às perguntas abaixo durante **15 dias**, levando em conta como você se sente a cada dia para cada item. Responda cada questão se você percebeu que teve um horário aproximado para o item ao longo de 24 horas. **Caso não existir um horário, circule o número da questão.**
Indique abaixo o horário aproximado no qual/em que....



ANEXO 7


ReGente
Bem-estar
Como você tem se sentido?
Quão ansiosa(o) eu estou me sentindo
nada ansiosa(o)
muito ansiosa(o)
Quão triste eu estou me sentindo
nada triste
muito triste
Acompanhe
Salvar
Acompanhamento Mensal