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O DESENVOLVIMENTO DA CRONOMEDICINA:

ESTUDO DOS RITMOS BIOLÓGICOS E SONO APLICADOS À DEPRESSÃO

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Tese apresentada ao Programa de Pós-Graduação em Psiquiatria e Ciências do Comportamento da Universidade Federal do Rio Grande do Sul (UFRGS) como requisito parcial para a obtenção do título de Doutor em Psiquiatria e Ciências do Comportamento.

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Aos brasileiros que fazem ciência,
que pensam, e que amam.
Façamos sobressair a razão e o amor
acima do obscurantismo e do ódio.

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"A 24/7 environment has the semblance of a social world,
but it is actually a non-social model of machinic performance
and a suspension of living that does not disclose the human
cost required to sustain its effectiveness."

Jonathan Crary,
24/7: Late Capitalism and the Ends of Sleep

RESUMO

A crescente morbimortalidade da depressão justifica a busca pelo aprimoramento do seu diagnóstico e por subtipos do transtorno que apresentem peculiaridades em relação ao tratamento clínico. Uma melhor compreensão das múltiplas facetas biológicas e psicológicas da depressão é fundamental para o desenvolvimento de terapêuticas mais eficazes. Neste contexto, a pesquisa em ritmos biológicos, incluindo o sono, representam linhas recentes e promissoras na investigação da etiologia, melhora do prognóstico e abordagem de tratamento no curso clínico da depressão. A ritmicidade das funções biológicas em mamíferos é mantida por um maquinário molecular complexo, e o principal estímulo ambiental que sincroniza os relógios biológicos é o ciclo claro-escuro. No entanto, as rotinas sociais também interferem nesse mecanismo, pois determinam a exposição à luz e os tempos de alimentação, bem como os padrões de atividade de repouso. Evidências clínicas descrevem que disfunções desse sistema temporizador estão presentes em uma variedade de condições clínicas mórbidas, como obesidade, diabetes, hipercolesterolemia, doenças cardiovasculares e câncer. As alterações de humor também foram amplamente estudadas em modelos de “desalinhamento” circadiano ou cronorruptura. Por exemplo, pacientes deprimidos apresentam um padrão distinto de movimento físico, tempo de reação motora e atividade psicomotora. Além disso, até 90% dos pacientes deprimidos reportam alterações de sono, como dificuldades em adormecer, permanecer dormindo e acordar de manhã mais cedo que o desejado.

Esta tese explora as associações destes parâmetros de atividade motora e sono medidos subjetivamente (por questionários quantitativos) e objetivamente (pela actimetria) com a depressão. Inicialmente, descrevem-se dois estudos metodológicos que objetivam a validação de um instrumento quantitativo sobre higiene do sono, e o estudo dos impactos de períodos de não-uso (“off-wrist”) dos actímetros nos cálculos de parâmetros de atividade motora. A seguir, são descritos os estudos desenvolvidos que associam a avaliação subjetiva e objetiva do sono, dos ritmos de atividade e da exposição à luz com sintomas depressivos e com o transtorno depressivo maior. Esta tese documenta avanços metodológicos e provas de conceito que possibilitam a criação de novas hipóteses em estudos da associação entre ritmos biológicos e depressão. Além disso, os estudos descritos fornecem provas de que a depressão (e outros transtornos psiquiátricos) deve ser estudada sob a ótica da Cronomedicina, o estudo dos impactos do "tempo" na fisiologia, endocrinologia, metabolismo e comportamento em diferentes níveis organizacionais.

Palavras-chave: Cronobiologia, Ritmos Circadianos, Saúde Mental, Psiquiatria, Transtornos de Humor, Actimetria, Actigrafia, Séries Temporais

ABSTRACT

The increasing morbidity and mortality of depression justifies the search for improvement of its diagnosis and of subtypes of the disorder that present peculiarities in terms of clinical treatment. A better understanding of the multiple biological and psychological facets of depression is essential for the development of more effective therapies. In this context, research on biological rhythms, including sleep, representes a recent and promising line of investigation of the etiology, improvement of the prognosis and treatment approach in the clinical course of depression. The rhythmicity of biological functions in mammals is maintained by complex molecular machinery, and the main environmental stimulus that synchronizes biological clocks is the light-dark cycle. However, social routines also interfere with this mechanism, as they determine exposure to light and feeding times, as well as resting activity patterns. Clinical evidence describes that dysfunctions of this timing system is present in a variety of morbid clinical conditions, such as obesity, diabetes, hypercholesterolemia, cardiovascular disease and cancer. Mood alterations have also been widely studied in models of circadian "misalignment" or “disruption”. For example, depressed patients have a distinct pattern of physical movement, motor reaction time and psychomotor activity. In addition, up to 90% of depressed patients report changes in sleep, such as difficulty falling asleep, staying asleep and waking up in the morning earlier than desired.

This thesis explores the associations of these parameters of motor activity and sleep measured subjectively (by quantitative questionnaires) and objectively (by actimetry) with depression. Initially, two methodological studies are described that aim to validate a quantitative instrument on sleep hygiene, and to study the impacts of off-wrist periods of actimeters in the calculations of motor activity parameters. The following are the studies developed that associate the subjective and objective assessment of sleep, activity rhythms and exposure to light with depressive symptoms and major depressive disorder. This thesis documents methodological advances and proofs of concept that enable the creation of new hypotheses in studies of the association between biological rhythms and depression. In addition, the studies described provide evidence that depression (and other psychiatric disorders) should be examined from the perspective of Chronomedicine, the study of the impacts of "time" on physiology, endocrinology, metabolism and behavior at different organizational levels.

Keywords: Chronobiology, Circadian Rhythms, Mental Health, Psychiatry, Mood Disorders, Actimetry, Actigraphy, Time Series

LISTA DE ABREVIATURAS E SIGLAS

DLMO – início da secreção de melatonina sob luz fraca (*dim light melatonin onset*)

ELAN – exposição à luz artificial à noite

HCPA – Hospital de Clínicas de Porto Alegre

HPA – eixo hipotálamo-hipófise-adrenal (*hypothalamic–pituitary–adrenal*)

ipRGC – células ganglionares intrinsecamente fotossensíveis

JLS – jet lag social

MCTQ – Questionário de Cronotipos de Munique

MEQ: Questionário de Matutinidade-Vespertinidade

MSF: ponto médio de sono em dias livres

PSG: polissonografia

SCN – núcleo supraquiasmático (*suprachiasmatic nucleus*)

SHI-BR – Índice de Higiene do Sono, versão em português brasileiro

THS – transtorno de humor sazonal

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CAPÍTULO 1 – REFERENCIAL TEÓRICO, JUSTIFICATIVA E OBJETIVOS

1. INTRODUÇÃO

"In a world of mere flux, change would not be cumulative; it would not move toward a close. Stability and rest would have no being. Equally it is true, however, that a world that is finished, ended, would have no traits of suspense and crisis, and would offer no opportunity for resolution. Where everything is already complete, there is no fulfillment… The live being recurrently loses and reestablishes equilibrium with his surroundings. The moment of passage from disturbance into harmony is that of intensest life. In a finished world, sleep and waking could not be distinguished. In one wholly perturbed, conditions could not even be struggled with. In a world made after the pattern of ours, moments of fulfillment punctuate experience with rhythmically enjoyed intervals. (…) Inner harmony is attained only when, by some means, terms are made with the environment."

John Dewey in "Art as Experience"

A pesquisa em ritmos biológicos, incluindo o sono, representa uma linha recente e promissora na investigação da etiologia, melhora do prognóstico e abordagem de tratamento no curso clínico da depressão(1). A ritmicidade das funções biológicas em mamíferos é mantida por um maquinário intracelular e uma rede de osciladores centrais cerebrais e em diversos outros sistemas. O principal estímulo ambiental que sincroniza os relógios biológicos ao ambiente é o ciclo claro-escuro(2,3). No entanto, as rotinas sociais também interferem nesse mecanismo, pois determinam a exposição à luz, a ocupação de espaços (*indoors* ou *outdoors*), os tempos de alimentação, bem como os padrões de atividade de repouso. Evidências clínicas descrevem disfunções desse sistema temporizador estão presentes em uma variedade de condições clínicas mórbidas, como obesidade, diabetes, hipercolesterolemia, doenças cardiovasculares e câncer(4,5). As alterações de humor e de comportamentos complexos como o sono também foram amplamente estudadas em modelos de perturbações agudas e crônicas deste sistema.

Pacientes deprimidos apresentam um padrão distinto de atividade motora e psicomotora(6). Além disso, até 90% dos pacientes deprimidos reportam alterações de sono, como dificuldades em adormecer, permanecer dormindo e acordar de manhã mais cedo que o desejado(7).

O crescente corpo de evidências que descreve a complexa regulação dos ritmos biológicos revela semelhanças existentes entre os sistemas envolvidos no controle do ritmo circadiano e aqueles que se acredita estarem envolvidos na manifestação dos transtornos do humor. Esses avanços científicos introduziram novas tecnologias para o estudo dos transtornos do humor, como a actimetria. O estudo clínico dos ritmos e do papel do tempo em diversos níveis organizacionais da fisiologia, endocrinologia, metabolismo e comportamento, ganhou o nome de Cronomedicina, uma ciência médica dedicada a prevenção, busca de causalidade, diagnóstico e tratamento de doenças em humanos(8).

Esta tese explora o paradigma da Cronomedicina em Psiquiatria, objetivando descrever as associações de parâmetros de atividade motora e sono medidos subjetivamente e objetivamente (pela actimetria) com a depressão. Inicialmente, descrevem-se dois estudos metodológicos que objetivam a validação de um instrumento quantitativo sobre higiene do sono, e o estudo dos impactos de períodos de não-uso (“off-wrist”) dos actímetros nos cálculos de parâmetros de atividade motora. A seguir, são descritos os estudos desenvolvidos que associam a avaliação subjetiva e objetiva do sono, dos ritmos de atividade e da exposição à luz com sintomas depressivos e com o transtorno depressivo maior.

2. CRONOBIOLOGIA: O ESTUDO DOS RITMOS BIOLÓGICOS

2.1. Regulação Fisiológica dos Ritmos Biológicos

Humanos e outros seres vivos possuem um sistema temporizador pelo qual garantem uma ritmicidade a diversas funções fisiológicas, desde a expressão de genes até a organização de sistemas(9). Atualmente, a regulação rítmica de uma miríade de aspectos da fisiologia humana e de outros mamíferos já é descrita, incluindo metabolismo, imunidade, sono e outros comportamentos. (Figura 1).

A busca pelas bases moleculares dos ritmos biológicos levou a identificação de um mecanismo retroalimentar de transcrição de genes (Figura 1), posteriormente conhecidos como genes do relógio (i.e., *clock genes*) e outros genes controlados a partir destes (i.e., *clock-controlled genes*)(2). Essa maquinaria foi identificada em todo o tipo de célula, desde procariotos, até eucariotos unicelulares e animais mais complexos. Em mamíferos, o mecanismo molecular consiste nos ativadores CLOCK e seu heterodímero BMAL1. Ao ser formado, o complexo CLOCK-BMAL1 se liga a elementos reguladores em um conjunto de genes rítmicos que codificam as proteínas repressoras PERIOD (i.e., *Per1, Per2, Per3*) e CRYPTOCHROME (i.e., *Cry1, Cry2*). Em animais noturnos, a ativação de CLOCK-BMAL1 ocorre durante o dia, levando à transcrição dos genes *Per* e *Cry* à tarde e ao acúmulo de suas proteínas, PER e CRY, no final da tarde ou noite. As proteínas PER e CRY exercem feedback negativo, interagindo com o complexo CLOCK-BMAL1 para reprimir sua própria transcrição. Com a consequente diminuição da transcrição de *Per* e *Cry*, a transcrição por CLOCK-BMAL1 pode começar novamente para iniciar um novo ciclo de transcrição na manhã seguinte(10).

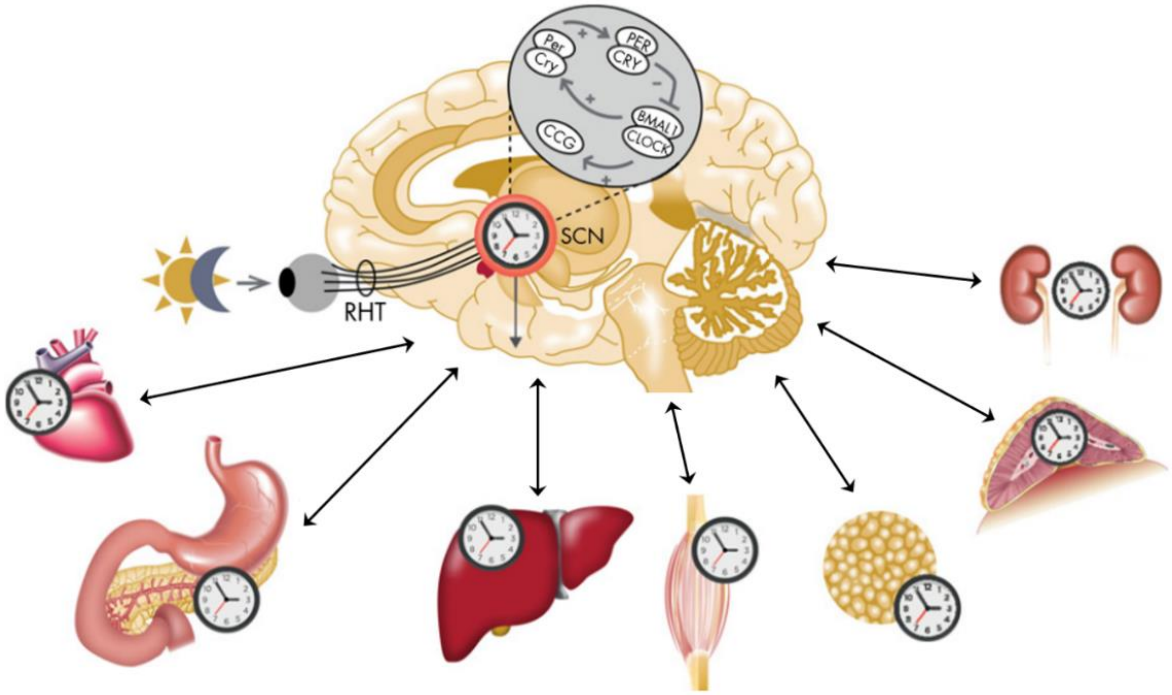

Figura 1. O sistema temporizador e a regulação dos ritmos biológicos. O mecanismo molecular que gera uma oscilação circadiana autossustentada em neurônios SCN é um ciclo de feedback transcricional-translacional complexo que compreende ativadores transcricionais centrais BMAL1 / CLOCK e dois conjuntos de repressores PER e CRY. Os ativadores transcricionais centrais BMAL1 / CLOCK regulam vários genes, conhecidos como "genes controlados por relógio" (CCGs). A mesma maquinaria molecular foi encontrada em outros tecidos centrais, bem como em quase todos os tecidos periféricos examinados até agora. O ciclo claro-escuro é o principal sincronizador externo do marcapasso circadiano central (via trato retino-hipotalâmico [RHT]), mas outros estímulos externos podem afetar a fase e a amplitude dos osciladores periféricos. Por exemplo, o tempo de ingestão de alimentos foi reconhecido como um importante sincronizador externo para a ritmicidade circadiana de órgãos periféricos envolvidos no controle do metabolismo energético. Outro importante estímulo externo são as rotinas sociais que influenciam os horários de exposição a iluminação e a outros *zeitgebers*. O relógio central no núcleo supraquiasmático (SCN) serve para sincronizar os osciladores centrais e periféricos para otimizar a função do organismo em relação às periodicidades de 24 horas no ambiente. Os sinais dos tecidos periféricos podem afetar a fase e a amplitude do marcapasso central. Figura adaptada de Oster e colaboradores, publicado no periódico *Endocrine Reviews* (11).

O sistema temporizador também é responsável pelo encarrilhamento (do inglês, *entrainment*) dos ritmos endógenos com as transições temporais ambientais, especialmente ao ciclo claro-escuro(12,13). Estes ritmos que acompanham as transições dos dias e das noites são denominados circadianos (i.e., um ritmo de quase 24 horas, do latim *circa* = “cerca de”, *diem* = “dia”). Embora existentes constitutivamente, é a interação dos osciladores internos principais com o ambiente que regula o período (i.e., a duração de um ciclo) e outras características (e.g., a fase de um ciclo) das diversas funções rítmicas.

O principal sinalizador ambiental que regula o sistema circadiano (i.e., *zeitgeber*, "doador de tempo" em alemão) é o ciclo claro-escuro. O núcleo supraquiasmático (SCN), uma pequena coleção de ~20,000 neurônios hipotalâmicos logo acima do quiasma óptico, recebe informações fóticas ambientais coletadas por células ganglionares intrinsecamente fotossensíveis (ipRGC) na retina. As ipRGC expressam o fotopigmento melanopsina, que traduz comprimentos de onda de luz em estímulo neural através do trato retino-hipotalâmico para o SCN (Figura 1). Este núcleo possui projeções para diversas estruturas do corpo, coordenando tecidos periféricos por meio da modulação de outros osciladores de acordo com as transições claro-escuro(14). Embora os neurônios do SCN individualmente atuem como marcapassos circadianos autônomos, eles exibem variações aleatórias na duração do período e se comunicam para manter a duração do período e as relações de fase estáveis para controle de dos ciclos diários(15,16). A destruição das células do SCN de roedores abole seus ritmos de atividade e repouso, e o transplante de células do SCN de outro roedor faz com que ele adquira os ritmos de atividade e repouso do seu doador(17). Deste modo, o sistema temporizador atua na manutenção da homeostase dos processos fisiológicos (incluindo comportamento e funções cognitivas) para que estas funções aconteçam no momento mais oportuno do ciclo circadiano(18,19) – i.e., o ser humano, evolutivamente um animal diurno, dorme à noite e se alimenta durante o dia.

Uma das conexões basilares do SCN é a glândula pineal (Figura 2), que é uma projeção do epitálamo ao terceiro ventrículo responsável pela produção e liberação da melatonina. A melatonina (N-acetil-5-metoxitriptamina) é uma indolamina anfifílica conservada de organismos unicelulares a plantas, até animais. Em mamíferos, tanto síntese pineal, quanto extra-pineal foram descritas (20). A escuridão da noite desencadeia uma via polissináptica que começa com o trato retino-hipotalâmico projetando-se para os SCN, e então ao núcleo

paraventricular hipotalâmico, e termina como uma entrada simpática para a glândula pineal (Figura 2). A síntese de melatonina é estimulada pela norepinefrina e requer serotonina como precursor(21). Ganhando a corrente sanguínea, a melatonina pineal, uma saída primária do marcapasso circadiano (i.e., o SCN), transmite informações circadianas e sazonais do ciclo claro-escuro para todo o corpo, regulando funções fisiológicas e comportamentos diários. Sendo assim, a sinalização da melatonina pineal é o braço hormonal da regulação dos ritmos biológicos(22). A melatonina também é sintetizada pela pele, intestinos e pulmões de forma constitutiva e sob demanda por células imunocompetentes ativadas, como macrófagos residentes e derivados de monócitos, além da microglia e de linfócitos(20,23). Apesar de estar sobre a influência do sistema temporizador, a melatonina extra-pineal não apresenta variação circadiana e acredita-se que suas ações se deem apenas em nível local de onde é produzida(24).

Por fim, uma conexão importante do sistema temporizador de distinta relevância para modelos neuropsiquiátricos é o eixo hipotálamo-hipófise-adrenal (HPA). A ritmicidade diária dos níveis plasmáticos de glicocorticoides (GC; em humanos, o cortisol) é um forte modulador de muitos processos fisiológicos e psicológicos, embora seu significado funcional seja mal compreendido. Os efeitos fisiológicos ideais do GC ocorrem quando o sinal central que controla o ritmo de liberação do GC e os ritmos periféricos nos tecidos que expressam os receptores do GC estão alinhados (11). Os ritmos de 24 horas do cortisol e da melatonina são relacionados temporalmente, mesmo no caso de trabalhadores de turno expostos à iluminação artificial à noite (25). Em uma noite escura, a melatonina plasmática em seres humanos começa a subir quando o cortisol plasmático está em seu nível mais baixo, atinge seu pico quando o cortisol começa a subir, descrevendo um atraso de fase de uma média de 5h entre o pico de melatonina noturno e o de cortisol no início

da manhã (26). O HPA também está envolvido na regulação fisiológica dos ciclos de sono-vigília. Assim, qualquer condição que perturbe o HPA também tem o potencial de modificar outros ritmos biológicos como os padrões de sono(24, 25).

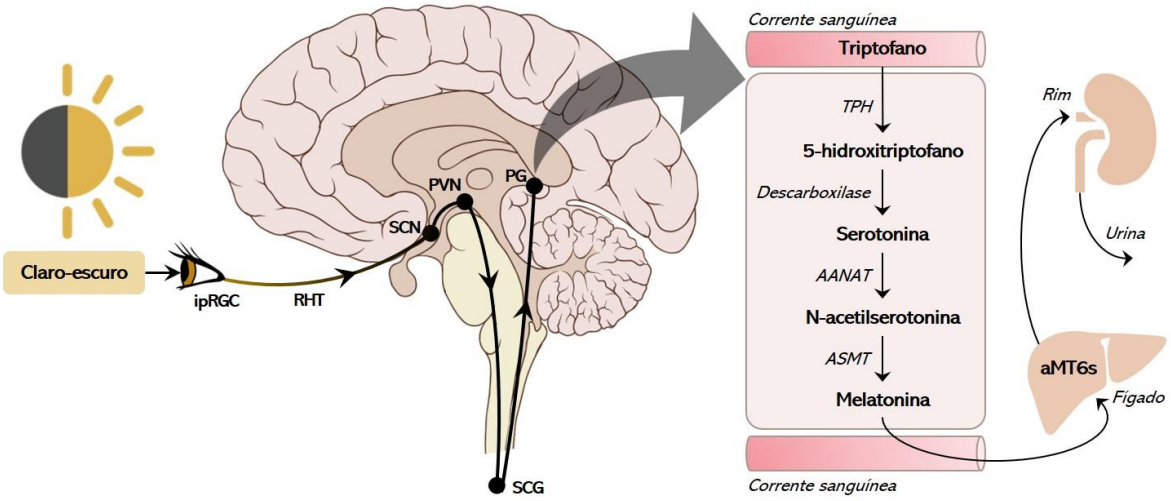

Figura 2. Via canônica da produção de melatonina pineal. O núcleo supraquiasmático (SCN) recebe informações fóticas ambientais coletadas por células ganglionares intrinsecamente fotossensíveis (ipRGC) na retina. As ipRGC expressam o fotopigmento melanopsina, que transduz comprimentos de onda de luz em entrada neural através do trato retino-hipotalâmico (RHT) para o SCN. O SCN inibe constitutivamente o núcleo paraventricular hipotalâmico (PVN) por meio da projeção GABA-érgica. Na ausência de luz, o PVN ativa os núcleos cervicais ganglionares (SCG, via coluna intermediolateral da medula), desencadeando fibras noradrenérgicas que inervam a glândula pineal (PG), liberando em última instância os co-transmissores noradrenalina e ATP. Este estímulo simpático desencadeia a ação da arilalquilamina N-acetiltransferase (AANAT), convertendo a serotonina (5TH) em N-acetilserotonina (NAS) dentro do pinealócito. Com a ação constitutiva da N-acetilserotonina O-metiltransferase (ASMT), a NAS é então convertido em melatonina e imediatamente liberado no líquido cefalorraquidiano e na corrente sanguínea. Por meio do metabolismo de primeira passagem no fígado, a melatonina é convertida em 6-sulfatoximelatonina (aMT6s), que é então excretada na urina. Figura adaptada de Tonon e colaboradores, aceito para publicação no periódico *Frontiers in Psychiatry* (**Anexo 1**).

2.2. O Ciclo Sono-Vigília

Dormir e acordar são manifestações evidentes do sistema circadiano dos mamíferos. Em 1982, Borbely e colegas propuseram um modelo para a regulação dos ciclos de sono, dividindo didaticamente o sistema regulatório nos processos homeostático (S) e cicadiano (C)(29). Segundo o processo homeostático, o momento fisiológico ideal de sono depende do transcorrer de tempo desde o último episódio de sono, onde se entenderia que os processos responsáveis por levar o organismo a "adormecer" se acumulam ao longo da vigília e criam uma pressão ou impulso gradual ao sono. Por outro lado, o processo circadiano descreveria uma oscilação padrão (e dependente do processo de encarrilhamento) que indica a propensão do organismo ao sono em alguns momentos do dia, independentemente do horário do último episódio de sono. Segundo a proposta original deste modelo, o sono aconteceria a partir da intersecção de uma pressão alta do sistema homeostático com a fase específica do processo circadiano. Embora por muito tempo os dois processos tenham sido estudados à parte, atualmente é bem aceita a hipótese de que são parte do mesmo sistema, com influência mútua, além de mecanismos complementares interligados fisiologicamente(30).

O controle do ciclo sono-vigília é complexo e envolve várias áreas e vias cerebrais (Figura 3). A excitação cortical e a vigília são mantidas por meio da atividade neuronal sustentada das vias colinérgicas e aminérgicas do tegmento pontino, *locus coeruleus* e núcleos da rafe, mediadas por orexina/hipocretina(31,32). Por outro lado, os neurônios da área pré-óptica ventrolateral (VLPO) são um grupo das poucas células cerebrais que participam na indução e manutenção do sono. A VLPO tem vias de saída para os centros de estimulação do hipotálamo e do tronco cerebral através de vias diretas ao tálamo. Estas

células realizam a inibição do sistema monoamérgico do tronco cerebral e do sistema hipotalâmico orexina/hipocretina via ácido γ-aminobutírico (GABA) e galanina(33).

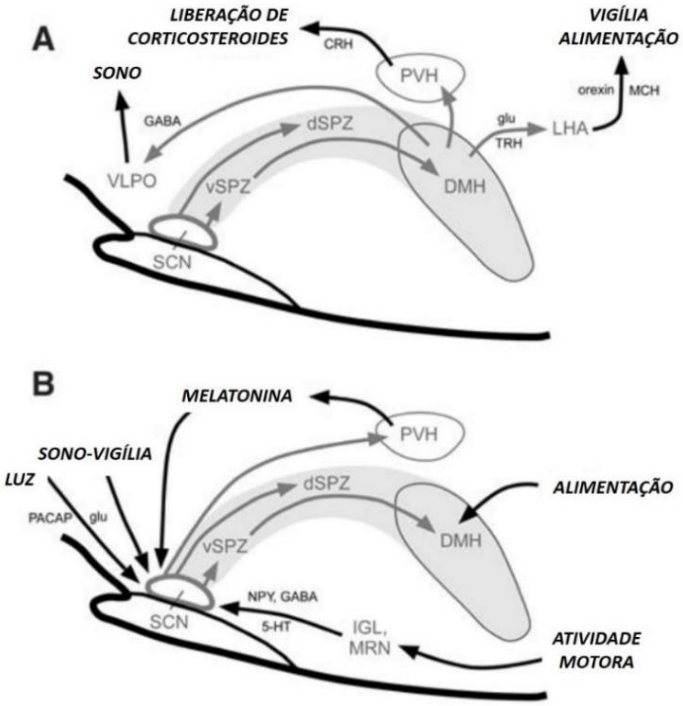

Figura 3. Regulação do ciclo sono-vigília com foco em regiões hipotalâmicas e suas conexões. A demonstração visual é das áreas descritas em roedores. (A) mostra vias originárias do hipotálamo e algumas das vias regulatórias do sono, da liberação de corticosteroides, da manutenção da vigília e do estímulo do apetite. (B) mostra as vias pelas quais atuam alguns dos fatores reguladores deste sistema e que convergem no SCN hipotalâmico, i.e., atividade motora, alimentação, melatonina, o estado de sono ou vigília e a luz. 5-HT: 5-hidroxitriptamina (serotonina); CRH: hormônio liberador de corticotrofina; GABA: ácido γ-aminobutírico; glu: glutamato; DMH: núcleo hipotalâmico dorsomedial; dSPZ: zona sub-paraventricular dorsal; IGL: folheto intergeniculado; LHA: área hipotalâmica lateral; MRN : núcleo mediano da rafe; MCH: hormônio concentrador de melanina; NPY: neuropeptídeo Y; PACAP: polipeptídeo hipofisário ativador de adenilato-ciclase; PVH: núcleo paraventricular hipotalâmico; SPZ: zona sub-paraventricular; TRH: hormônio liberador de tireotrofina; VLPO: área pré-óptica ventrolateral; vSPZ: zona sub-paraventricular ventral. Figura adaptada de Fuller e colaboradores(34), publicada no periódico *Journal of Biological Rhyhtms.*

A reorganização diurna do equilíbrio excitatório-inibitório de neurônios liberadores de orexina/hipocretina e as variações circadianas nas interconexões entre as áreas cerebrais descritas determinam os ciclos dos episódios de sono(35,36). Tanto a duração quanto a fase do sono – i.e., o momento em que o episódio de sono acontece em relação ao dia de 24h – se correlacionam com a fase circadiana do ritmo de temperatura(37). Do mesmo modo, outras descobertas em humanos mostram a associação entre a fase do sono e a fase

de outros ritmos, como frequência cardíaca(38) secreção de cortisol(39) e de melatonina à noite(40). Estas e outras evidências comprovam a integração entre os sistemas regulatórios do sono e de outros ritmos. Estes ritmos sofrem a influência constante de pistas temporais do ambiente, modo pelo qual se alinham a um período de 24h.

2.3. Encarrilhamento dos Ritmos Biológicos, Saúde e Comportamento

O arrastamento ou encarrilhamento (*entrainment*) fótico é o processo pelo qual o SCN é alinhado a um período de 24 horas pela luz e escuridão diária(26, 27). Sem o estímulo fótico ambiental, os organismos entram em um estado circadiano de livre curso (*free-running*), cujo período varia entre as espécies(43,44). Quer dizer, mesmo na ausência de um *zeitgeber*, o sistema temporizador conta com marcapassos biológicos autossustentáveis. Isso faz com que, em escuro constante, desde as cascatas de retrocontrole dos genes *clock*, até a temperatura corporal e os ciclos de sono e vigília existam em períodos de aproximadamente 24h (Figura 4)(45).

Um corpo sólido de evidências mostra que os efeitos do encarrilhamento fótico no sono variam dependendo do momento do dia em que se tem o estímulo luminoso. A exposição à luz mais tarde no dia (próxima à noite) se associa a um sono mais tardio e com mais despertares, além de influenciar a arquitetura do sono, especificamente, diminuindo a latência para o sono REM, e reduzindo o percentual total de sono em REM(46).

A visão tradicional na pesquisa de ritmos biológicos é a de que os relógios circadianos controlam os ciclos de sono-vigília e outras funções fisiológicas, considerando que os marcapassos centrais e os ritmos periféricos estão em uma relação mestre-escravo (*master-slave*). Nesta visão, o estímulo fótico é o clássico *zeitgeber*, capaz de modificar o período e a fase do marcapasso central – o SCN – e, por consequência, mudar a fase de

outros ritmos periféricos(47). Embora seja amplamente aceito que o encarrilhamento pela luz envolve elementos celulares dentro do SCN que são exclusivamente responsivos a pistas fóticas, já existem evidências suficientes de pistas não-fóticas que podem regular os marcapassos centrais. Por exemplo, manipulações comportamentais em modelos animais envolvendo atividade locomotora, excitação/alerta ou estados correlacionados são capazes de redefinir (*reset*) a fase do relógio circadiano(48–50). Em humanos, *zeitgebers* sociais incluem acordar pela manhã, fazer exercícios e outras atividades da rotina diária típica. Estes estímulos notavelmente interagem com o sistema de encarrilhamento fótico, na medida em que podem determinar os horários de exposição à luz, além de também promoverem estímulos temporais próprios(51,52). Horários de alimentação também já foram descritos como *zeitgebers*, sendo possivelmente resultado do encarrilhamento de órgãos metabolicamente ativos por meio da sinalização intestinal(53). Por fim, a mudança no padrão de secreção de melatonina endógena ou a sua administração exógena potencialmente alteram a fase do sistema temporizador(54,55).

Desafios agudos ao sistema, como a exposição à luz artificial à noite (ELAN), um turno de trabalho à noite e o deslocamento transmeridional rápido por viagens aéreas são frequentemente acompanhados por consequências adversas a curto prazo. Por exemplo, a síndrome de “jetlag” das viagens transmeridionais cursa com queixas de sono, fadiga, problemas cognitivos, humor melancólico e sintomas gastrointestinais(56). Ainda assim, espera-se a ressincronização do sistema também a curto prazo, caso sejam retomados os estímulos ambientais habituais. No entanto, quando tais desafios são vivenciados cronicamente, a adaptação pode não ser alcançada, por exemplo no caso de pessoas com escalas de trabalho de turno à noite. As perturbações transitórias e crônicas do sistema temporizador ficaram conhecidas como “cronodisrupção” ou “cronorruptura” (em inglês,

circadian disruption), mas muitos outros termos foram propostos e usados para se referir a situações semelhantes(57). Apesar de ainda não haver consenso acerca do melhor indicador para se quantificar a cronorruptura, um número expressivo de condições clínicas foi associado a alterações diversas do sistema temporizador. Entre elas, destacam-se as alterações hormonais e metabólicas (e.g., diabetes, obesidade)(5,58), cardiovasculares (e.g., perda do descenso noturno da pressão arterial)(59), câncer (em especial, o câncer de mama em mulheres trabalhadoras de turno)(60), alterações de sono e transtornos psiquiátricos(61).

Como a diferença no tempo de sono entre o trabalho e os dias livres lembra a situação de viajar por vários fusos horários entre as noites de sexta-feira e manhãs de segunda-feira, cunhou-se o termo "jetlag social" (JLS) para descrever essas mudanças semanais no tempo de sono(62). Esta medida, resultado do cálculo da diferença entre o ponto médio do sono em dias livres e em dias de semana. Algumas pessoas podem sentir mais os efeitos destas alternâncias de fase devido à sua tipologia circadiana.

Define-se tipologia circadiana como a manifestação da fase do relógio biológico em humanos que os condiciona a alocar comportamentos em determinados períodos do dia(63), Nesta classificação, matutinos são os indivíduos que tem a fase de seus ritmos adiantada no período das 24h do dia; assim, são indivíduos que relatam preferência por horário de dormir e de acordar mais cedo. Já os vespertinos apresentam atraso de fase de seus ritmos (Figura 4) e, portanto, preferem dormir e acordar mais tarde. A epidemiologia da tipologia circadiana mostra que os tempos de sono e vigília apresentam uma distribuição quase gaussiana – com os matutinos e vespertinos nas pontas – e que depende da idade e do sexo(64). Por exemplo, do final da infância até o início da vida adulta (aproximadamente aos 21 anos) há uma tendência progressiva à maior vespertinidade (identificada início dos

episódios de sono progressivamente mais tarde). Essas preferências individuais na atribuição de tarefas do dia-a-dia, incluindo tempos de sono, atualmente não são vistas apenas como traço psicológico, mas como constructo biológico, tendo respaldo em estudos genéticos(65) e relação com o início da secreção de melatonina sob luz fraca (*dim light melatonin onset*; DLMO(66). A tipologia circadiana influencia notavelmente a fisiologia e o metabolismo, mas também aspectos de interesse para a psicologia e a psiquiatria, i.e., desempenho cognitivo e sintomas psiquiátricos (63,67,68).

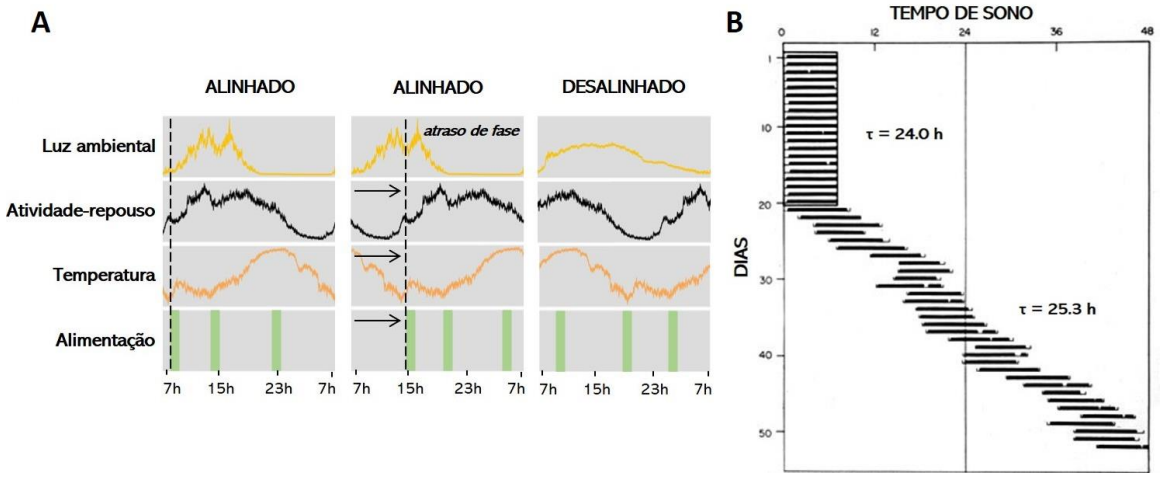

Figura 4. O processo de encarrilhamento dos ritmos biológicos. As linhas em (A) apresentam ritmos de um ser humano em um período de 24 horas. Na primeira coluna, a fase entre os ritmos está alinhada ao estímulo luminoso. Quer dizer, o período de maior atividade ocorre durante o dia, bem como as refeições principais, e a temperatura corporal é maior à noite. A segunda coluna também mostra ritmos alinhados, mas com um atraso de fase em relação ao estímulo luminoso. A terceira coluna apresenta ritmos não encarrilhados ao sinal de luz e que também não têm relação de fase entre si, determinando um desalinhamento (i.e. um processo de cronorruptura). A figura (B) mostra o padrão de sono-vigília de um em condições normais e corrida livre de um sujeito jovem do sexo masculino que ficou em isolamento de pistas temporais externas a partir do 20º dia. O eixo do tempo horizontal é referenciado à hora habitual de dormir do sujeito (hora zero), conforme registrado em um diário de sono-vigília em casa durante a semana anterior. Nota-se que, ao isolar-se de pistas temporais (incluindo a iluminação), a duração do seu ciclo sono-vigília (τ), que era de 24h, passou a ocorrer a cada 25.3 horas. Figura (A) adaptada de Tonon e colaboradores, aceito para publicação no periódico *Frontiers in Psychiatry* (**Anexo 1**); Figura B adaptada de Czeisler e colaboradores, publicada no periódico *Science* (37).

3. RIITMOS BIOLÓGICOS E DEPRESSÃO

3.1. Evidências Experimentais de Modelos Psicopatológicos

As relações entre o comportamento do tipo depressivo e os ritmos biológicos foram amplamente documentadas em modelos animais. Ratos da espécie *Arvicanthis niloticus*, um roedor diurno também conhecido como rato do capim-do-Nilo, expostos a fotoperíodos curto (5h de luz e 19h de escuro por dia) por seis semanas, apresentaram preferência de sacarina reduzida e maior tempo de imobilidade no teste de nado forçado, apesar de não apresentarem alterações e no tempo gasto no lado da luz da caixa claro/escuro em comparação aos controles(69). As mesmas espécies submetidas a uma baixa intensidade de luz durante o dia (ciclos de penumbra de 50lux durante 12h e de escuro durante 12h) por 4 semanas apresentaram aumento da imobilidade e diminuição da escalada no teste de nado forçado; novamente, nenhum efeito foi observado no teste de da caixa claro/escuro ou na locomoção em campo aberto em comparação com controles submetidos à luz brilhante comum de biotério(70).

Em camundongos noturnos, a luz fraca à noite (16h de luz [∼150lux] e 8 horas de escuro [5lux]) por 4 semanas aumentou a imobilidade no teste de nado forçado e reduz a preferência por sacarose(71). Além disso, roedores noturnos sob luz constante por 3-4 semanas, um regime de luz conhecido como modelo de dessincronização do comportamento de atividade e repouso(72), exibiram um número significativamente maior de eventos de *grooming* durante o teste de campo aberto e diminuíram a preferência de sacarose ao longo do estudo(73). Ainda, sob dessincronização forçada (ciclos claro-escuro de 22 horas), os ratos desenvolvem alguns fenótipos tipo-depressivos, quais sejam, anedonia, disfunção sexual, aumento da imobilidade no teste de natação forçada(74).

Landgraf e colegas(75) mostraram que ratos nocaute para o gene *Bmal1* (um dos genes do relógio que participam da manutenção da periodicidade circadiana dentro das células) no SCN de camundongos levou a latências de escape aumentadas e número de falhas de escape no paradigma de desamparo aprendido, induziu maior tempo de imobilidade no teste de suspensão pela cauda e diminuiu o tempo gasto no compartimento claro do teste claro/escuro. Esses resultados indicam que a interrupção dos ritmos centrais das células do SCN causa comportamento de desamparo, desespero e ansiedade. Este experimento sustenta uma relação causal porque os ciclos de claro-escuro ambientais e as vias de entrada de luz permaneceram inalteradas. Portanto, os resultados não poderiam ser explicados pela luz que afeta o comportamento do tipo depressivo por meio de outras vias (ou seja, sem perturbar o sono ou ritmicidade circadiana, ou por meio de vias não relacionadas ao SCN(76)). Ao contrário dos nocautes globais, nos quais os camundongos também podem apresentar comportamento alterado relacionado às funções pleiotrópicas dos genes do relógio, o modelo tem a vantagem de induzir seletivamente ritmos perturbados no SCN, evitando outros danos neurais causados por lesões do SCN. Além disso, o comportamento anedônico medido pelo teste de preferência de sacarose, a preferência espacial no teste de campo aberto e a aversão a comer em um ambiente novo permaneceram inalterados, sugerindo que apenas aspectos específicos do comportamento tipo depressivo são influenciados pela interrupção do relógio central(75).

Por fim, é importante ressaltar que os sistemas neurotransmissores monoaminérgicos alterados na depressão (e.g., serotonina, noradrenalina, dopamina) apresentam oscilações circadianas nos níveis sanguíneos (77,78), embora não esteja claro se ou como os estados de humor podem afetar esse padrão circadiano.

3.2. Evidências Clínicas: Uma Perspectiva Histórica

As primeiras evidências clínicas empíricas da relação entre desalinhamento de ritmos biológicos e depressão (e, portanto, o maior corpo de evidência até o momento) versam sobre o transtorno de humor sazonal (THS). O relato de surgimento de sintomas depressivos – ou piora dos sintomas já existentes – no período do outono e inverno(79) ganhou atenção dos pesquisadores da Cronobiologia em função da chamada hipótese de mudança de fase circadiana. Esta teoria tem por base o conceito de que alguns transtornos afetivos podem surgir, pelo menos em parte, devido a uma incompatibilidade nos ritmos circadianos, especialmente aqueles relacionados ao ciclo sono-vigília(80,81). Segundo a hipótese, os indivíduos ficam deprimidos no outono e inverno em função da exposição à luz no final do amanhecer, fazendo com que seus ritmos circadianos atrasem em relação à hora do relógio e em relação ao seu ciclo sono-vigília. O THS é relatado em locais onde há marcadas mudanças sazonais na duração natural do dia (fotoperíodo), o que reforçou a busca por explicações fisiopatológicas relacionadas à exposição à luz e a mudança de fase da secreção de melatonina.

Como a duração da produção de melatonina é o sinal neuroquímico para a mudança circa-anual na duração da noite(22), a administração de melatonina próximo ao horário de dormir seria capaz de induzir mudanças de fase circadiana semelhantes às causadas pela luz. Apesar da plausibilidade biológica desta teoria – considerando que a melatonina é sabidamente é suprimida mesmo com baixos estímulos luminosos(82) – as evidências até o momento são controversas. Há estudos que relatam tanto um atraso de fase quanto redução na duração de tempo de secreção de melatonina de indivíduos diagnosticados com THS comparados a controles(83,84). Porém, não há evidências suficientes que comprovem a redução da amplitude da melatonina diária durante inverno nestes

indivíduos(83,85). Além disso, o tratamento preventivo ou profilático com melatonina não é capaz de reduzir a sintomatologia do THS(86–88).

Por outro lado, a exposição terapêutica à iluminação artificial(89) é capaz de promover o alinhamento de ritmos circadianos (por exemplo, adiantando a fase do DLMO(90)) e induzir remissão do episódio depressivo em indivíduos com THS. Uma possível explicação para este benefício clínico a despeito da manutenção dos níveis melatonina surgiu nos últimos anos com a evidência de circuitos que conectam as ipRGC na retina a estruturas cerebrais relacionadas ao humor, independentemente do SCN e da regulação circadiana(91). Segundo estes estudos recentes, a regulação do humor pela luz requer uma via independente de SCN ligando ipRGC a uma região talâmica previamente não reconhecida, denominada núcleo perihabenular(92).

O interesse clínico na melatonina não se deu apenas pelo seu potencial efeito terapêutico no TSH, mas também na busca pela etiopatologia da depressão. Como a produção de melatonina ocorre a partir da serotonina após a estimulação dos adreno-receptores(21), foi levantada a hipótese de que um distúrbio na secreção de melatonina poderia estar presente na fase aguda da doença depressiva e possivelmente estar relacionado à sua fisiopatologia. Além disso, a melatonina também controla a sinalização da dopamina nas regiões do lobo frontal hipotálamo e hipocampo(93,94), o que indicaria uma possível relação com estados de humor.

No entanto, as evidências até agora são altamente controversas. Enquanto alguns estudos anteriores relataram níveis mais baixos de melatonina no soro ou saliva noturno em indivíduos deprimidos, outros relataram nenhuma diferença, ou mesmo níveis mais elevados em comparação com os controles(95). Um estudo relata que indivíduos com níveis de melatonina nos dois quartis mais baixos da amostra estavam em maior risco de

sintomatologia grave(96). Estudos que investigaram a produção noturna de 6-sulfatoximelatonina não encontraram diferenças comparando indivíduos deprimidos e controles antes do tratamento(97,98). Um estudo encontrou níveis mais baixos de melatonina plasmática na hora de dormir em indivíduos deprimidos com características melancólicas(99). Alguns outros estudos indicam que subgrupos de indivíduos deprimidos que não suprimem o cortisol matinal após o teste de estimulação com dexametasona têm maior probabilidade de apresentar melatonina noturna mais baixa(100,101).

A falta de concordância nos achados sobre os níveis de melatonina em indivíduos deprimidos pode ser resultado de 1) a diversidade de fenômenos comportamentais e etiológicos em estados depressivos; 2) heterogeneidade metodológica, como inconsistência nos métodos de avaliação da melatonina, dificuldades no cumprimento do protocolo de coleta de melatonina e distinções entre os desenhos dos estudos; e 3) a premissa errônea de que a melatonina pode contribuir (falta ou excesso) ou neutralizar (como antidepressivo) a depressão precisa evoluir para o entendimento de que a melatonina é uma molécula integrativa que potencialmente afeta o humor por meio da regulação da fisiologia e do comportamento em vários níveis. Uma discussão detalhada sobre as intersecções da melatonina e depressão se encontra em um artigo aceito para publicação no periódico *Frontiers in Psychiatry,* e se encontra no Anexo 1.

3.3. Evidências Clínicas de Alterações de Ritmos Biológicos em Estados Depressivos

A regulação circadiana do sistema temporizador modula os padrões diários na maioria dos sistemas comportamentais e fisiológicos, incluindo comportamentos complexos como o ciclo sono-vigília. Como descrito nas seções anteriores, problemas na organização temporal interna e no alinhamento aos estímulos ambientais estão relacionados a uma

série de patologias clínicas e alterações comportamentais. Ainda assim, os mecanismos exatos pelos quais se observam os desfechos adversos agudos ou crônicos de desafios impostos ao sistema temporizador ainda não é compreendido. Além disso, muitas das evidências produzidas até o momento são advindas de estudos transversais ou ecológicos, não sendo possível descartar a hipótese de que a cronorruptura ou desalinhamento sejam consequências ou mesmo desfechos concomitantes e não necessariamente desencadeadores destas patologias.

Os padrões diários de fatores imunológicos e respostas ao patógenos são modulados pelo sono e pela fase circadiana(102). Por exemplo, algumas semanas de um protocolo clínico experimental de desalinhamento circadiano aumentaram os níveis da citocina anti-inflamatória IL-10 e das proteínas pró-inflamatórias TNF-α e proteína C reativa, além de modificar a fase e a amplitude da secreção de cortisol(103). A secreção de cortisol de 24 horas parece ser mais variável(104) e menos fortemente relacionada a *zeitgebers* sociais(105) e à fase de secreção de melatonina(106) em pacientes deprimidos. Em um estudo, a diminuição de amplitude de secreção do cortisol não foi observada em todos os pacientes, mas apenas naqueles com diagnóstico de subtipo psicótico(107), indicando que mesmo dentro do constructo global de depressão maior, existem variações pela natureza complexa e multifatorial dos transtornos de humor.

Muitos pacientes com depressão unipolar (de padrão não-sazonal) mostram variações no padrão diurno de sintomas, tendo alguns estudos mostrado uma piora dos sintomas pela manhã(108,109) e outros uma piora no final da tarde(110), embora já tenha sido reportada uma grande variabilidade entre indivíduos em termos da oscilação circadiana destes sintomas(109). Além das variáveis intrinsecamente ligadas ao diagnóstico do transtorno de humor, já foram reportadas uma série de outras alterações de ritmos em

indivíduos deprimidos. Entre elas, observam-se amplitudes atenuadas da temperatura corporal(111,112) e dos ritmos de atividade motora(113), alterações de fase do cortisol e de noradrenalina(114), além de avanços de fase de outros marcadores do ciclo sono-vigília (115,116). Foram relatadas variações no metabolismo de glicose cerebral nas regiões límbico-paralímbica ventral, parietal, temporal e frontal que se relacionam com uma melhora dos sintomas no final da tarde(117). Por fim, entre os distúrbios circadianos associados à depressão, alterações do ciclo sono-vigília são muito comuns e são observados de forma robusta na literatura.

3.4. O Ciclo Sono-Vigília e a Depressão

"*Depois de amanhã, sim, só depois de amanhã...*
Levarei amanhã a pensar em depois de amanhã,
E assim será possível; mas hoje não...
Não, hoje nada; hoje não posso.
A persistência confusa da minha subjectividade objectiva,
O sono da minha vida real, intercalado,
O cansaço antecipado e infinito,
Um cansaço de mundos para apanhar um eléctrico...
Esta espécie de alma...
Só depois de amanhã... (...)"

"Adiamento" – Álvaro de Campos, 1928

Uma das condições clínicas mais frequentes dentre as perturbações dos ciclos sono-vigília é a insônia. Queixas de insônia aparecem em até 90% dos indivíduos deprimidos(7) e precedem o início ou a recorrência de um episódio depressivo em até 40% dos casos(118). Alguns estudos ainda reportam altos índices de suicídios entre indivíduos deprimidos com insônia e outros transtornos de sono(119,120). Alterações de arquitetura do sono em indivíduos deprimidos também foram registradas por diversos estudos, por exemplo, a redução da latência entre o início do sono e o primeiro episódio de sono REM, o aumento

da duração do sono REM, o aumento do número de movimentos oculares durante o sono REM e a diminuição de sono de ondas lentas (115,121).

Os extremos do espectro da tipologia circadiana (i.e., matutinos ou vespertinos extremos) são sujeitos a importantes avanços ou atrasos de fase em relação ao relógio social. Sendo assim, é sabido que muitos indivíduos apresentam insônia e/ou sonolência excessiva diurna, que prejudicam substancialmente suas atividades diárias. Esse grupo de indivíduos enquadra-se nos diagnósticos conhecidos como transtornos do sono relacionados ao ritmo circadiano (TSRRC)(122). Estes incluem o transtorno da fase sono-vigília avançada – característica de matutinos extremos – e o transtorno da fase sono-vigília atrasada – característica de vespertinos extremos –, que são mais prevalentes em indivíduos mais velhos e mais jovens, respectivamente(123). Além destes, também são classificados o transtorno de sono-vigília não-24 horas – quando o indivíduo é incapaz de se encarrilhar ao ambiente, seguindo uma periodicidade dos ritmos biológicos distinta de 24h – e o distúrbio do sono decorrente do trabalho de turno – resultado da alternância constante das fases de sono.

Os TSRRC são frequentemente associados a depressão(124). Além disso, este grupo de indivíduos também tende a ser restringido por fatores sociais, como rotinas de trabalho ou escola, que encurtam o sono, resultando em um acúmulo de "débito de sono". Isso causa sonolência excessiva durante o dia e comprometimento do funcionamento cognitivo(123). A privação do sono é considerada um estressor fisiológico e um desafio metabólico que costuma estar associado a níveis elevados de cortisol e escores subjetivos de estresse(103). Em um estudo prospectivo, mostrou-se que não apenas a depressão é causa de redução do tempo total de sono, mas também que a privação de sono pode preceder o desenvolvimento de um episódio depressivo após um ano(125). Por fim, a ocorrência de

alterações de sono também traz implicações ao tratamento da depressão. Maiores índices de distúrbio subjetivo do sono durante o tratamento farmacológico são associados a uma menor probabilidade de remissão de um episódio depressivo(126).

3.5. Higiene do sono: um novo paradigma de estudo

Práticas e hábitos inadequados de sono abrangem vários fatores biológicos e ambientais, refletindo na qualidade e duração do sono, sensação de sono inquieto ou não reparador, e sonolência excessiva diurna(127). Fatores de estilo de vida pouco saudáveis, por exemplo, falta de exercício físico e consumo de substâncias (e.g., cafeína, álcool e nicotina), afetam negativamente o sono(128). Além disso, o uso de dispositivos eletrônicos, como telefones celulares, computadores e televisores também demonstraram afetar a qualidade subjetiva do sono e sua duração(129). No intuito de mudar os desfechos relacionados aos hábitos inadequados, cunhou-se o termo higiene do sono, postulado como um conjunto de estratégias que incorporam ajustes diários em ambos comportamento e ambiente(130). Recomendações de higiene do sono são universalmente incluídas como parte de programas de tratamento cognitivo-comportamental para insônia e outros distúrbios do sono(131).

Não existe uma única recomendação formal de higiene do sono. Diferentes sociedades e instituições vinculadas a pesquisa e clínica em Medicina do Sono traçam suas diretrizes e recomendações seguindo um padrão semelhante baseado no nível de evidência atual. O Box 1 apresenta um resumo destas recomendações embasados na American Alliance for Healthy Sleep(132), American Sleep Association (133) e na Sleep National Foundation(134).

Box 1. Recomendações de Higiene do Sono	
OTIMIZAÇÃO DAS ROTINAS DE SONO	• Dormir e acordar no mesmo horário (± 20 min), mesmo em finais de semana • Ajustar as rotinas gradualmente (máxima diferença de 1-2h por noite) • Evitar sonecas

PROMOÇÃO DE ROTINAS RELAXANTES NA HORA DE DORMIR	• Apenas ir para a cama quando se está com sono
	• Sair da cama se não adormecer depois de 10-20 min
	• Evitar atividades estimulantes na cama
	  • Assistir à televisão
	  • Usar computador, smartphone ou tablet
	  • Planejar, ler
	• Diminuir a ingestão de líquidos antes de dormir
	• Criar um ritual antes de dormir
	  • Tomar banho quente
	  • Utilizar técnicas de relaxamento

MANUTENÇÃO DE UM QUARTO QUE PROMOVA O SONO	• Manter o quarto calmo e silencioso • Atentar para ronco de companheiro(a) ou barulho de animais • Manter uma temperature agradável • Manter o quarto escuro ou usar tapa-olhos • Cama e colchão confortáveis
EXPOSIÇÃO À LUZ ADEQUADA	• Expor-se à luz natural durante o dia (preferencialmente pela manhã) • Reduzir a exposição a iluminação artificial à noite • Desligar aparelhos eletrônicos pelo menos 30min antes de dormir
HÁBITOS DIURNOS PROMOTORES DE SONO	• Exercitar-se regularmente e manter uma dieta saudável • Evitar exercícios físicos vigorosos antes de dormir • Evitar refeições copiosas antes de dormir; preferir um lanche saudável • Evitar o consume de cafeína a partir do final da tarde • Evitar o consume de álcool ou cigarro perto da hora de dormir • Controlar o uso de medicações ou outras substâncias que promovam alerta • Usar a cama apenas para dormir ou para fazer sexo

A relação entre má higiene do sono e depressão foi identificada em uma série de contextos, sendo a maioria das evidências compostas de amostras de estudantes universitários(135,136) e pacientes com apneia obstrutiva do sono(137). Em um estudo, adolescentes com problemas de restrição crônica de sono que receberam instruções sobre higiene do sono conseguiram dormir mais cedo, acordar mais cedo, e aumentar seu tempo de sono ao mesmo tempo. Este protocolo de três semanas foi capaz de reduzir os sintomas de insônia e de depressão(138). Efeitos similares foram produzidos em protocolos semelhantes com populações de estudantes universitários(139).

O efeito benéfico das estratégias de higiene do sono se relaciona ao componente homeostático da regulação do sono, por exemplo, ao recomendar a não ingestão de

substâncias estimulantes próximo da hora de dormir. Também há recomendações relacionadas ao componente circadiano, através do controle de estímulos ambientais como a exposição à luz artificial à noite (ELAN).

3.6. Encarrilhamento Circadiano, Cronorruptura e Depressão

Os desafios ao sistema temporizador, como a exposição à iluminação à noite e o trabalho de turno, são novos na história da humanidade. O advento da eletricidade e a acessibilidade constante à luz artificial criaram um ambiente que é fundamentalmente distinto do que o homem vivia 200 anos atrás. A exposição à luz artificial à noite (ELAN) – por vezes denominada “poluição luminosa” – e a redução da exposição à luz durante o dia fazem parte da rotina de muitos indivíduos. Este padrão de diminuição da amplitude do sinal luminoso diário, especialmente às custas da ELAN, mostra associações com depressão em crianças e adolescentes (140,141), adultos(142), e idosos(143,144).

Figura 5. **“Arkwright’s Cotton Mills by Night” de Joseph Wright of Derby.** c.1782-3. Óleo sobre tela. 99.7 x 125.7 cm. “(...) a luz artificial não surgiu na história do homem apenas com as lâmpadas incandescentes. A pintura “Arkwright’s Cotton Mills by Night”, de Joseph Wright of Derby, é um dos primeiros retratos feitos pelo homem da luz tomando conta do escuro. Desde 1772, essa fábrica localizada em Cromford, na Inglaterra, funcionava 24 horas por dia em 2 turnos de 12 horas. À noite, ela era iluminada por velas. Já nessa época, antes de Thomas Edison e de James Bowman Lindsay, nossas noites tinham o escuro sequestrado em prol das necessidades do mundo moderno”. Trecho citado da postagem “O Sequestro do Escuro” no blog “Seu Corpo, Seu Tempo”, disponível em: https://seucorposeutempo.wordpress.com/.

Não obstante, as rotinas sociais são organizadas em períodos arbitrários (por exemplo, horário comercial das 8h00 às 18h00 na maioria das cidades do Brasil). Essas rotinas são influenciadas por vários fatores, que incluem a demanda por aumento de produtividade e competitividade, segurança pública e outros aspectos culturais, como religião e etnia. Considerando que cada pessoa apresenta uma fase individual de encarrilhamento ao ambiente (i.e., cronotipo), que reflete em horários diários ideais para diferentes atividades, entende-se que nem sempre os indivíduos estarão ativos e aptos a desempenhar funções sociais em horários convencionados. Existem significativas variações diurnas nas medidas

de desempenho cognitivo e físico entre os fenótipos circadianos(145), o que explica por que vespertinos frequentemente têm dificuldades em se ajustar às horas de trabalho tradicionais.

Nesta linha, diversas evidências documentam que indivíduos vespertinos apresentam escores mais altos para depressão(146), diminuição do desempenho cognitivo matinal, sonolência diurna excessiva(147), bem como aumento dos riscos de morbidade e mortalidade geral(148). No entanto, evidências recentes mostram que os resultados adversos relacionados à saúde mental em vespertinos não são intrínsecos a este cronotipo, mas um reflexo de uma incongruência fisiológica da própria ritmicidade biológica do indivíduo com o tempo social(61). Nosso arquétipo de organização social favorece aqueles que tendem a acordar e dormir mais cedo (tipos matutinos) e obriga aqueles que tendem a acordar e dormir mais tarde (tipos vespertinos) a seguir rotinas que diferem de sua organização temporal interna(149). Assim, entende-se que pessoas vespertinas mais frequentemente apresentam desafios ao seu sistema temporizador. Por exemplo, estes indivíduos restringem sua duração de sono em dias de trabalho ou de escola, compensando este débito em dias livres. Na adolescência, este problema se acentua, pois há uma progressiva tendência à vespertinidade nesta faixa etária.

Um ensaio clínico randomizado testou o impacto de estratégias não-farmacológicas de higiene do sono – modificação da exposição luminosa, rotinas fixas de alimentação, restrição de cafeína e exercícios físicos – nos horários do sono, no DLMO e na resposta de cortisol ao despertar. Surpreendentemente, quando se observa um avanço de fase em indivíduos vespertinos, eles apresentam melhorias significativas nos sintomas depressivos e estresse auto-relatados, bem como melhores medidas de desempenho cognitivo e físico

pela manhã, quando reportavam que seu desempenho é, geralmente, "abaixo do ideal"(150).

Independentemente do cronotipo, um percentual significativo da população mostra médias de horas de sono em dias de trabalho ou de escola muito inferiores comparadas às médias dos dias livres(151). Além da duração, também se observa um atraso significativo da fase de sono nestes dias livres, determinando um maior jetlag social (JLS). Esta medida já foi associada com diversos desfechos de saúde, incluindo dislipidemia, epilepsia, agressão e problemas de conduta, transtornos de humor, comprometimento cognitivo no trabalho e desempenho acadêmico e uso de substâncias(152). Alguns estudos mostram que o aumento do risco de apresentar sintomas depressivos acontece quando esta medida do JLS é superior a 2h(146,153). Uma revisão destes desfechos de saúde física e comportamento associados ao JLS foi publicada pelo nosso grupo no periódico *ChronoPhysiology and Therapy*(152).

4. AVANÇOS NA PESQUISA DE RITMOS BIOLÓGICOS E SONO: POR QUE ESTUDAR SUA RELAÇÃO COM DEPRESSÃO?

4.1. A pluralidade da avaliação das rotinas de sono

Tendo em vista a base teórica construída nas seções anteriores, ainda vale mencionar que paradigmas recentes de estudo de doenças mentais como o *Research Domain Criteria* (RDoC) do National Institute of Mental Health (NIHM) propõe a integração de diversos níveis de informação objetivando explorar as dimensões básicas dos fenômenos mentais que abrangem todo o espectro do comportamento humano, do normal ao anormal(154).

Em uma vasta quantidade de pesquisas o sono é estudado pela ótica de diagnósticos psicopatológicos (e.g., insônia, apneia do sono), de sintomas associados (e.g., sonolência excessiva diurna) ou do constructo de "qualidade de sono", que engloba a percepção subjetiva de sono reparador e, por vezes, outros parâmetros como duração de sono e necessidade de uso de medicações para dormir. O sono, entretanto, é um comportamento complexo, e necessita de um olhar multidimensional para que seja compreendida a maneira como se associa aos sintomas depressivos e aos transtornos de humor. Para tal, atualmente é possível identificar muitos parâmetros pelos quais se pode estudar os padrões de sono-vigília e suas consequências(155).

Figura 6. Sobre o estudo das rotinas de sono para além da sua duração e da qualidade percebida. Quadrinhos de Quino, 2003.

Diversos parâmetros são particularmente úteis para determinar a maneira como cada indivíduo dorme, e cada parâmetro único é relevante para um distúrbio, doença do sono, ou condição clínica de interesse. Por exemplo, o tempo de início do sono, a latência do sono e o tempo total de sono são essenciais para diagnosticar pacientes com insônia. Por outro lado, um excesso nos índices de despertar sugere aumento da fragmentação do sono. Diários de sono podem ser construídos para o acompanhamento dia a dia dos padrões de dormir e acordar, além da possível adição de outros fatores de interesse, como despertares, idas ao banheiro no período da noite, dentre outros. Instrumentos como o Quesitonário de CronoTipo de Munique (MCTQ) permitem a identificação de distintos aspectos das rotinas de sono separadamente para dias de trabalho ou estudo e para dias livres (Figura 7). A partir do MCTQ, também se pode calcular as fases de sono e o JLS, medidas que têm ganhado atenção na pesquisa de ritmos biológicos em Psiquiatria(61,146). Por fim, também se podem estudar práticas e hábitos do dia-a-dia que interferem na qualidade, duração e eficiência do sono. Neste contexto, surge o conceito de higiene do sono.

Existem poucos instrumentos quantitativos validados projetado para avaliar higiene do sono. Consequentemente, é essencial desenvolver escalas que visam avaliar estes comportamentos, a fim de melhorar a confiabilidade e reprodutibilidade dos estudos sobre o tema. Entre os poucos instrumento, o *Sleep Hygiene Index* (SHI), desenvolvido por Mastin e colegas(156), é um instrumento validado para o inglês com ótimas propriedades psicométricas. Um crescente número de estudos investigou o sono e problemas relacionados ao sono em nações lusófonas (157–159). Portanto, há a necessidade de uma escala de higiene do sono na língua portuguesa. Tal instrumento contribuiria para a

pesquisa e a prática clínica de tratamento e acompanhamento de problemas de sono e de sua associação com outras condições comórbidas.

4.2. Avaliação objetiva das rotinas de sono e dos ritmos de atividade e repouso

Dentre os exames propostos atualmente para avaliação objetiva do sono, a polissonografia (PSG) é o que recebeu maior destaque até o momento na clínica, sendo capaz de diagnosticar a maior parte dos transtornos de sono descritos pela Classificação Internacional dos Distúrbios do Sono (*International Classification of Sleep Disorders*)(160). A PSG é um procedimento médico composto por vários testes simultâneos, mas independentes, que monitoram diferentes funções corporais durante o sono e que são registrados para seu estudo posterior usando canais diferentes. Entre estes testes, estão incluídos eletroencefalograma, eletro-oculograma, eletromiograma, eletrocardiograma, oximetria de pulso e capnografia(155). Uma modalidade de PSG portátil já foi descrita para possibilitar um exame no domicílio dos pacientes, com validade semelhante à PSG tradicional para detecção de indivíduos com transtornos de sono graves(161). Mais recentemente, a técnica da actimetria surgiu como oportunidade de aferição de padrões de sono em estudos naturalísticos(162). Cada dispositivo tem vantagens, portanto, a consideração principal para os pesquisadores será determinar qual é o mais adequado para um determinado projeto de pesquisa(163).

A actimetria (ou actigrafia) é um método de monitoramento contínuo da atividade motora por meio de um acelerômetro usado no pulso ou quadril(164). Esta tecnologia fornece dados que permitem análises completas de séries temporais de atividade e repouso. Alguns dispositivos disponíveis também monitoram a exposição à luz e a temperatura do pulso. Actímetros são dispositivos portáteis não invasivos que podem ser

usados fora de instalações clínicas, o que permite a coleta contínua de dados durante vários dias ininterruptos. Os dados são coletados em intervalos fixos (*bin*) que variam de 30 segundos a alguns minutos ou horas.

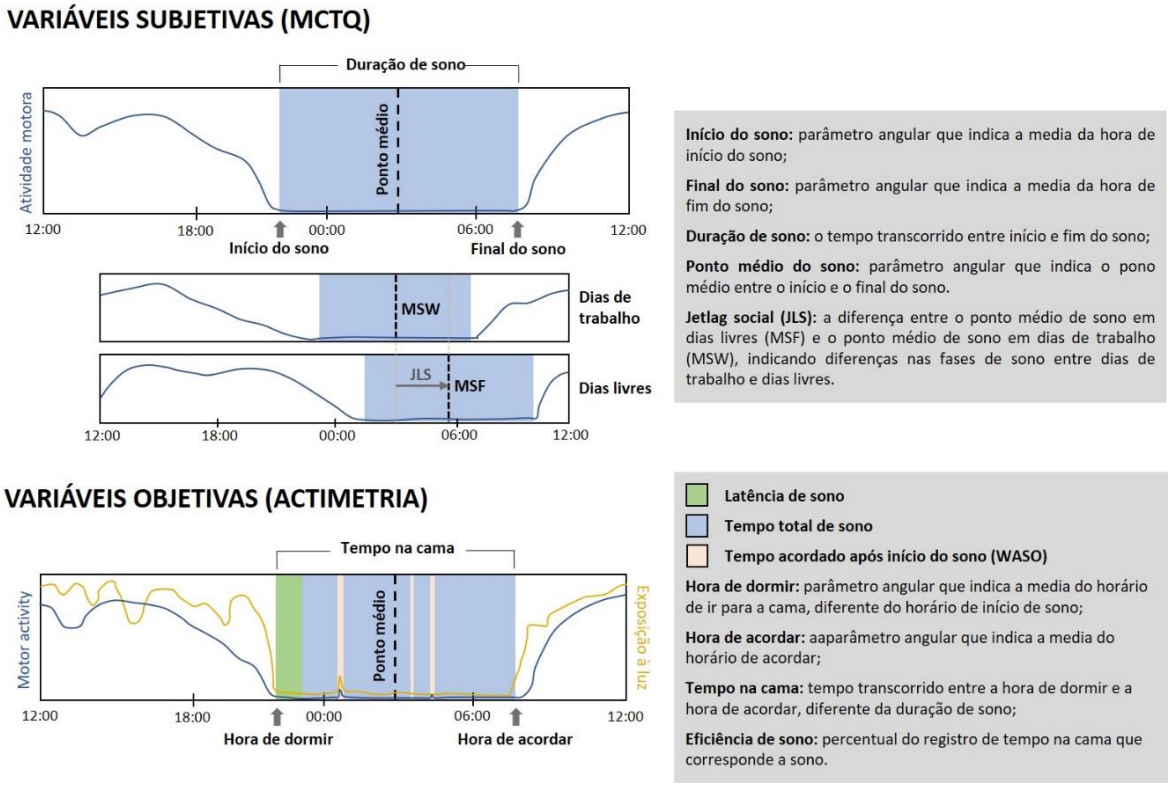

Figura 7. Parâmetros subjetivos de sono aferidos pelo Questionário de Cronotipos de Munique (MCTQ) e objetivos aferidos pela actimetria. Figura adaptada do artigo de Tonon e colaboradores que faz parte do corpo desta tese (Capítulo 6).

A maioria das evidências clínicas disponíveis usa dados de actimetria para monitorar padrões de sono de acordo com parâmetros de atividade-repouso (Figura 7)(165,166). Alguns estudos mostram que estes parâmetros possuem correlações satisfatórias àqueles avaliados por polissonografia(167,168). No entanto, outros estudos mostram que os actígrafos registraram uma latência de sono mais curta, tempo de início de sono avançado, aumento do número e duração dos despertares noturnos, aumento da duração do sono noturno e aumento do número e duração dos cochilos em comparação com os registros subjetivos do sono(169). Sendo assim, considerando estes baixos valores preditivos e a

superestimação do sono, alguns autores atualmente desqualificam a actigrafia como um indicador de sono-vigília preciso. A actigrafia pode, entretanto, ser útil para medir o período circadiano e tem validade de face como medida de atividade-repouso(170), variável fundamental para caracterizar o ritmo biológico de um ser vivo.

A possibilidade de monitoramento do comportamento de atividade (Figura 8) ganhou atenção recente nas principais áreas das ciências biomédicas, incluindo disciplinas médicas, Fisiologia, Farmacologia, Psicologia e Nutrição(171–173). Além disso, alguns estudos têm apontado recentemente as aplicações clínicas da actigrafia para avaliar a eficácia de tratamentos para distúrbios do sono, detectar padrões de atividade em distúrbios do humor e distinguir algumas psicopatologias(174–177). Por exemplo, diversos relatos descrevem que indivíduos deprimidos são mais ativos durante o período de repouso, e, por vezes, menos ativos durante o dia, traduzindo uma menor amplitude dos ritmos de atividade motora ao longo do dia(113,178).

Apesar da crescente utilização dessa técnica em estudos clínicos e experimentais, algumas questões metodológicas podem limitar a confiabilidade e generalização dos resultados. Em particular, uma vez que a coleta de dados ocorre fora do laboratório e depende da adesão dos sujeitos em usar o dispositivo em todos os momentos, sempre há o risco de se encontrar registros incompletos. Esses períodos fora do pulso são registrados como imobilidade pelo sensor de actimetria. No entanto, eles devem ser identificados como dados ausentes junto com dados resultantes de falha técnica e qualquer outro valor incongruente (normalmente chamado de “ruído” na coleta). Não há consenso atual sobre o melhor procedimento de manuseio após defini-los como 'NA' (não disponível). Várias abordagens para dados ausentes na actimetria são usadas, incluindo: 1) a manutenção da saída de actimetria, 2) a exclusão de um dia inteiro quando as perdas excedem uma certa

duração naquele dia, e 3) substituição de perdas por imputações de novos dados (ou seja, média e mediana dos pontos de tempo semanais(179,180)). No entanto, os artigos publicados muitas vezes não relatam claramente o processamento de dados e não existe uma técnica padronizada para lidar com estes episódios de dados faltantes.

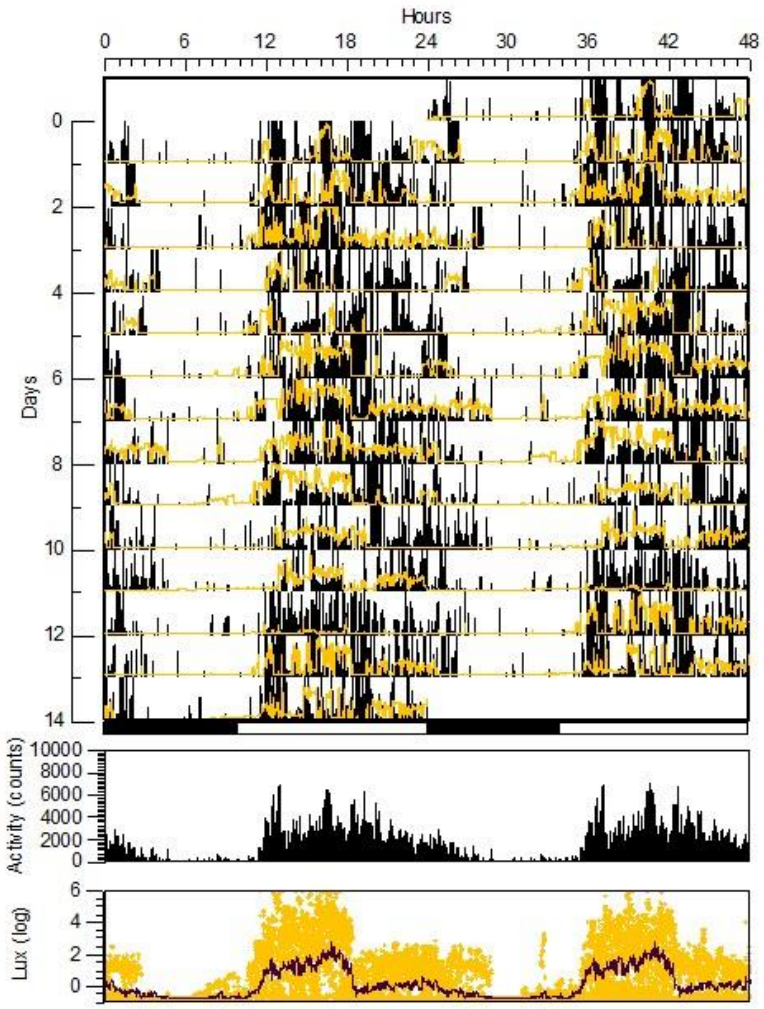

Figura 8. Ritmos de atividade-repouso e de exposição à luz medidos pela actimetria. A figura superior corresponde a um actograma que ilustra os ritmos de atividade-repouso (em barras pretas) e de exposição à luz (linhas amarelas) de 14 dias de registro. Cada dia se repete na próxima linha como uma forma de visualizar a transição entre os dias. Os dois gráficos da parte inferior representam a onda resultante da média de todos os dias de atividade motora e exposição à luz (em log de lux, onde os pontos amarelos representam os pontos de dados e a linha preta representa a onda média). Esta onda também está duplicada para que se possa observar a transição entre maiores e menores valores Figura adaptada do artigo de Tonon e colaboradores que faz parte do corpo desta tese (Capítulo 3).

5. JUSTIFICATIVA

“100

91

84

81

72

69

58

44

37 *38*

42

21 *28*

12

7 ”

“4.48 Psychosis” – Sarah Kane

A crescente morbimortalidade da depressão justifica a busca pelo aprimoramento do seu diagnóstico e por subtipos do transtorno que apresentem peculiaridades em relação ao tratamento clínico. Uma melhor compreensão das múltiplas facetas biológicas e psicológicas da depressão é fundamental para o desenvolvimento de terapêuticas mais eficazes(181). Neste contexto, algumas iniciativas internacionais surgem para desenhar diretrizes da pesquisa em saúde mental(154).

Dada a onipresença dos ritmos biológicos na fisiologia humana, entende-se por que em 75% dos ensaios clínicos desenhados sob a ótica da Cronomedicina publicados nos últimos 50 anos observa-se uma dependência do horário de tratamentos médicos para diversas condições clínicas(182). Isso nos leva a afirmar que o efeito do resultado de muitas intervenções terapêuticas pode ser altamente dependente do horário do dia. Embora muitas ciências médicas tenham avançado neste conhecimento de Cronomedicina (e.g., Cardiologia e Endocrinologia), ainda não se construiu um grupo sólido de evidências em

Psiquiatria. E quais seriam variáveis candidatas para o estudo da depressão na ótica da Cronomedicina?

Para que se estude um ritmo biológico, algumas características são essenciais. A facilidade de obtenção dos dados e a repetitividade são duas características importantes na escolha da variável de estudo. Padrões de sono-vigília e atividade motora são variáveis que satisfazem essas características e que têm sido estudadas no contexto de depressão. Perturbações nestes sistemas – tanto mudança de padrões de sono, quanto da ritmicidade de atividade motora – são consistentemente associadas a transtornos de humor.

O descobrimento das diversas facetas dos padrões de sono-vigília – além da clássica determinação duração e da qualidade subjetiva do sono – determina novas maneiras de olhar para o sono em diversos níveis. Entre estas facetas, encontra-se a higiene do sono e o jetlag social. Do mesmo modo, o início da aplicação clínica de tecnologias como a actimetria no início da década de 1990 abriu um novo campo de descobertas relevantes sobre padrões de atividade e repouso de indivíduos deprimidos. A partir do aprimoramento destes avanços, garantindo sua aplicabilidade e validade na população brasileira, pode-se propor sua aplicação na prática clínica psiquiátrica.

Para tanto, é fundamental aprimorar duas importantes ferramentas utilizadas no estudo de ritmos biológicos e sono, tanto para as medidas subjetivas como o desenvolvimento de uma escala de higiene do sono em português brasileiro, quanto para medidas objetivas, através do aprimoramento da aferição do ritmo de atividade-repouso pela actimetria. Além disso, dadas as teorias de intersecção entre sono, ritmos biológicos e depressão, é necessário que sejam desenvolvidos estudos de prova de conceito. Para este fim, escolhemos o estudo três situações distintas para serem incluídas nesta tese, quais sejam 1) uma população de homens jovens da comunidade, 2) uma amostra clínica de

pacientes com transtorno depressivo grave, 3) grupos de adolescentes com risco para depressão ou com diagnóstico de transtorno depressivo atual.

Portanto, as hipóteses a serem testadas nesta tese são:

1. A adaptação de um instrumento de higiene do sono para o português brasileiro é útil por revelar fatores de risco modificáveis associados a sintomas depressivos em nossa comunidade;
2. Dados faltantes nas séries temporais coletadas por actimetria impactam significativamente os resultados de parâmetros calculados a partir delas;
3. Cronotipo vespertino e má qualidade de sono se associam a sintomas depressivos, sendo que alguns componentes que determinam qualidade de sono podem ser modificáveis;
4. A análise de padrões de atividade-repouso e de exposição à iluminação aferidos pela actimetria é capaz de diferenciar subtipos melancólico e não-melancólico em pacientes com depressão grave;
5. Adolescentes em episódio depressivo apresentam maior irregularidade de rotinas de sono, maior exposição à luz à noite e menor amplitude dos ritmos de atividade comparado a adolescentes não deprimidos; algumas alterações destes ritmos também estão presentes em indivíduos com alto risco para depressão.

6. OBJETIVOS

Objetivo geral

Identificar a associação entre rotinas de sono e ritmos de atividade-repouso em estados depressivos.

Objetivos específicos

- Traduzir para o português e validar para uma população brasileira um questionário de avaliação quantitativa de higiene do sono (Capítulo 2, referente à hipótese 1);
- Descrever a associação de higiene do sono com sintomas depressivos (Capítulo 2, referente à hipótese 1);
- Descrever os impactos de simulações de dados faltantes no cálculo de parâmetros derivados de séries temporais de atividade motora medidos pela actimetria (Capítulo 3, referente à hipótese 2);
- Determinar quais aspectos da qualidade de sono que se associam com sintomas depressivos clinicamente significativos em uma população de adultos jovens (Capítulo 4, referente à hipótese 3);
- Testar a validade do uso de actimetria para a diferenciação de subtipos melancólico e não melancólico de depressão (Capítulo 5, referente à hipótese 4);
- Identificar padrões de sono e de atividade motora que diferenciem adolescentes com baixo risco para depressão, alto risco para depressão, e diagnóstico de transtorno depressivo maior (Capítulo 6, referente à hipótese 5).

CAPÍTULO 2 – O DESENVOLVIEMENTO DO ÍNDICE DE HIGIENE DO SONO

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Title: The Sleep Hygiene Index (SHI): Brazilian-Portuguese translation, cultural adaptation, and psychometric properties

Running title: The Brazilian-Portuguese Sleep Hygiene Index (SHI)

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ABSTRACT

Objective: To translate the Sleep Hygiene Index (SHI) to Brazilian Portuguese, to describe its psychometric properties and to show its association with sleep quality, daytime sleepiness, risk for sleep apnea and depressive symptoms.

Methods: Thirty subjects participated in the cultural adaptation and the item clarity evaluation. Twenty subjects answered the instrument in three different time-points for test-retest reliability. Eighty adult workers completed the SHI, the Pittsburgh Sleep Quality Index (PSQI), the Epworth Sleepiness Scale (ESS), the Beck Depression Inventory (BDI) and the STOP-BANG (S-B).

Results: SHI shows an acceptable internal consistency (Cronbach's $\alpha = 0.75$), as well as a high reproducibility (intraclass correlation = 0.972, $P < 0.01$). The three final factors of confirmatory factor analysis extract an average of 48.22% of the total sample variance. SHI score positively correlated with total PSQI ($r = 0.398$, $P < 0.001$), ESS ($r = 0.406$, $P < 0.001$) and BDI ($r = 0.324$, $P = 0.003$) scores. No correlations with S-B were found.

Conclusions: SHI presents satisfactory-to-optimal psychometric properties. This instrument is useful for treatment planning and management of sleep hygiene practices. Thus, it represents a reliable way of assessing sleep hygiene quantitatively in both research and clinical settings.

Keywords: Good Sleep Habits; Psychiatry; Depression; Sleep Apnea; Sleepiness; Sleep Quality; Sleep Hygiene

INTRODUCTION

Inadequate sleep practices and habits encompass multiple biological and environmental factors, reflecting on sleep quality, sleep duration, unrestful sleep sensation, and excessive daytime sleepiness(1). Unhealthy lifestyle factors, for instance lack of physical exercise and consumption of substances (i.e., caffeine, alcohol, and nicotine), negatively affect sleep(2). Moreover, the use of electronic devices such as cellphones, computers, and televisions also have demonstrated to impact sleep quality and duration(3).

In this scenario, sleep hygiene (SH) is postulated as a set of strategies that incorporate daily adjustments in both behavior and environment aiming to improve sleep(4). SH recommendations are almost uniformly included as part of cognitive-behavioral treatment programs for insomnia and other sleep disorders(5). Furthermore, sleep problems are commonly associated with mental health issues, such as depressive mood(6) and other psychological status(7), but the exact mechanisms of this bidirectional interaction remain unknown.

There are few validated quantitative instruments designed to evaluate SH. Consequently, it is essential to develop scales that aim to assess SH in order to improve the reliability and reproducibility of studies regarding the topic. Amongst these few, the Sleep Hygiene Index (SHI), developed by Mastin and colleagues(1), is a valid and reliable instrument made to assess sleep-related practices and behaviors.

Literature review highlights the necessity for the SHI to be translated to Portuguese, as increasing studies have investigated sleep and sleep-related problems in lusophone nations(8–10). Therefore, the instrument would contribute to the research and clinical practice of treatment and follow-up of sleep issues (9,10). Henceforth, this study aims to

develop a Portuguese translation and cross-cultural validation of the SHI into Brazilian Portuguese, as well as describe its psychometric properties and its association with sleep quality, daytime sleepiness, risk for sleep apnea and depressive symptoms.

MATERIAL AND METHODS

The process of translation, cultural adaptation and establishment of construct validity of the Brazilian Portuguese version of the SHI was based on the recommendations and methodology of Guillemin et al.(11), Brito et al.(12) and Terwee et al.(13)

Sample selection and study procedures

The Research Ethics Committee of the Hospital de Clínicas de Porto Alegre approved all procedures (protocol number #2015-0263 GPPG/HCPA). Individuals that accepted participation in the research protocol signed the informed consent after full explanation of study objectives.

For all sample size calculations (i.e., cross-cultural adaptation and clarity, internal consistency, and validity) the previous recommendations by Anthoine and colleagues (14) were used. Initially, the process of translation of the original scale was developed, followed by the cultural adaptation and evaluation of clarity of items by primary healthcare professionals (n=12) as well as patients attending family doctor appointments (n=18). With that, the final version of the scale was used for the assessment of test-retest reliability by professionals from primary healthcare settings (n=20). Finally, for the establishment of the Cronbach's alpha, the construct validity and the correlations of the instrument with depressive symptomatology and sleep questionnaires, adult hospital workers (nurses,

nurse assistants, and security staff; n=80) from both sexes were invited to answer study instruments.

The Sleep Hygiene Index

The Sleep Hygiene Index (SHI) is a self-reported instrument containing 13 items designed to assess behaviors and habits related to maladaptive sleep-related practices(1). Participants are asked to report how often they engage in such behaviors on a Likert scale ("always", "frequently", "sometimes", "rarely" and "never"). A total continuous score is derived from a sum of all questions, representing a global assessment of sleep hygiene, being higher scores an indicative of poor sleep hygiene (i.e., maladaptive sleep hygiene status). Yet, there is no cut-off established to define groups based on the total score(1,15,16). This instrument was based on a robust sleep hygiene model in a large nonclinical population.

Development of the Brazilian Portuguese version of the SHI

Two separate processes of translation were developed. Two groups of native Portuguese speakers with proficient English independently translated the original version of the SHI into Brazilian Portuguese. These translations were assessed by two panels composed of eight scientists with backgrounds in psychology (1), medicine (4), and biomedical science (3). This revision aimed to identify translation errors and to certify content validity. The revised drafts were translated back into English by two independent translators who are fluent in Brazilian Portuguese and are English native speakers. The translators had not been informed about the original scale or about the study objectives. The same revision teams from the previous step compared the back-translation with the

original instrument in English. The back-translation process was used to find and correct any errors in the Portuguese draft that would have led to inconsistencies with the original version.

A conciliatory version was determined by analyzing both translated versions. For this version, the research team considered the Brazilian linguistic and cultural context, aiming to achieve an instrument that was accessible to individuals with different social backgrounds, also preserving the rationale of the original scale. The conciliatory version underwent the following steps: cultural adaptation, evaluation of the clarity of items, analysis of reliability and reproducibility, and construct validity. The process from the original translation to the final conciliatory version is available in the **Table A**, available as an appendix.

Cultural adaptation and evaluation of the clarity of items

The conciliatory version was given to graduate students and individuals with higher education (n=12) and patients from the primary healthcare setting (n=18) for the assessment of clarity and adjustment to different cultural backgrounds. These participants evaluated the clarity of all items based on a visual analog scale (VAS; **Table 1**) ranging from “not at all clear/comprehensible” to “very clear/comprehensible”. The participants were also given the opportunity to provide further feedback and suggestions on how to adapt the instrument. These remarks were taken and reviewed by the research team, in order to achieve the final consensus version of the SHI in Brazilian Portuguese (see **Supplementary File A,** available as an appendix).

Internal consistency and reproducibility

The Cronbach's alpha was used as an internal consistency estimate. Furthermore, 20 individuals answered the instrument in three different time points: baseline, three hours later, and two weeks later. This process, called test-retest reliability, examines the reproducibility of the self-reports of the SHI in different moments, without any intervention with the tested subjects.

Validity

Face validity is defined as the capacity of an instrument to assess what it was designed to measure. In this study, face validity was determined by the multidisciplinary committee responsible for the Brazilian version of the SHI.

Content validity is defined as the rate to which either item applies to measure the target content. The content of the Brazilian Portuguese SHI is entirely based on the original validated instrument. Still, in order to establish the content validity of the content, each item of the SHI was compared to the SH recommendations of four different international societies (e.g. American Alliance for Healthy Sleep(17), American Sleep Association(18), Healthy Sleep – Harvard Medical School(19), Sleep National Foundation(20)).

Construct validity is the process by which the correlation of a measure with other variables is tested, aiming to analyze theoretical consistency. Considering previous evidence on the associations of SH with sleep-related outcomes(1,2,16), this study's hypothesis is that sleep quality and daytime sleepiness associate with SH. In addition, the SHI was correlated with risk for sleep apnea, expecting no significant associations, as this latter assessment is mainly based on constitutional variables (e.g., body mass index, age, gender, neck circumference), which are not expected to vary according to the modification

of sleep-related habits. Finally, previous studies report that sleep hygiene practices seem to be associated with depressive symptomatology(6,7). Therefore, we expanded our correlation analyses aiming to verify this association with the Brazilian Portuguese version of the SHI.

The construct validity was also tested using exploratory factor analysis.

Instruments

Sleep quality

The Pittsburgh Sleep Quality Index (PSQI) is a self-reported questionnaire that assesses sleep quality over the past month(21). The PSQI contains 19 items and seven components (i.e., sleep duration, sleep latency, habitual sleep efficiency, sleep disturbances, daytime disturbance, subjective sleep quality, and use of sleep-promoting substances). For this study, the validated Brazilian Portuguese version of the PSQI was used(22).

Daytime sleepiness

The Epworth Sleepiness Scale (ESS) is a questionnaire designed for subjective assessment of daytime sleepiness(23) (Johns MW., 1991). Individuals are asked to answer their likelihood to fall asleep in 8 daily hypothesized situations. For this study, the validated Brazilian Portuguese version of ESS was used(24).

Sleep apnea

The STOP-BANG (S-B) is a self-reported questionnaire that measures the risk of obstructive sleep apnea (OSA)(25). This questionnaire consists of 8 dichotomous items in a yes/no structure. The version used in this study is a validated version for Brazilian-Portuguese populations(26).

Depressive symptoms

The Beck Depression Inventory (BDI) is self-reported 21-item inventory that assesses symptoms and attitudes related to depression symptomatology(27). For this work, the validated Brazilian Portuguese version was used(28).

Statistical Analyses

The Shapiro-Wilk test assessed normality in data distribution. Student's t test for independent samples was used for group comparisons of parametric data. Intraclass correlation analyses were used for test-retest reliability, aiming to compare items and total score within the timepoints. For the exploratory factorial analyses, the Varimax rotation method extracted principal components and the number of factors suggested to be retained was based on Kaiser criteria (Eigenvalues > 1) and the scree plot. Pearson's correlation analyses compared the continuous SHI score with other study instruments. The values for Pearson's correlation were considered: weak (0 – 0.3), moderate (0.3 – 0.7), strong (0.7 – 0.9) and very strong (0.9 – 1).

The analyses were performed using SPSS for Windows (version 19; SPSS Inc., Chicago, IL) and all graphs were generated using GraphPad Prism version 7.0 for Windows (GraphPad Software, San Diego, CA). Values of $P < 0.05$ were considered statistically significant.

RESULTS

The presented version of the Sleep Hygiene Index (SHI) showed no significant changes in the structure of the questionnaire according to the translation process into

Brazilian Portuguese. The main alterations were linguistic adaptations that aimed to guarantee the adequate cross-cultural validation of the instrument.

The final version of the Brazilian-Portuguese SHI (see **Supplementary File A,** available as an appendix) was filled by eighty hospital workers (descriptive statistics shown in **Table 2**). The scale shows an acceptable internal consistency, as measured by a Cronbach's α of 0.75, as well as a high reproducibility estimate for the total score (intraclass correlation = 0.972, 95% confidence interval = 0.941-0.988, $P < 0.01$).

The construct validity of the Portuguese SHI version was tested using exploratory factor analyses. The Kaiser-Meyer-Olkin measure of sampling adequacy was 0.71. The Bartlett's chi-square value (214.76; $P < 0.001$) indicates the appropriateness of the data for the analyses. The Kaiser criteria (Eigenvalues > 1) and the scree plot suggested five factors to be retained. However, according to the theoretical sense of the items in each of the five factors, the model that provided the most desirable rotated factor structure was the three-factor model. Thus, a confirmatory analysis was conducted with the three defined factors (**Table 3**). The three factors extract an average of 48.22% of the total sample variance (F1= 26.80%, F2= 11.67% and F3= 9.76%), that explained a variance for each factor to the total variance among all SHI questions.

The final SHI score positively correlated with total PSQI (r = 0.398, $P < 0.001$), ESS (r = 0.406, $P < 0.001$) and BDI (r = 0.324, $P = 0.003$) scores (**Figure 1**). No significant associations were found between the SHI final score and the risk for sleep apnea measured by S-B. Sex differences were only significant for S-B data (t = -4.8, $P < 0.001$).

DISCUSSION

The Brazilian Portuguese version of the Sleep Hygiene Index (SHI) is an easy-to-use, self-administered instrument that can complement the evaluation of sleep issues both in clinical and research settings. This study used international recommendations and based its methods on previous high-quality studies to ensure that the Brazilian Portuguese translation of the SHI were both reliable and appropriate to the cultural context. The SHI is based on a robust SH model tested for its original version. Hence, in this study, the instrument was compared to the SH practices recommended by four different international societies, corroborating its content validity.

Individuals that were asked to answer the scale on every step of the methodology found the scale easy to comprehend. This quality is also reflected in the high levels of reported clarity of all items (**Table 1**). The instrument shows acceptable internal consistency ($\alpha = 0.75$), which is higher than the original English version ($\alpha = 0.66$)(1). Similarly, a satisfactory internal consistency was found in a nonclinical sample of Nigerian students ($\alpha = 0.64$)(16) and in a Korean sample with chronic pain ($\alpha = 0.75$)(15). Even though sleep habits constantly vary in an individual's life course, this study shows a high reproducibility for the Brazilian version of the SHI, as measured by intraclass correlations.

The construct validity of the Portuguese SHI version was tested using exploratory factor analyses, and three factors were obtained. The first factor in this Brazilian sample, defined as “sleep disturbing behavior and environment,” was composed by most items. The second factor was defined as “bedtime proceedings.” Resembling the results from a sample of Nigerian undergraduate students(16), the items “regular bedtime” and “regular get-up time” were grouped in the third factor, defined as “Irregular sleep-wake schedule.” For both samples, this factor demonstrates a high value for each item loaded. Additionally,

Chehri et al.(29) and Ozdemir et al.(30), found that the items loaded differently in the general Persian population and Turkish clinical and non-clinical samples, respectively. This difference can be attributed to cultural differences and sample characteristics.

The average score for the SHI is slightly lower compared to the one found in the original scale (30.02 ± 6.82 in this study compared to 34.66 ± 6.6 in the original(1)), with no significant sex differences. This study confirms the hypothesis that SH is associated to sleep quality and daytime sleepiness, but not to the risk of sleep apnea. The moderate correlations of the SHI with the PSQI and the ESS endorse the construct validity of the presented instrument. These results also point to the relevance of including assessments of SH in studies regarding sleep issues. Indeed, available evidence suggest that SH counseling(4) including sleep time regularity, avoidance stimulants beverage and daytime napping, improve sleep quality(7,31) and reduce daytime sleepiness(30,32). Sleep apnea is a medical condition commonly associated to constitutional factors that are not expected to change with better sleep practices. Thus, the absence of a significant correlation strengthens the construct validity of the SHI. Furthermore, a moderate correlation between inadequate sleep hygiene and depressive symptoms was observed. This relationship is in line with recent reports indicating that sleep hygiene strategies are somewhat related to depressive symptomatology (6,30,33,34).

A homogeneous convenience sample size of hospital workers was chosen to guarantee internal consistency to our findings. However, this may be a limitation of this study because 1) a convenience sample represents a risk for selection bias and 2) we only studied hospital workers from a community in south Brazil and it is not possible to control for socio-cultural and work-related aspects of this population. Moreover, our study was primarily based on self-reported measurements, and no diagnostic interview was

performed. Nevertheless, the results are similar to other studies that assessed SH using the SHI (1,16,30). In addition, even though we calculated our sample size *a priori*, it might have underestimated the correlation analyses. Future studies would highly contribute to the topic by exploring clinical samples and different settings.

CONCLUSIONS

The SHI is a simple self-report measure which presents satisfactory-to-optimal psychometric properties. This report shows moderate significant correlations of inadequate sleep hygiene with poor sleep quality, daytime sleepiness and depressive symptoms in an adult nonclinical population. This instrument can be useful in the treatment planning and in the management of sleep hygiene practices. Thus, it represents a feasible and reliable way of assessing sleep hygiene quantitatively in both research and clinical settings.

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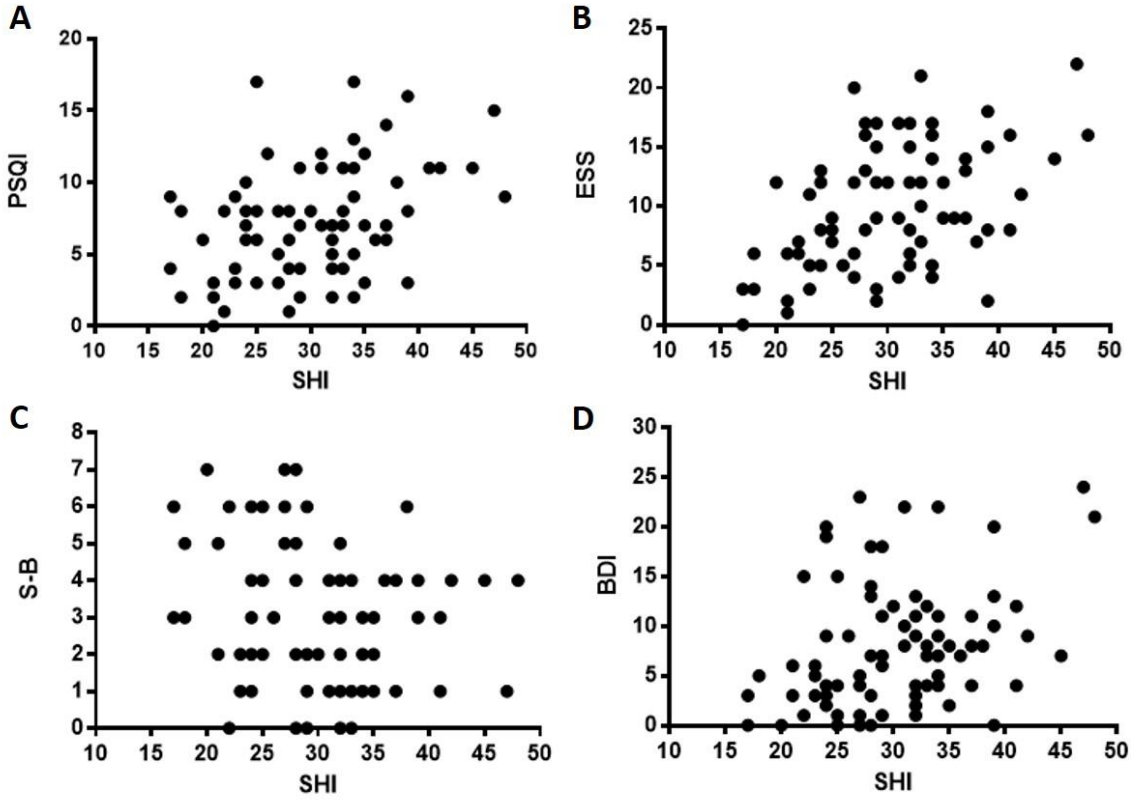

Figure 1. Correlations of sleep hygiene scores (Sleep Hygiene Index, SHI) with sleep quality (Pittsburgh Sleep Quality Index, PSQI, A), daytime sleepiness (Epworth Sleepiness Scale, ESS, B), risk for sleep apnea (STOP-BANG, S-B, C), and depressive symptoms (Beck Depression Inventory, BDI, D). Values are for Pearson's correlation coefficient and significance levels.

Table 1. Psychometric properties and content validity of the Brazilian Portuguese version of the SHI

SHI items	Item clarity (VAS from 0 to 10)	Internal consistency and reliability		International recommendations [1]			
		Cronbach's alpha if item is deleted	Intraclass correlation (95% CI)	SNF	AAHS	HS-HMS	ASA
Cronbach's alpha = 0.752							
1. Daytime naps	9.83	0.747	0.908 (0.807-0.961)	x		x	x
2. Regular bedtime	9.62	0.741	0.949 (0.889-0.979)	x	x	x	x
3. Regular get-up time	9.22	0.746	0.861 (0.707-0.941)		x	x	x
4. Nighttime physical exercise	9.79	0.767	0.859 (0.706-0.939)			x	x
5. Prolonged time in bed	9.64	0.744	0.907 (0.806-0.960)				
6. Use of stimulants close do bedtime	9.90	0.734	0.966 (0.930-0.986)	x	x	x	x
7. Doing activities that promote wakefulness prior to sleeping	9.66	0.722	0.919 (0.831-0.965)	x	x	x	x
8. Distressed emotional states at bedtime	9.75	0.719	0.899 (0.789-0.957)		x	x	x
9. Use of bed for activities other than sleeping or sex	9.85	0.728	0.979 (0.957-0.991)		x	x	x
10. Inadequate bed conditions	9.88	0.744	0.786 (0.545-0.909)	x	x		x
11. Inadequate room conditions (e.g. light, temperature and noise)	9.87	0.738	0.941 (0.876-0.975)	x	x		x
12. Dealing with important matter at bedtime	9.84	0.724	0.931 (0.857-0.971)	x		x	x
13. Worrying in bed/ nervousness in bed	9.88	0.715	0.911 (0.812-0.962)	x		x	x

[1] For each Sleep Hygiene Index item, an "x" represents the presence of sleep hygiene advice in the recommendations of these four international societies. Clarity items are represented as mean values of all responses. AAHS = American Alliance for Healthy Sleep; ASA = American Sleep Association; HS-HMS = Healthy Sleep - Harvard Medical School; SNF = Sleep National Foundation; VAS = Visual-analog scale.

Table 2. Descriptive statistics.

	Female (n=37)	**Male (n=43)**	**Total (n=80)**
Age	44.78 ± 8.77	44.74 ± 7.29	44.76 ± 7.95
Sleep quality (PSQI)	7.35 ± 4.33	6.81 ± 3.39	7.06 ± 3.84
Daytime sleepiness (ESS)	11.08 ± 5.2	9 ± 5.1	9.96 ± 5.23
Risk for OSA (S-B)	1.92 ± 1.91	3.79 ± 1.58	2.93 ± 1.97
Depressive symptoms (BDI)	9.32 ± 6.37	8.03 ± 6.23	8.04 ± 6.24
Sleep hygiene (SHI)	30.84 ± 5.58	29.42 ± 7.75	30.08 ± 8.83

BDI = Beck Depression Inventory; ESS = Epworth Sleepiness Scale; PSQI = Pittsburgh Sleep Quality Index; S-B = STOP-BANG; SHI = Sleep Hygiene Index.

Table 3. Factor loadings for the Sleep Hygiene Index (SHI) items.

SHI items	**Factor 1** Sleep disturbing behavior and environment	**Factor 2** Bedtime proceedings	**Factor 3** Irregular sleep-wake schedule
1. Daytime naps	0.027	0.641	0.058
2. Regular bedtime	0.203	0.054	0.749
3. Regular get-up time	-0.042	0.222	0.828
4. Nighttime physical exercise	-0.120	0.241	0.094
5. Prolonged time in bed	0.078	0.705	0.114
6. Use of stimulants close do bedtime	0.424	0.082	0.408
7. Doing activities that promote wakefulness prior to sleeping	0.649	0.031	0.202
8. Distressed emotional states at bedtime	0.678	0.265	0.037
9. Use of bed for activities other than sleeping or sex	0.453	0.584	-0.078
10. Inadequate bed conditions	0.694	-0.217	-0.115
11. Inadequate room conditions	0.549	-0.165	0.298
12. Dealing with important matter at bedtime	0.671	0.113	0.079
13. Worrying in bed/ nervousness in bed	0.583	0.486	0.105
Eigenvalues	3.48	1.52	1.27
% of variance	0.27	0.12	0.10

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CAPÍTULO 3 – IDENTIFICAÇÃO E MANEJO DE DADOS FALTANTES EM ACTIMETRIA

Artigo a ser submetido para o periódico *Journal of Clinical Sleep Medicine***.**

Title: Handling missing data in rest-activity time series measured by actimetry

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ABSTRACT

Background: Despite the increased utilization of actimetry in clinical and experimental studies, some methodological issues are still common. Since data collection occurs in a naturalistic environment and is dependent on an interrupted use of the device, there is always a risk for incomplete records, which are automatically recorded as immobility ("zeroes"). There is no current consensus on the best handling procedure after setting them as missing episodes ("NA"). We aimed to describe the effects of missing data in common parameters calculated from time series of motor activity derived from acrimetry. **Method:** We used two complete records (regular *vs* irregular sleep-wake and daytime activity) of 14 consecutive days sampled in 1-minute epochs using Proportion Integration Mode (PIM) as an estimator of motor activity. We have generated single NA intervals of 1h, 2h, 4h, 6h, 12h and 24h as well as random multiple NA episodes following probabilistic rules. The, we replaced these episodes with "zeroes" (the standard output for immobility), the mean or the median of the remaining 13 days corresponding to the missing bins. **Results:** Parameters showed less than 5% variation within groups by NA length and original series. With 24h exclusions the irregular series show a higher variability in acrophase ($F_{(13)}$=9.2, *P*<.01), MESOR ($F_{(13)}$=7.5, *P*=.01), L5 ($F_{(13)}$=8.02, *P*<.001), M10 ($F_{(13)}$=35.40, *P*<.001) and RA ($F_{(13)}$=5.19, *P*=.03) compared to the regular series. No differences were found comparing distinct lengths of total NA values in the random probabilistic simulations. The imputation of zeroes greatly increased the variance, but the substitution of by mean or median resulted in similar patterns of compared to the simulations with NA. **Conclusion:** Based on these results, the default undetected (i.e., the "zeroes") data should not be considered in any of the cases described above. If for some reason the parameters cannot be computed in the presence of NAs, we recommend the use of the weekly mean of corresponding timepoints.

Keywords: actigraphy, chronobiology, biological rhythms, motor activity, method

1. INTRODUCTION

Humans and other living beings have a temporal system that produces rhythms in various physiological functions, from gene expression to systemic organization[1]. Under natural conditions, these rhythmic physiological events synchronize to environmental cyclic signals they are entrained. Studies of biological rhythms require long periodic sampling of data to adequately characterize them. Usually, the biological rhythms phenomena are represented through a series of data values indexed according to time. This data format is called time series.

In the studies of biological rhythms, the ease of obtaining the data and the repetitiveness are two important characteristics when choosing the study variable. Motor activity and temperature are variables that satisfy these characteristics. Actimetry (or actigraphy) is a method of continuous monitoring of motor activity using an accelerometer worn on the wrist or hip[2,3]. This technology provides data that allow for thorough analyses of time series of rest-activity. Some available devices also monitor light exposure and wrist temperature. Actimeters are typically noninvasive portable devices that can be worn outside clinical facilities, which allows continuous data collection during several uninterrupted days. Data is collected in fixed intervals (bin) ranging from 30 seconds to a few minutes or hours.

Most available clinical evidence uses actimetry data (assessed by actigraphy devices) to monitor sleep patterns according to rest/activity parameters[4,5], which is comparable to polysomnography[6,7] and has gained recent attention in major areas of the Biomedical sciences, including medical disciplines, Physiology, Pharmacology, Psychology, and Nutrition[8–11]. Besides, some studies have recently pointed to the clinical applications of actigraphy for confirming diagnostics and evaluating the efficacy of treatments for sleep

disorders, detecting and preventing mood disorders and distinguishing some psychopathologies [12–15]. Despite the increased utilization of this technique in clinical and experimental studies, some methodological issues might limit the reliability and generalizability of results. In particular, since data collection occurs outside the lab and is dependent on the adherence of the subjects in wearing the device at all times, there is always a risk for incomplete records. These off-wrist periods are registered as immobility by the actimetry sensor. However, they should be identified as missing data along with data resulting from technical glitch and any other incongruent value (typically called “noise” in recording).

There is no current consensus on the best handling procedure after setting them as ‘NA’ (Not Available). Several approaches to missing data in actimetry are used, including: 1) the maintenance of the actimetry output, 2) the exclusion of a whole day when missings exceed a certain length on that day, and 3) substitution of missings by imputations of new data (i.e. mean and median of weekly time points[16,17]). Nonetheless, published articles often do not clearly report data processing and there is no standardized technique to handle missing episodes.

Since adequately dealing with missing data can ensure the reproducibility of the analysis[18], we aimed to describe the effects of missing data in common parameters calculated from time series of motor activity derived from acrimetry. For that, we describe the effects of simulated missing intervals on Cosinor, variance and amplitude measures. Additionally, we test the performance of different imputation procedures applied to simulated missing gaps.

2. METHODS

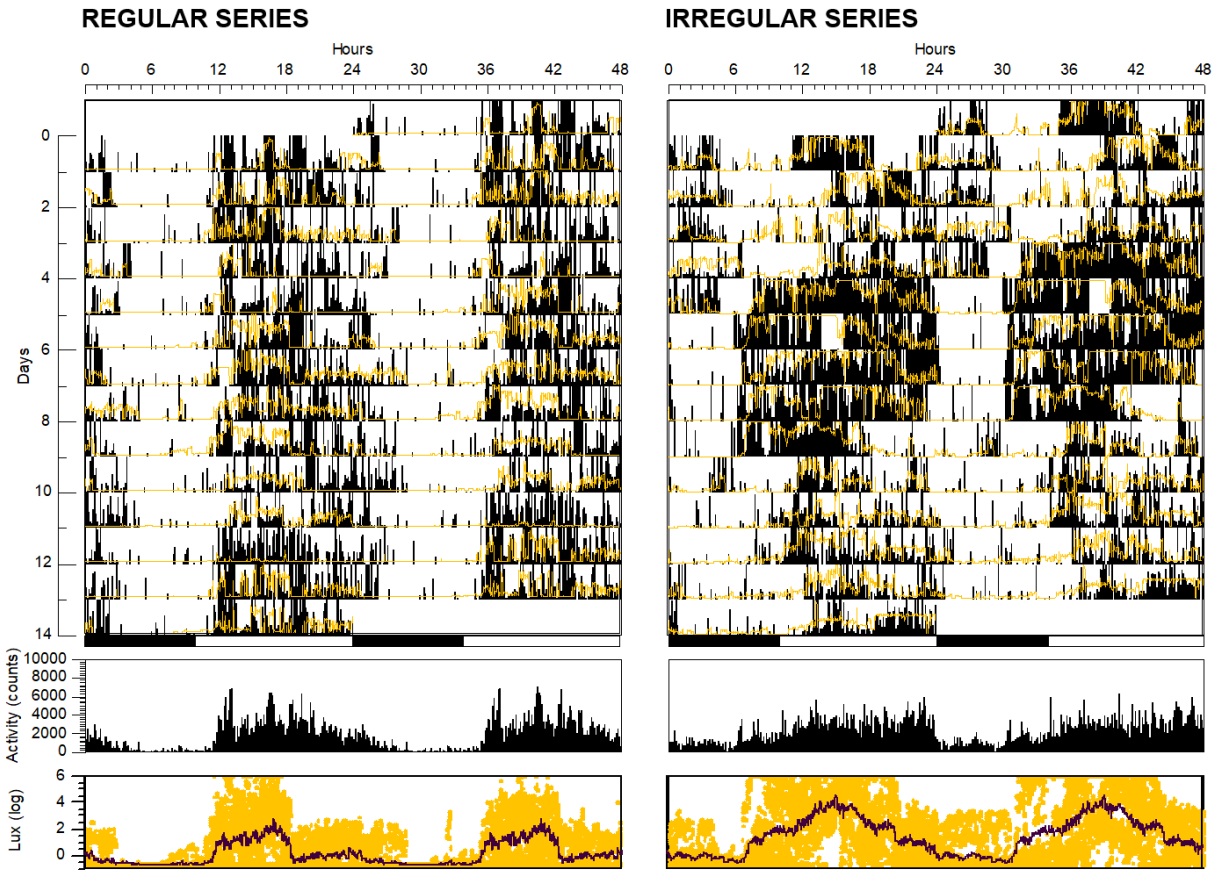
2.1. Data collection and series characteristics

The devices used for this study were ActTrust Condor™, equipped with an accelerometer, a luxmeter and a thermometer, allowing for the collection of motor activity, light exposure, and wrist temperature, respectively. The actimetry data was selected from another study with adolescents aged between 15 and 17, approved by the Institutional Review Board of the Hospital de Clínicas de Porto Alegre (number: 2018-0489). Upon agreement to participate, participants' parents signed a written informed consent. These subjects were asked to use the actimeters on the non-dominant arm for at least 14 consecutive days. Data were sampled in 1-minute epochs using Proportion Integration Mode (PIM), which calculates the area under the curve for each epoch. The ActStudio software (Condor™, Brazil, v. 1.0.12.0.1) was used to export data from the actimeters into data frames. All simulations of missing data were performed in two time series of two different subjects selected based on the distinct patterns visualized in their actograms: one had regular sleep-wake and daytime activity levels, and the other showed an irregular pattern of motor activity (**Figure 1)**.

Both time series were 14 days long and had originally no missing episodes. Missing episodes due to off-wrist were ruled out using two methods: 1) visual inspection of the actogram; 2) we used an algorithm by which data would be set to NA due to off-wrist if more than 55 in 60 consecutive data points were zeros, and if the difference between wrist temperature and environment temperature was lower than 0.5ºC. It is important to note that this technique for off-wrist detection is an in-house algorithm that was specifically designed for our dataset, considering the device we used (e.g., which collects data on wrist and ambient temperature with sensors). To maintain a fixed amount of data points per day,

the first and the last day of recordings were excluded – as they did not have data for the whole 24 h period. Series onsets were set at 0:00. Each time series had 20160 data points (1440 data points times 14 consecutive days with no missing episodes) (**Figure 1**).

Figure 1. The top figures are the double plotted actograms for activity (black bars) and log of light exposure (yellow line) of the 14 days of the regular and irregular series. Each day repeats itself in the next line as a way of visualizing the transition between days. Horizontal black bars represent the mean night period and horizontal white bars represent the mean photoperiod of the period. The two piled plots in the bottom part represent the double plotted mean wave of motor activity and light exposure (in log of lux, where yellow dots represent the data points and the black line represent the mean wave).



2.2. Actimetry-based parameters (outcomes)

To test the consequences of simulated missing intervals, we selected common reported parameters derived from actigraphic time series: Cosinor parameters (MESOR

and acrophase), and linear parameters derived from the meanwave (interdaily stability, intracycle variability, L5, M10, and relative amplitude).

2.2.1. Cosinor measures

From the Cosinor analysis, we chose the MESOR (Midline Estimating Statistic Of Rhythm), which is the constant after fitting data to a sinusoidal function and is close to the mean, and the acrophase, that indicates the time within the rhythm when the maximum value occurs.

The Cosinor analysis is a regression method that fits a cosine curve to the data series $\{t_i, y_i\}$ with $i = 1, \ldots, n$, where *n* is the number of data points. In other words, it considers that the values y_i at time t_i can be written as

$$y_i = M + A\cos\left(\frac{2\pi}{T}\, t_i - \,\phi\,\right) + \,\varepsilon_i$$

where the MESOR (M), the acrophase (φ), and the amplitude (A) are data adjustable parameters. The period (T) is set according to the expected period of the rhythm, usually 24h. The random variables ε_i represent the deviation between the data and the model and are expected to have a Gaussian distribution.

In the general case in which the times t_i are arbitrary, M, φ and A are determined by a full regression analysis, but when measuring times are regularly distributed in the period interval, for instance $t_i = i\,T/n,$ the MESOR is calculated simply by:

$$M = \frac{1}{n}\sum_{i=1}^{n} y_i$$

and the acrophase by

$$\phi = arctan\left(\frac{\sum_{i=1}^{n} y_i \sin\left(\frac{2\pi i}{n}\right)}{\sum_{i=1}^{n} y_i \cos\left(\frac{2\pi i}{n}\right)}\right)$$

and the amplitude by

$$A = \frac{1}{2}\sqrt{\left(\sum_{i=1}^{n} y_i \sin\left(\frac{2\pi i}{n}\right)\right)^2 + \left(\sum_{i=1}^{n} y_i \cos\left(\frac{2\pi i}{n}\right)\right)^2}$$

2.2.2. Variance Measures

The interdaily stability (IS) quantifies the repetitiveness of daily patterns and is defined by

$$IS = \frac{1}{p}\sum_{h=1}^{p}(\underline{y}_h - \underline{y})^2 \;/\; \frac{1}{n}\sum_{n=1}^{n}(y_i - \underline{y})^2$$

where $\underline{y}$ is the average of the time series, $\underline{y}_h$ is the average of the time series in the daily interval h across different days, and p is the number of these intervals (if hour by hour p=24, if minute by minute, p=1440). IS represents the variance explained by the daily rhythm. If every day is exactly the same, we should observe $IS = 1$, if the rhythm is completely uncorrelated from day to day, $IS \approx 0$.

The intracycle variability (IV) estimates the fragmentation of the rest-activity cycle and it is defined as

$$IV = \frac{1}{n-1}\sum_{i=2}^{n}(y_i - y_{i-1})^2 \;/\; \frac{1}{n}\sum_{n=1}^{n}(y_i - \underline{y})^2$$

It compares the total variance of the time series with the variance observed in consecutive data points. If the time series is smooth with few very well-defined transitions between rest and activity (for instance a perfect sinusoidal) we should observe $IV \approx 0$, otherwise if the signal approaches a Gaussian noise we could observe a $IV \geq 2$.

2.2.3. Amplitude Measures

The M10 represents the average value of the continuous 10 hours interval of the day with highest values of activity. The L5 represents the average of the continuous 5 hours interval of the day with lowest values of activity.

The relative amplitude (RA) between M10 and L5 is defined as

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

and it measures how different M10 and L5 are.

2.3. Simulations of missing data

2.3.1. Single missing episodes of up to 12 hours

To test if the removal of a single interval of data could significantly impact actimetry parameters, we have excluded single intervals of 1h, 2h, 4h, 6h or 12h, starting each 10 min of the original series, generating a new simulated series with every removal. The values excluded were set to “NA” (not available) in the data frame. This process resulted in 2016 different series for each episode length. Actimetry parameters were then estimated for all simulations, from which the coefficient of variation was calculated for each time interval.

2.3.2. Exclusion of a whole day

A standard procedure in studies with actigraphy when a day presents a couple of hours (usually above 4 hours) of missing data is the exclusion of that specific day from the analyses. To test the impacts of this strategy, we have generated series with single-day exclusions of a whole period from 00:00 to 23:59 every 24 hours for each of the 14 days.

2.4. Random missing allocation simulating real off-wrist episodes

Finally, to test the impacts of realistic off-wrist episodes, we derived from each original datasets 300 activity time series with simulated episodes of off-wrist, with the respective data points set to NA following probabilistic rules. These rules were formulated based on common patterns observed in actigraphy recordings. Off-wrist periods were simulated distinctly for day and night: daytime missing episodes range from 2 to 6 hours and appear randomly with a minimum interval of 10 hours between them; nighttime missing simulations were always a fixed 9h period, considering that, when participants in real life remove the device prior to bedtime, they rarely put it on before waking up. This period was set from midnight to 9am, according to the usual bedtime of the two subjects included. Thus, all 600 series derived from these simulations have a different proportion of missing data, varying from 11.45 to 93.55 hours. Detailed information on the probabilistic rules follow below:

1) Probability of a daytime missing was p = 1/2. We allowed simulations to create only one missing episode per day during daytime;
2) A daytime missing episode could occur from 9am to 11h59pm;
3) The length of daytime missing episodes was uniformly distributed between 2 and 6 hours, and they were clipped to 12am so daytime missing did not overlap with nighttime missings;
4) The probability of a nighttime missing was of 1/7;
5) Nighttime missings were always from midnight to 9am.

For all analyses, the series were grouped into categories of hours of NA. The categories are: **(1) series with less than 36 hours of NA:** 103 series derived from the regular and 94 from the irregular original series; **(2) series with between 36 and 48 hours of NA:**

100 derived from the regular and 102 from the irregular original series; **(3) series with between 48 and 60 hours of NA:** 54 derived from the regular and 71 from the irregular original series; **(4) series with more than 60 hours of NA:** 43 from the regular and 33 from the irregular original series.

2.5. Imputations of values to the periods set to NA (zeros, mean and median)

Available actimetry devices do not indicate off-wrist periods with desirable reliability. Thus, the usual output of actimetry data for no compliance (i.e., off-wrist) are records with a remarkably high prevalence of zeros. Often these periods can be confused with inactivity (i.e., rest periods). To test the impacts of off-wrist periods left as "zeroes" (undetected), we have replaced the "NA" values of the random simulations of off-wrist episodes with "zeroes". We also replaced zeros using a few imputation procedures aiming to determine whether the substitution of missing values would correctly approximate the series with simulated NAs to the original, and therefore minimize the difference and variance observed in the parameters derived from them. A common approach to handle missing data is the substitution of missing data bins for the average of all other bins corresponding to the same instant in other days[17]. To test the impacts of such handling, we have replaced the missing episodes of the probabilistic simulations with the mean of the remaining 13 days corresponding to the missing bins. We have also computed the median value as a more conservative approximation of the week activity average.

2.6. Statistical analysis

Single missing episodes simulations: Series with simulations of single missing episodes were grouped according to length and series of origin. A single coefficient of variation was calculated per group. Series with simulations of whole day exclusions were

grouped into those derived from regular vs. irregular series. One-sample t-tests were conducted to evaluate if the sample distributions are different from the original parameter estimates. The equality of variances of the parameters estimated from these groups was assessed with the Levene's Test for Homogeneity of Variance.

Random missing episodes simulations: For each parameter, an analysis of variance (ANOVA) assessed differences between categories of NA length (i.e., < 36h of NA, 36-48h of NA, 48-60h of NA and >60h of NA). The same parameters estimated from the series after treatment with imputation procedures were compared with ANOVA likewise. All analyses were performed using the *R* software version 4.0.2.

3. RESULTS

3.1. A single missing episode of up to 12 hours do not significantly affect actimetry parameters

Overall, parameters showed less than 5% variation within groups by NA length and original series (i.e., regular or irregular) (**Figure 2**). The regular series showed nearly no variability in acrophase and RA. The irregular series showed the greatest variance in all parameters. The greatest Cv can be seen for the L5 of the irregular series with 12h of missing episodes (Cv = 4.3%).

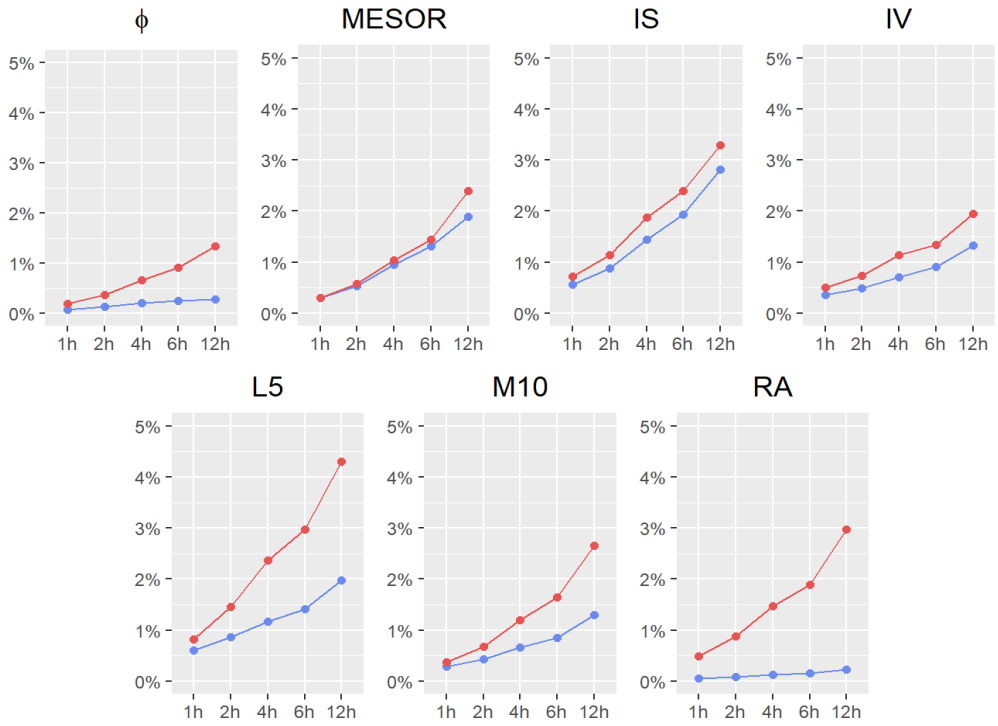


Figure 2. Coefficient of variation (Cv) of the actimetry parameters calculated form 2016 single missing episode simulations for the regular (blue) and irregular (red) series. The y-axis represents the Cv in decimals. Φ = acrophase (in hours), MESOR = midline-estimating statistic of rhythm, IS = interdaily stability, IV = intracycle variability, M10 = most active 10 hours, L5 = least active 5 hours, RA = relative amplitude.

3.2. Parameter estimates vary depending on time of day that data was excluded

Figure 3 shows the deviation of the estimated actigraphy parameters for 2016 simulations of single missing episodes of 12h. The graphs represent daily means and standard deviations of the actimetry parameters calculated from the series with missing episodes starting at the specific times of the day (i.e. the first data point represents the mean of the 14 series with the missing episode starting at 00:00, then the second data point represents the mean of the 14 series with the missing episode starting at 00:10, and so on).

By visual inspection, it seems time of day of the missing episodes impacts the estimation variability of most parameters. For example, daytime but not night time missing episodes impact the M10 value, whereas night time missings impact L5 and RA. Furthermore, the irregular series seems to have been more affected than the regular series.

The IS is the only parameter for which the regular series showed greater variance. The simulated values of IS are overestimated with respect to the original ones for both series around midday.

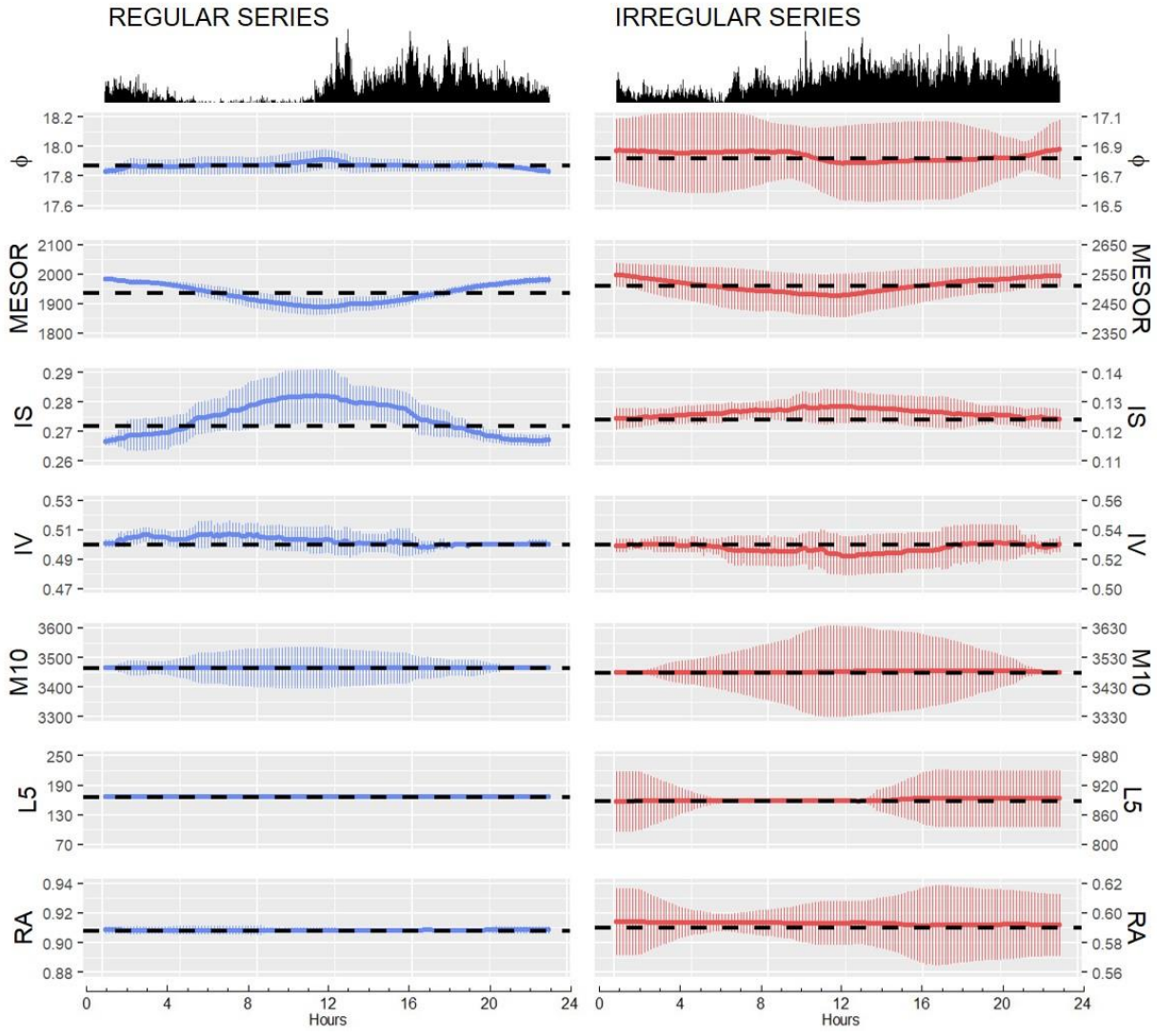


Figure 3. Variations in actimetry parameters with a single 12-h episode of missing data. Each data point on the "x" axis represents the start time of NA episodes (6 per hour). These data points are the average of the estimates derived from 14 series with NA-intervals set at specific times of the day (start of the NA-episode). The "y" axis represents mean and standard deviation of parameter estimates at the specific data point. Dashed black lines represent the parameter estimate for the original series. Black bars on top represent the meanwave for each subject from 00:00 to 23:50. Φ = acrophase (in hours), MESOR = midline-estimating statistic of rhythm, IS = interdaily stability, IV = intracycle variability, M10 = most active 10 hours, L5 = least active 5 hours, RA = relative amplitude.

3.3. Exclusion of a whole day might significantly influence some parameters.

Figure 4 presents the results for 14 simulations of exclusion of an entire day. Results from one sample t-tests show that none of the parameters show distributions that are significantly different from the original estimates (all $P > 0.05$, data not shown). Highest or lowest values did not correspond to any specific day of the week (data not shown).

The irregular series with exclusions show a higher variability in the estimation of acrophase (Φ; $F_{(13)}$=9.2, P<.01) and MESOR ($F_{(13)}$=7.5, P=.01).We observed more than 1h of difference between the lowest and highest Φ values of simulations from the irregular series. Both regular and irregular series slightly overestimated IS, but variances are similar comparing regular and irregular series ($F_{(13)}$=4.1, P=.05). The IV s of series with simulated NAs is on average close to that of the original series. No significant differences were observed in the variance of series derived from the regular and irregular original series ($F_{(13)}$=0.02, P=.88). The L5 and M10 estimations also present greater variability for the irregular series ($F_{(13)}$=8.02, P<.001; $F_{(13)}$=35.40, P<.001, respectively). The estimations generated from the irregular series had a greater variance in RA compared to those derived from the regular series ($F_{(13)}$=5.19, P=.03).

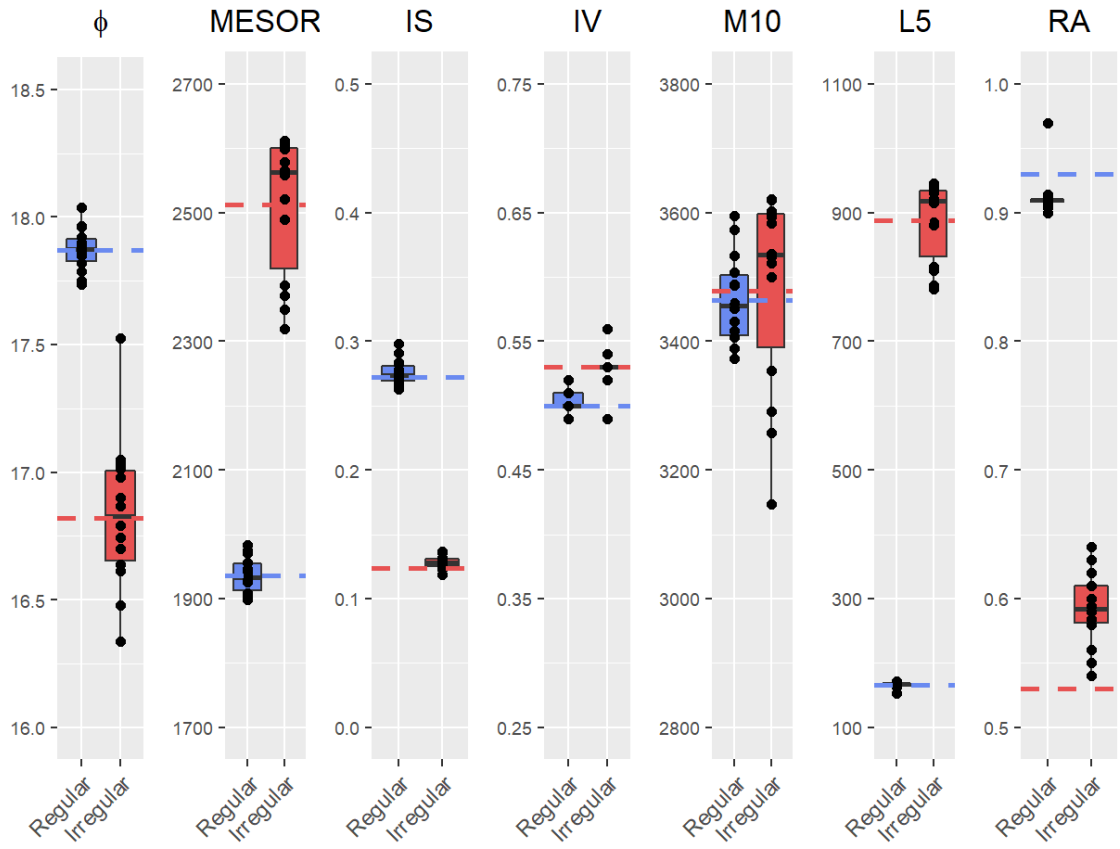


Figure 4. Boxplot representations of motor activity parameters for regular (blue) and irregular (red) series after simulated whole-day exclusions. Each boxplot represents the summary statistics for the median and interquartile range of the parameters derived from 14 simulated series (black dots) with the exclusion of an entire 24h period (from 00:00 to 23:59). Boxplot limits represent the first (Q1) and third (Q3) quartiles, and the vertical black line indicates the range of Q1 or Q3 times 1.5 the interquartile range. Dashed lines represent the parameter value derived from the original series. Φ = acrophase (in hours), MESOR = midline-estimating statistic of rhythm, IS = interdaily stability, IV = intracycle variability, M10 = most active 10 hours, L5 = least active 5 hours, RA = relative amplitude.

3.4. Random missing allocation simulating real off-wrist episodes results in significant changes in most parameters.

Most actimetry parameters show some dispersion around the original value (**Table 1** and **Figure 5**). We could not observe statistical differences comparing categories of total NA-length in any of the parameters.

3.5. Imputation of values to missing data distinctly impact actimetry parameters.

The imputation of zeroes greatly increased the variance of estimated parameters around the original value (**Table 1** and **Figure 5**). MESOR, IS and M10 were underestimated. We could not observe statistical differences comparing the categories of total NA-length of any of the parameters.

Table 1. Descriptive statistics for the random missing allocation simulating off-wrist episodes (NA) and the three methods of imputation of values (Zero, Mean, Median).

Regular (n=300)									
	Original	**NA**		**Zero**		**Mean**		**Median**	
		mean (sd)	***P***	**mean (sd)**	***P***	**mean (sd)**	***P***	**mean (sd)**	***P***
Φ	17,87	17.94 (0.11)	< 0.001	17.83 (0.24)	< 0.001	17.89 (0.09)	0,62	17.84 (0.12)	< 0.001
MESOR	1936,49	1932.52 (65.35)	0,29	1685.78 (82.08)	< 0.001	1938.45 (35.35)	0,34	1843.05 (43.98)	< 0.001
IS	0,27	0.28 (0.02)	< 0.001	0.24 (0.02)	< 0.001	0.31 (0.02)	< 0.001	0.29 (0.01)	< 0.001
IV	0,50	0.50 (0.01)	0,001	0.48 (0.01)	< 0.001	0.49 (0.01)	< 0.001	0.5 (0.01)	< 0.001
M10	3461,19	3468.73 (79.03)	0,10	3021.14 (180.52)	< 0.001	3468.73 (79.03)	0,10	3323.54 (90.89)	< 0.001
L5	166,69	166.56 (7.92)	0,78	145.78 (14.34)	< 0.001	166.56 (7.92)	0,78	145.89 (14.27)	< 0.001
RA	0,908	0.905 (0.005)	0,37	0.905 (0.012)	0,49	0.908 (0.005)	0,37	0.916 (0.008)	< 0.001
Irregular (n=300)									
	Original	**NA**		**Zero**		**Mean**		**Median**	
		mean (sd)	***P***	**mean (sd)**	***P***	**mean (sd)**	***P***	**mean (sd)**	***P***
Φ	16,84	16.89 (0.36)	0,10	16.67 (0.57)	< 0.001	16.86 (0.34)	0,91	16.77 (0.37)	< 0.001
MESOR	2512,52	2515.51 (85.36)	0,54	2190.11 (104.05)	< 0.001	2504.08 (75.27)	0,05	2357.31 (84.29)	< 0.001
IS	0,12	0.13 (0.01)	< 0.001	0.12 (0.01)	< 0.001	0.15 (0.01)	< 0.001	0.14 (0.01)	< 0.001
IV	0,53	0.53 (0.02)	0,003	0.5 (0.02)	< 0.001	0.52 (0.02)	< 0.001	0.52 (0.02)	< 0.001
M10	3478,75	3473.74 (136.42)	0,5253	3033.12 (167.03)	< 0.001	3473.74 (136.42)	0,53	3312.9 (131.54)	< 0.001
L5	887,82	881.53 (94.1)	0,25	747.36 (115.66)	< 0.001	881.53 (94.1)	0,25	768.96 (113.07)	< 0.001
RA	0,593	0.596 (0.037)	0,32	0.605 (0.053)	< 0.001	0.596 (0.037)	0,32	0.624 (0.048)	< 0.001

P values result from one sample t-tests comparing sample distribution with the original parameter. *Φ = acrophase (in hours), MESOR = midline-estimating statistic of rhythm, IS = interdaily stability, IV = intracycle variability, M10 = most active 10 hours, L5 = least active 5 hours, RA = relative amplitude*

Substitution of missing values by mean or median resulted in similar patterns of distribution of actimetry parameters compared to the random simulations of missing values themselves (**Table 1** and **Figure 5**). For MESOR, M10 and L5 the imputation with median values resulted in overall lower values. We did not observe statistical differences comparing categories of total NA-length in any of the parameters.

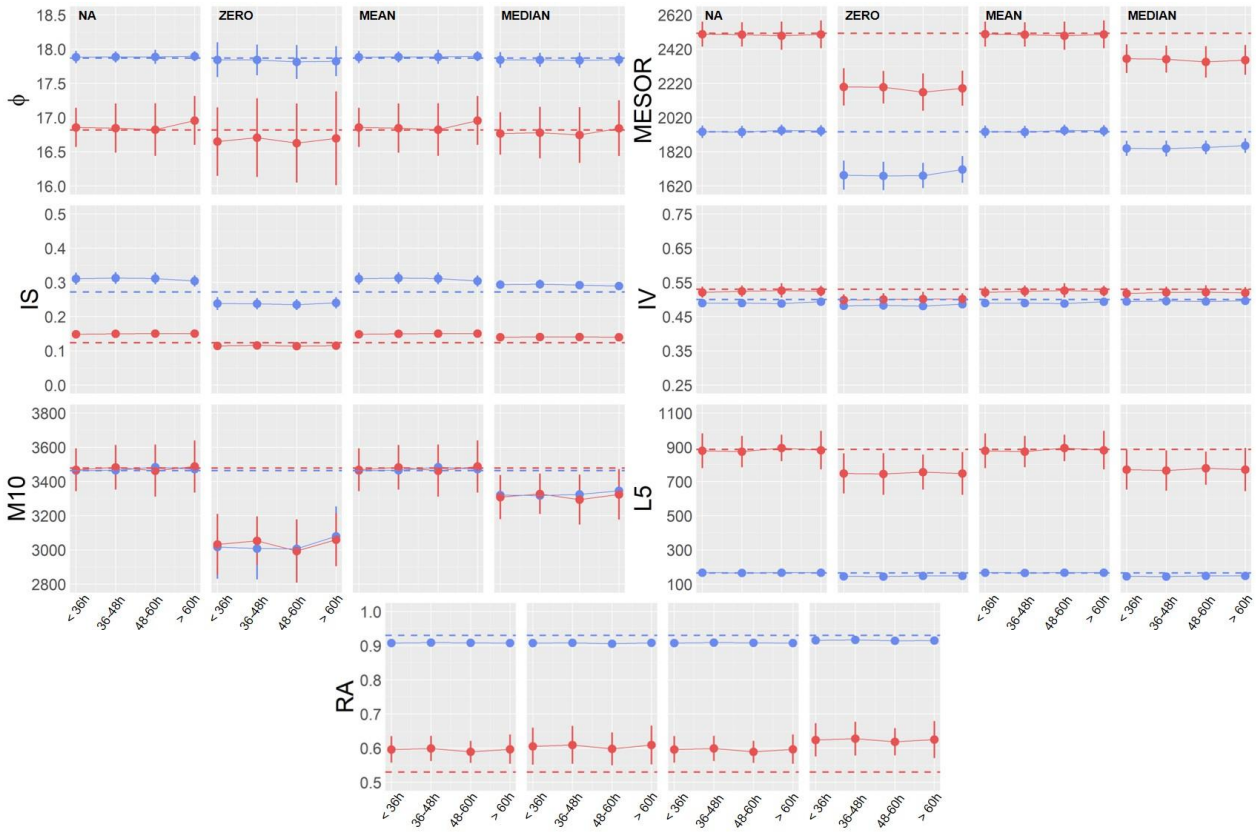


Figure 5. Actimetry parameters for regular (blue) and irregular (red) series after simulated probabilistic removal data (n = 600) grouped in intervals of hours of removed data. For each parameter, the first graph represents the mean and standard deviation of actimetry parameters by categories of hours of excluded data (missing data set as "NA", left panel). The graphs on the right show the results from the imputation with zeroes, and with the weekly mean or median replacing the excluded time point. Dashed lines represent the parameter value computed using the original series. Φ = acrophase (in hours), MESOR = midline-estimating statistic of rhythm, IS = interdaily stability, IV = intracycle variability, M10 = most active 10 hours, L5 = least active 5 hours, RA = relative amplitude.

4. DISCUSSION

Here we aimed to shed some light on the longstanding issue of missing data in actimetry. This study analyzed a few parameters with different mathematical characteristics as well as clinical and research implications. We show that missing data potentially impacts actimetry data depending on (1) the regularity of the time series in terms of day-to-day variability, (2) the nature of the computed parameter, (3) the length of the missing episode, and (4) the time of day of the excluded data. We could also demonstrate the effects of common handling of missing data on actimetry parameters: the exclusion of an entire day, the maintenance of off-wrist data as they are (undetected and included in the analysis; here mimicked by replacing simulated NAs with zeros), the replacement of missing data with weekly mean or median of the respective time point.

The irregular series show a greater variability in all simulations for the Cosinor parameters (Φ and MESOR), the M10, the L5 and the RA. This indicates a lower reliability of such estimates from irregular series with missing data, especially with more than 12h of NAs. However, the mean values of these parameters estimated from time series with simulated stretches of NA are similar to those of the original series (i.e., complete, with no missing data). Hence, a series with several days of collection (at least 14, as per our data) possibly maintains average values closer to the expected, whereas only a small percentage of patterns of missing data might lead to the misinterpretation of parameters. The IS is the only parameter that shows greater albeit small variability in the regular series. One possible explanation is that, since IS is a measure of daily repetitions of the activity patterns, the removal of data from an irregular series leads to a reduction in the variability, thus increasing the IS value. This hypothesis is supported by the fact that both series (i.e. regular and irregular) show an overestimation of IS in all simulations. The IV does not change

significantly comparing regular and irregular subjects, possibly because it is calculated based on hourly transitions of activity counts. Therefore, it does not depend on day-to-day regularity.

Even though we observe variations in the parameter values with up to 12h of missings, the estimates are still overall close to the original parameter. However, the results of exclusions of 12h clearly show a distinct impact of the missing episode depending on the period of the day where data was excluded. As expected, the circular parameter (Φ) and other parameters that depend on higher activity levels (MESOR and M10) are more impacted by daytime missing episodes, whereas L5 (the average activity of the 5 consecutive hours with the lowest levels of activity) are more affected by nighttime missing episodes. The RA follows the trend of L5 and seems to be more impacted by nighttime missing episodes. IS and IV vary more with daytime missings, possibly because these are often periods of greater variability.

Contrary to our hypothesis, the exclusion of a whole day does not show significant impact on any parameter. According to our data, Φ, MESOR and M10 parameters might be either overestimated or underestimated in irregular time series with a whole day excluded. However, the remaining parameters estimated from irregular series might still be reliable, as well as any of the parameters derived from regular series. Taken together, the results of simulating up to 24h single missing episodes indicate that it is not necessary to exclude the entire day when there are gaps up to 12h long when computing the same parameters as we did. The calculation of these parameters from simulations with missing episodes of 4, 12 or 24h show similar results. Although we could not observe an influence of any specific day of the week (i.e., work/school day or free day), we highlight that we only have two weekends in each series, with limited possibilities of studying this hypothesis.

We also hypothesized that the more missing data, the greater would be the variance of the estimates. However, the results of our random missing allocation simulating participants' non-compliance showed the same variability from about 12h to about 90h of total missing data. No statistical difference could be found comparing categories of total NA-length.

Finally, the imputation of zeros to missing episodes considerably distance the simulated parameter values from the original. MESOR and M10 are underestimated in both regular and irregular series. L5 is underestimated solely in the irregular series, which presents more non-zero values of activity during the rest period. This imputation also resulted in an increased variability of the Φ and RA estimates, with no significant impact to the IS or IV parameters. The use of the weekly means of the corresponding time points shows overall similar results compared to the distribution of the missing values set as "NA". This is expected as these parameters are calculated from a mean wave derived from the 14 days of data. The use of the weekly median of the corresponding time points is similar to the mean estimation, except for the MESOR, M10 and L5 measures, in which an overall underestimation of values was observed. Based on these results, the default undetected (i.e., left as is) missing data should not be considered in any of the cases described above. If for some reason the parameters cannot be computed in the presence of NAs, we recommend the use of the weekly mean of corresponding timepoints.

Our study has several strengths, including the use of two complete and distinct time series (i.e., irregular, and regular), the calculation of widely used actimetry parameters, and the choice of the most common patterns of missings that we observe in real-life time series. However, there are also some limitations to consider. First, our study did not encompass all patterns of rhythmic activity. For example, we did not assess the effects of missing data

when characterizing free running rhythms. We believe it would be interesting to test the effects of missing data on the characterization of ultradian and infradian rhythms as well. In addition, we cannot extrapolate these results to other elements of actigraphy, like light exposure and temperature since they represent time series of different characteristics. Other time series parameters that are not based on the mean wave and/or 24h-period (e.g., Rayleigh test, harmonics, stationarity properties and complexity analyses) might be differently affected by missing data. This is also the case of sleep parameters automatically calculated by actimetry softwares, as the algorithms used are based on periods of inactivity, which were not evaluated in this study. For example, studies with the purpose of detecting naps or short sleep periods might find a much more significant impact of off-wrist NA values which could be detected as immobility. Finally, we performed the analyses on 7 and 14 days of actimetry data binned in 1-minute epochs. Different results might have been obtained with other series' lengths and resolutions.

Our study has valuable implications for research in actimetry. The impact of missing data in studies of rodent models might be extrapolated from the results of simulations of missing data in the regular series of the present study. This assumption derives from the fact that most activity data obtained from animals in standard housing conditions present is aligned to light-dark cycles. Human actimetry data should be treated as "irregular" when a conservative approach is chosen. Alternatively, studies can consider accounting for regularity of participants' routines in order to foresee the impacts of missing data. Of course, ensuring a complete data collection avoiding missing episodes is preferable. When recordings have missing episodes of greater significance (i.e. more than 12h of missing data within a day or among days), and variables derived from the activity phase (e.g. Φ, MESOR and M10) are desired, a new data collection should be considered.

To our knowledge, this is the first study that tested and proposed different imputation approaches to apply on missing gaps. Despite the growing body of research employing actimetry, there is no current consensus about some methodological aspects. It is unclear how many days of recording are necessary for an optimal analysis of sleep and rhythmic parameters. Reports found in the literature vary from a few days to several weeks, even when the same analysis is performed[19–21].

We chose 14 days of recordings to include two subsequent weekends in the analysis, as we hypothesized this could impact our results. However, we also performed all analyses for the first 7 days of our time series (data not shown), obtaining similar results, except for a larger variability. These results could help increase reliability and validity of parameters estimated from actimetry in research and clinical settings.

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CAPÍTULO 4 – CRONOTIPO, SONO E ESTRESSE EM JOVENS COM SINTOMAS DEPRESSIVOS

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ORIGINAL ARTICLE

How do stress, sleep quality, and chronotype associate with clinically significant depressive symptoms? A study of young male military recruits in compulsory service

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Abstract

Objective: Although studies have shown an association between poor sleep and chronotype with psychiatric problems in young adults, few have focused on identifying multiple concomitant risk factors.

Methods: We assessed depressive symptoms (Beck Depression Inventory [BDI]), circadian typology (Morningness-Eveningness Questionnaire [MEQ]), sleep quality (Pittsburgh Sleep Quality Index [PSQI]), perceived stress (Perceived Stress Scale [PSS]), social rhythm (Social Rhythm Metrics [SRM]), and salivary cortisol (morning, evening and night, n=37) in 236 men (all 18 years old). Separate analyses were conducted to understand how each PSQI domain was associated with depressive symptoms.

Results: Depressive symptoms were more prevalent in individuals with higher perceived stress (prevalence ratio [PR] = 6.429, $p < 0.001$), evening types (PR = 2.58, $p < 0.001$) and poor sleepers (PR = 1.808, $p = 0.046$). Multivariate modeling showed that these three variables were independently associated with depressive symptoms (all $p < 0.05$). The PSQI items “subjective sleep quality” and “sleep disturbances” were significantly more prevalent in individuals with depressive symptoms (PR = 2.210, $p = 0.009$ and PR = 2.198, $p = 0.008$). Lower levels of morning cortisol were significantly associated with higher depressive scores ($r = -0.335$; $p = 0.043$).

Conclusion: It is important to evaluate multiple factors related to sleep and chronotype in youth depression studies, since this can provide important tools for comprehending and managing mental health problems.

Keywords: Depression; circadian rhythm; circadian typology; cortisol; psychological stress; eveningness

Introduction

The prevalence of mood disorders, such as major depression, is growing in young populations, and the impairment they cause may affect development and functionality.[1,2] Such psychiatric disorders are essentially multifactorial, encompassing a set of mood and anxiety symptoms, as well as neurochemical balance, which is also related to altered sleep patterns and impaired circadian rhythm entrainment.[3,4]

Disrupted sleep increases vulnerability to psychiatric symptoms.[5,6] Recent evidence from correlational and experimental studies in healthy adults suggests that a loss of sleep results in poorer emotional response, such as heightened reactivity to negative emotional experiences.[7,8] Biological rhythmicity in mammals is generated by complex molecular machinery, and the main environmental cue that synchronizes biological clocks is the light-dark cycle.[9] However, social routines also interfere in this mechanism, since they determine light exposure and feeding times, as well as rest-activity patterns.[10] Such changes in social routines have been considered risk factors for developing depressive symptoms.[11] Furthermore, theoretical models indicate that physiological response to chronic stress may function as a vulnerability factor, linking environmental stress or trauma to the etiology of depression.[12] Poorer mental health outcomes, such as depression, are associated with flatter diurnal cortisol rhythm,[13,14] lack of control, negative affective reactions, and less ability to deal with stressors, which is measured by perceived stress.[15]

We hypothesized that subjective factors related to stress, sleep, and circadian rhythm are independently linked to clinically significant depressive symptoms in young men. To test this, we assessed circadian typology, sleep quality, perceived stress, and social rhythm (both the regularity and volume of activities) in a homogenous sample of 18-year-old male recruits in compulsory military service. To further classify sleep quality in this population,

we conducted a separate analysis aiming to understand how each domain of the Pittsburgh Sleep Quality Index (PSQI) was associated with depressive symptoms. We also hypothesized that the higher the depressive symptoms scores, the flatter the diurnal cortisol variation. To test this we measured salivary cortisol three times of a day: upon awakening, in the afternoon, and at bedtime.

Material and methods

Subjects and study protocol

The investigation was conducted in accordance with the latest version of the Declaration of Helsinki (2013). The research ethics committee of the Hospital de Clínicas de Porto Alegre (HCPA), Brazil, approved all procedures (case 2015-0263 GPPG/HCPA).

Eligible participants were healthy young conscripts accepted for compulsory military service in the Brazilian Air Force (Canoas Air Force Base/ALA 3, Canoas, Rio Grande do Sul) between June and December 2015. Conscription is considered the period between compulsory enrollment and enlistment for duty, which in Brazil could last up to 12 months. The Regional Air Force Commander and other local authorities endorsed this study. All candidates had already passed the Air Force medical evaluation and physical fitness test. Exclusion criteria for this study were continuous medication, clinical comorbidities, or inability to understand the research instruments.

This study included 236 young male conscripts (all 18 years old) who were undergoing the standard psychological evaluation. Individuals who agreed to participate in the study protocol provided written consent after the nature of the procedures had been fully explained, and were led to a separate room where the research team guided self-completion of the study's instruments.

Study questionnaires

Beck Depression Inventory (BDI)

The self-reported 21-item BDI refers to symptoms and attitudes related to depression. In this study, individuals who scored >10 were considered to have clinically significant depressive symptoms, following the cutoff of Gomes-Oliveira et al.[16] for Portuguese-speaking non-clinical Brazilian populations, which demonstrated excellent internal consistency (Cronbach's alpha = 0.93).

Perceived Stress Scale (PSS)

This 10-item self-report questionnaire includes six items assessing lack of control and negative affective reactions and four positively stated items representing the ability to deal with stressors. The total score is obtained by reversing the responses to the four positively stated items (e.g., 0 becomes 4, etc.) and then summing all items. In this population, the 75th percentile of the total score was used to separate lower from higher perceived stress. The Brazilian Portuguese version of the PSS was validated by Luft et al.,[17] showing good reliability (Cronbach's alpha = 0.83).

Morningness-Eveningness Questionnaire (MEQ)

This self-report questionnaire includes 19 questions related to individual preferences in the temporal organization of activities throughout the day based on an optimal sleep-wake cycle.[18] The global score is used to classify circadian typology, i.e., individuals are classified as morning-type (66-86), evening-type (16-44), and intermediate-type (45-65). These cutoffs were established for Brazilian populations by Benedito-Silva et al.[19]

Pittsburgh Sleep Quality Index (PSQI)

This self-report questionnaire was developed to assess sleep quality and disorders over the past month. The PSQI includes seven components: (C1) subjective sleep quality; (C2) sleep latency; (C3) sleep duration; (C4) habitual sleep efficiency; (C5) sleep disturbances; (C6) use of sleeping medication; (C7) daytime dysfunction.

The total score, i.e., the sum of all seven components, describes a continuum of impaired sleep. Individuals scoring > 5 were considered to have poor global sleep quality. To analyze individual PSQI components, the first two (i.e., 0 and 1) and the last two (i.e., 2 and 3) scores were grouped to create separate cutoffs (described below).The Brazilian Portuguese version of the PSQI, which was validated by Bertolazi et al., presented good reliability (Cronbach's alpha = 0.82).[20]

Social Rhythm Metric (SRM)

SRM assesses social cues as a means of regularity in social routines and social activity level. The short version used in this study (SRM-6) consists of a list of six activities used to quantify an individual’s daily social rhythms.[21] The recruits completed the scale one week prior to reporting for military training. Those who were enlisted delivered it to the researchers in person on enlistment day, while those were discharged from service delivered it by mail.

Two parameters are obtained from the SRM: a “hit” score, which reflects the regularity of activities, and the Activity Level Index (ALI), which refers to the volume of different activities in that period. A total of 178 participants completed the SRM-6. The Portuguese version of the SRM-6 was developed by Schimitt & Hidalgo.[21]

Salivary cortisol measurement

After the questionnaire assessment, the research team provided three Eppendorf flasks with a 1.5 mL sampling capacity for saliva collection at three times of day: morning (upon awakening), afternoon (between 4:00 p.m. and 5:00 p.m.) and night (just before going to bed). A random sample of 37 conscripts provided all three salivary samples. The participants were instructed to avoid eating and to perform brief oral hygiene with water before sample collection. They were also instructed to refrigerate the samples overnight before returning them between 6:30 a.m. and 7 a.m. the next morning. All samples were returned on the same day to the HCPA and were stored at -80 ºC until further analysis.

Biochemical analysis began by homogenizing and centrifuging the salivary samples at 3000 *g* for 10 minutes to remove debris. The samples and reagents were handled at room temperature, and the assay was conducted according to manufacturer instructions. Each sample was analyzed in duplicate and the salivary cortisol levels (ng/mL) (DBC-Diagnostics Biochem Canada Inc., London, Canada) were determined by enzyme-linked immunosorbent assay (ELISA). The sensitivity of the ELISA kit was 1.0 ng/mL, with an inter-assay variability of 8% and an intra-assay variability of 8.7%. The presented results are the mean of the duplicates of each sample. We used a computerized method (My Assays; https://www.myassays.com/home.aspx) with a four-parameter curve fit to obtain the results.

Statistical analysis

The Shapiro-Wilk test was used to assess the normality of data distribution. For parametric variables, groups were compared using Student's *t*-test for independent

samples. ANOVA with Tukey post-hoc analysis was used to assess differences in mean cortisol levels between the three sampling times. These results are expressed as mean and 95% confidence interval (95%CI). Data with non-Gaussian distributions (i.e., ALI scores) were compared using the Mann-Whitney *U* test and are expressed as median ± interquartile range (IQR).

Univariate analysis with robust Poisson regression was performed to obtain prevalence ratios (PR) for continuous variables (as covariates) and categorical variables (as cofactors). Multivariate modeling with robust Poisson regression was also performed. Variables reaching a significance level of 0.2 in the univariate models were entered the multivariate analysis. The same significance levels were used for all steps of the multivariate models. Parametric tests were used to compare cortisol levels with BDI scores and obtain Pearson's correlation coefficient. A quadratic regression model was used to test for non-linear effects of the correlation between cortisol and BDI scores.

Statistical analysis was performed using SPSS version 19 for Windows and all graphs were generated using GraphPad Prism version 7.0 for Windows (GraphPad Software, San Diego, USA). Values of $p < 0.05$ were considered statistically significant.

Results

The average BDI score in this population was 6.93 (standard deviation [SD] ± 6.79). Using the cutoff established for non-clinical populations (i.e., BDI > 10), 44 (18.6%) individuals had clinically significant depressive symptoms and 192 (81.4%) did not.

Associations between depressive symptoms and perceived stress, chronotype, and sleep quality

Table 1 shows the descriptive statistics of questionnaire data for the total sample, as well as the distribution comparisons between these groups. Table 2 shows estimated PR of depressive symptoms based on the questionnaire data for each BDI group.

Table 1. Questionnaire data, descriptive statistics, and distribution comparisons between BDI groups

	Total sample		**BDI ≤ 10**		**BDI > 10**		
Variable	**n**		**n**		**n**		**p-value**
Perceived stress (PSS)	236	14.78 (13.98-15.58)	192	13.4 (12.25-14.05)	44	20.7 (18.46-22.91)	< 0.001*
Lower	177	0.75	163	84.9	14	31.8	
Higher	59	0.25	29	15.1	30	68.2	< 0.001*
Chronotype (MEQ)	236	51.18 (50.09-52.27)	192	51.9 (51.46-54.06)	44	47.9 (43.07-50.37)	0.004†
Morning-types	6	2.5	5	2.6	1	2.3	
Intermediate-types	176	74.6	152	79.2	24	54.5	0.002†
Evening-types	54	22.9	35	18.2	19	43.2	
Sleep quality (PSQI)	236	6.35 (5.98-6.72)	192	5.9 (5.4-6.23)	44	8.1 (6.51-8.93)	< 0.001*
Good (≤ 5)	108	45.8	94	49.0	14	31.8	0.040†
Poor (> 5)	128	54.2	98	51.0	30	68.2	
Social rhythm (SRM-6)	-	-	-	-	-	-	-
"Hit"	178	3.5 (3.29-3.7)	146	3.54 (3.32-3.37)	32	3.27 (2.78-3.75)	0.28
ALI	178	36 [33-40]	146	36.0 [34.0; 40.0]	32	35.5 [32.5; 39.0]	0.37

95%CI = 95% confidence interval; ALI = Activity Level Index; BDI = Beck Depression Inventory; MEQ = Morningness-Eveningness Questionnaire; PSQI = Pittsburgh Sleep Quality Index; PSS = Perceived Stress Scale; SRM-6 = Social Rhythm Metrics.

Data for continuous variables (i.e., questionnaire scores) are described as mean (95% confidence interval) if they presented Gaussian distribution; distributions were compared with Student's *t*-test. ALI scores were described as median interquartile range [IQR]; non-Gaussian distributions were compared with the Mann-Whitney *U* test. Data for categorical variables are presented as percentage of the total population and were compared with chi-square tests.
* $p < 0.001$; † $p < 0.05$.

Table 2 Prevalence ratio and multivariate regression for predicting depressive symptoms (BDI > 10) based on questionnaire scores

	Univariate			**Multivariate**		
Variable	**PR**	**95%CI**	**p-value**	**PR**	**95%CI**	**p-value**
Perceived stress (PSS)	1.136	1.099-1.173	< 0.001*	1.115	1.078-1.154	< 0.001*
Lower	1	-	-			
Higher	6.429	3.665-11.275	< 0.001*			
Chronotype (MEQ)	0.958	0.930-0.986	0.004†	0.964	0.934-0.995	0.024†
Morning types	1.222	0.197-7.6	0.83			
Intermediate types	1	-	-			
Evening types	2.58	1.536-4.335	< 0.001*			
Sleep quality (PSQI)	1.187	1.113-1.266	< 0.001*	1.085	1.007-1.169	0.033†
Good (≤ 5)	1	-	-			
Bad (> 5)	1.808	1.012-3.230	0.046†			
Social Rhythm (SRM-6)						
"Hit"	0.877	0.688-1.119	0.29			
ALI	0.984	0.930-1.040	0.55			

95%CI = 95% confidence interval; ALI = Activity Level Index; BDI = Beck Depression Inventory; MEQ = Morningness-Eveningness Questionnaire; PR = prevalence ratio; PSQI = Pittsburgh Sleep Quality Index; PR = PSS = Perceived Stress Scale; SRM-6 = Social Rhythm Metrics.
Univariate models were used to define the PR of all variables. A significance level (p-value) of 0.2 in the univariate analyses was the cutoff for including a variable in the multivariate model.
* $p < 0.001$; † $p < 0.05$.

Perceived stress was significantly different ($t = -7.828$, $p < 0.001$) between groups. In the BDI > 10 group, 68.2% of the participants were considered to be suffering from higher stress ($\chi^2 = 53.785$, $p < 0.001$), a PR of 6.429 compared to the lower perceived stress group (95%CI 3.665-11.275, $p < 0.001$). Crosstab analysis also showed significant between-group differences for chronotype ($\chi^2 = 12.666$, $p = 0.002$). Although 22.9% of the total sample

could be classified as evening types, 43.2% of the BDI > 10 group fall into this category. No differences were found between morning types and intermediate types. In addition, the total sleep quality score differed significantly between the BDI groups ($t = -3.828$, $p < 0.001$). Social rhythm (both "hit" and ALI scores) did not differ between the BDI groups.

Table 2 shows the multivariate model developed to determine the interactions between BDI scores ≥ 10 and perceived stress, circadian typology, and sleep quality.

Associations between Pittsburgh Sleep Quality Index (PSQI) components and depressive symptoms

A separate univariate analysis of variance was conducted to identify to what extent each PSQI component (PSQI-C) is associated with depressive symptomatology (Table 3). Five components were entered into the multivariate model. At the final step, subjective sleep quality (PSQI-C1; PR = 2.210, 95%CI 1.214-4.021, p = 0.009) and sleep disturbances (PSQI-C5; PR = 2.198, 95%CI 1.234-3.916, p = 0.008) were significantly associated with depressive symptoms.

Table 3 Prevalence ratio (PR) and multivariate regression for predicting depressive symptoms (BDI > 10) with PSQI components

		Univariate			Multivariate step 1			Multivariate step 2		
PSQI component	**n (%)**	**PR**	**95%CI**	**p**	**PR**	**95%CI**	**p**	**PR**	**95%CI**	**p**
1. Subjective sleep quality										
Very good or fairly good	192 (81.4)	1	-	-	1	-	-	1	-	-
Fairly poor or very poor	44 (18.6)	3.316	2.014-5.46	< 0.001	2.171	1.196-3.941	0.011 †	2.210	1.214-4.021	0.009 †
2. Sleep latency‡										
Up to 30 min	99 (41.9)	1	-	-	1	-	-			
More than 30 min	137 (58.1)	1.584	0.868-2.764	0.13	1.099	0.614-1.965	0.75			
3. Sleep duration										
Up to 6 hours	204 (86.4)	1	-	-						
Less than 6 hours	32 (13.6)	1.417	0.725-2.767	0.3						
4. Habitual sleep efficiency										
Up to 75%	166 (70.3)	1	-	-	1	-	-			
Less than 74%	70 (29.7)	1.493	0.871-2.559	0.14	1.027	0.602-1.750	0.92			
5. Sleep disturbance§										
Less troubled sleep	195 (82.6)	1	-	-	1	-	-	1	-	-
More troubled sleep	41 (17.4)	3.293	2.002-5.417	< 0.001	2.166	1.203-3.901	0.010 †	2.198	1.234-3.916	0.008 †
6. Use of sleeping medication										
Less than once a week	218 (92.4)	1	-	-						
Once a week or more	18 (7.6)	1.553	0.700-3.446	0.27						
7. Daytime dysfunction‖										
Less dysfunction	206 (87.3)	1	-	-	1	-	-	1	-	-
More dysfunction	30 (12.7)	2.575	1.498-4.426	0.001	1.485	0.828-2.664	0.18	1.507	0.859-2.644	0.15

95%CI = 95% confidence interval; BDI = Beck Depression Inventory; PR = prevalence ratio; PSQI = Pittsburgh Sleep Quality Index.

Univariate models were used to define PR of all variables. A significance level (p-value) of 0.2 in the univariate analyses was used to determine which variables would be included in step 1 of the multivariate model. The same criteria were applied in step 2. C1, C5 and C7 were entered in the second multivariate model (step 2).

* $p < 0.001$; † $p < 0.05$.

‡ Sleep latency encompasses two PSQI questions; one is related to the time (in minutes) it takes to fall asleep, while the other is related to the weekly frequency of occasions when the respondent cannot fall asleep within 30 min.

§ Sleep disturbances include weekly frequency of troubled sleep due to various factors.

‖ Daytime dysfunction includes PSQI questions related to trouble staying awake and problems keeping the necessary energy level to accomplish normal tasks.

Figure 1 shows all PSQI-C5 sleep disturbance questions. The questions that most directly contributed to higher PSQI-C5 scores were: “Wake up in the middle of the night or early morning”, “Have to get up to use the bathroom”, and “Feel too hot”. Positive responses to the other questions were less frequent in this population.

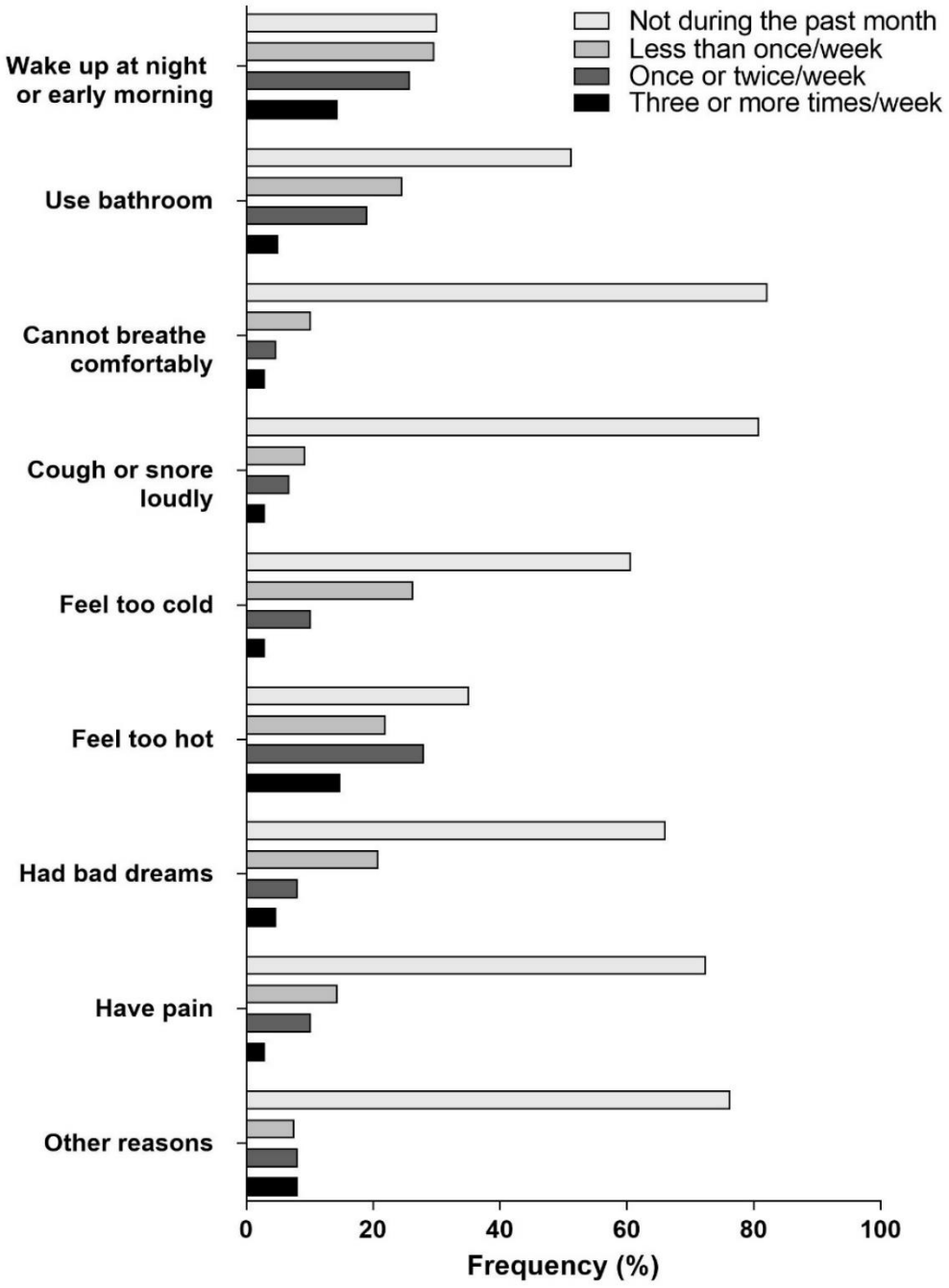

Figure 1 Questions from component 5 (sleep disturbances) of the Pittsburgh Sleep Quality Index (PSQI)

Correlation between Beck Depression Inventory (BDI) score and salivary cortisol level

Figure 2 shows salivary cortisol distribution (panel A) and Pearson's correlations of BDI score with morning (B), afternoon (C), and night (D) cortisol levels. Mean cortisol levels were significantly higher (F = 12.94, degree of freedom [df] = 2, p = < 0.001) in the morning (mean = 15.06, 95%CI 11.9-18.2) than the afternoon (mean = 10.27, 95%CI 8.19-12.34) or night (mean = 6.66, 95%CI 5.03-8.29). The higher the BDI score, the lower the morning cortisol level (r = -0.335; p = 0.043). No significant correlations were found between BDI score and afternoon or night cortisol level. The quadratic regression model (which included non-linear effects) was not significant (p = 0.911).

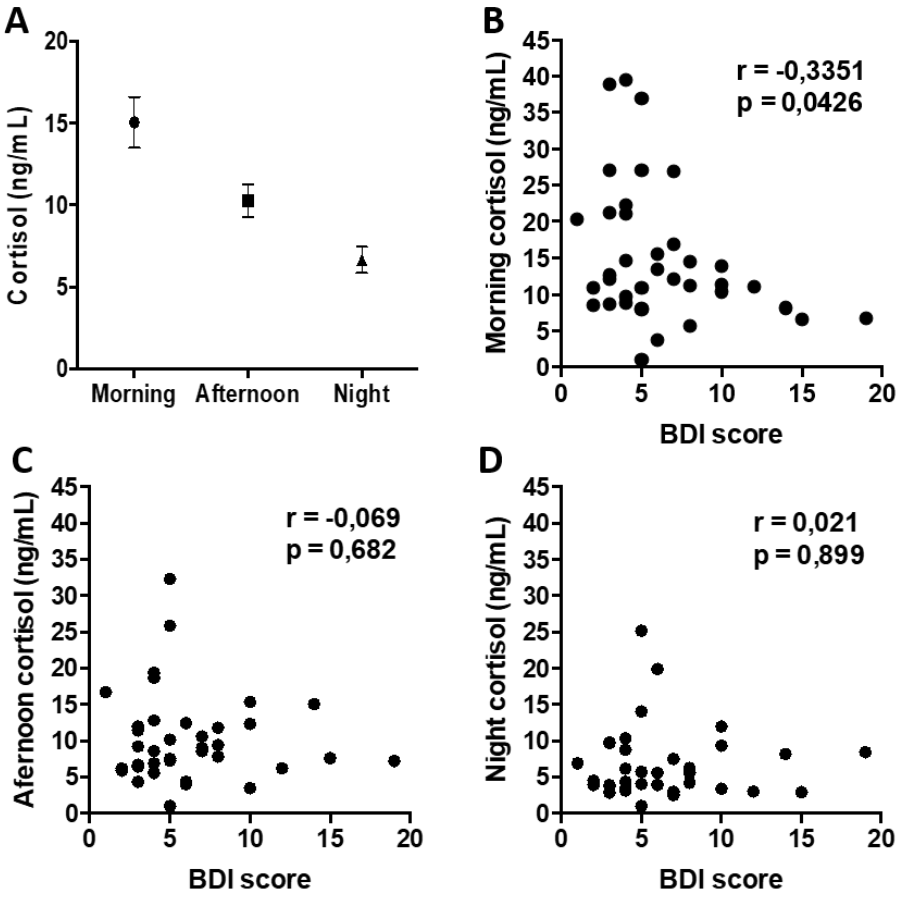

Figure 2 Salivary cortisol distribution (A) and Pearson's correlations of Beck Depression Inventory (BDI) scores with morning (B), afternoon (C) and night (C) cortisol levels

Discussion

This study found important associations between clinically significant depressive symptoms and perceived stress, circadian typology, and sleep quality in a homogenous non-clinical sample of young men. Our results provide further evidence about the multiple concomitant factors associated with depressive symptomatology in youth. We found a clinically significant depressive symptom prevalence of 18% in this sample, which is similar to the rate found in a previous population-based study of Brazilian adolescents.[22]

Perceived stress as a strong indicator of depression

Perceived stress was significantly higher in individuals suffering from depressive symptoms. Univariate analysis showed a considerable PR of 6.42, which remained significant in the multivariate model. This indicates that young men suffering from clinically significant depressive symptoms are prone to reporting less control and more negative affective reactions, as well as with less ability to deal with stressors. Evidence from previous studies suggests that there is a consistent relationship between perceived stress and depression, i.e., that pro-inflammatory response to an acute stressful event is increased by factors such as loneliness, subclinical depression, and major depression.[23] Furthermore, life stressors are associated with the recurrence of depression.[24] Our results strongly indicate that mental health professionals should further investigate depressive mood in youth who report high perceived stress.

Correlated between low morning cortisol and depressive symptoms

Cortisol distribution across times of day occurred as expected,[25] since average levels were significantly higher in the morning and lower at night. Our results demonstrate a

significant negative correlation between BDI score and morning salivary cortisol level. Internalizing problems, including depression, anxiety, and somatic complaints, were associated with gradual declines in cortisol production among adolescents.[26] A previous study with a sample of healthy medical students found that the area under the curve of daily cortisol secretion was significantly lower in an acute stress phase than in a control situation.[27]

Sleep quality, sleep timing, and circadian typology

Sleep quality was strongly associated with depressive symptoms and stress.[28,29] The literature suggests that, in young adults, poor sleep quality is associated with altered cortisol response following an acute stressor.[30] Moreover, misperception and poor insight regarding sleep problems are prevalent in clinical depression.[31] The high prevalence of poor sleep quality in our sample agrees with the findings of a previous large population study of older adolescents and young adults in which more than 60% had poor sleep quality according to the PSQI.[32]

In our sample, two components of the PSQI score seemed to play a major role in clinically significant depressive symptoms: subjective sleep quality and sleep disturbances. Among factors included in the "sleep disturbances" component, "wake up in the middle of the night or early morning", "have to get up to use the bathroom" and "feel too hot" occurred with significant frequency in our population. These are common factors modifiable through sleep hygiene awareness, in contrast with respiratory problems, pain or nightmares (Figure 1). Thus, sleep hygiene advice is an efficient behavioral intervention for improving sleep health and depressive symptoms in young populations.[33]

Moreover, there are different physiological predispositions regarding sleep timing over a 24-hour period.[34] In our sample, evening types were more prone to report depressive symptoms. However, recent studies indicate that the cognitive and affective impairment found in these individuals are not intrinsic to circadian typology itself but to chronodisruption. That is, due to the constant mismatch between their biological and social times, evening-types suffer more from sleep-related disturbances, including sleep deprivation, hypersomnia, and social jetlag.[35] Nevertheless, our results cannot corroborate this hypothesis, since we did not evaluate variables related to chronodisruption. No differences could be found for morning types. Nevertheless, we point out that these individuals correspond only to 2.5% of the sample, which hinders the statistical inference.

The relevance of social rhythm in mood dysfunctions

Previous studies have reported important relationships between social rhythm and depression. Stetler et al.[36] evaluated 50 depressed individuals and matched controls, showing intersections of activity regularity ("hit" score) and circadian variations in cortisol. The controls in their study presented significant correlations between "hit" scores and cortisol decline throughout the day, reflected in greater cortisol secretion. Another study from our group found that the regular activity of work days correlates with fewer minor psychiatric symptoms.[37] The results of the present study do not support an association between disrupted social rhythm and depressive symptoms. Certain methodological limitations might explain why we could not reproduce the previous findings. First, the data collection time was different from that of the previous study. In our sample, SRM data was collected during one week, although some studies have recommend a minimum of three weeks to provide a more accurate score.[38] Nevertheless, other studies have reported using

data from a single week.[37,39] Furthermore, despite the homogeneity of our population, no records of work activity were recorded. Therefore, we could not determine the influence of social routines as a social *zeitgeber* (time regulator). If some of the presented issues had been addressed as recommended by previous studies, the social rhythm variables might have been associated in our population.

Strengths and limitations

A recent review article by Pemberton & Fuller-Tyszkiewicz[4] describes a series of factors related to depressive mood states, which include poor sleep and stressful negative events. The authors point out that the evaluation of multiple factors in a single study is rare. Furthermore, we chose to examine cortisol at three different times of day, although most studies involve only one. Our study provides supportive evidence that multifactorial relationships are related to clinically significant depressive symptoms in non-clinical populations. It is important to point out that we studied a very homogenous sample (i.e., otherwise healthy 18-year-old men not on continuous medication), which provides internal consistency to our findings. We also emphasize that, even though we found strong associations, our study cannot determine causal relationships due to its cross-sectional design. Future studies could contribute to this topic by assessing similar multifactorial relationships before and after acute stressors in similar vulnerable populations. Moreover, our study was primarily based on self-reported measurements, and no diagnostic interview was performed.

Conclusions

There is an evident need in the biomedical literature for multifactorial approaches in the study of mood symptoms. Our study is relevant due to its high internal consistency (given the homogeneity of the sample), as well as for evaluating several concomitant variables. Although causal relationships could not be established, we highlight the strong associations between depressive symptoms and high perceived stress, cortisol secretion disturbances, poor sleep quality and eveningness. Investigating chronotype and sleep quality in adolescents and young adults can provide important tools for understanding and managing mental health problems, considering their significant associations with clinically significant depressive symptoms.

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Disclosure

The authors report no conflicts of interest.

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CAPÍTULO 5 – ATIVIDADE MOTORA À NOITE DIFERENCIA SUBTIPOS DE DEPRESSÃO

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Nocturnal motor activity and light exposure: objective actigraphy-based marks of melancholic and non-melancholic depressive disorder. Brief report.

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Abstract

Differentiation of melancholic (MEL) and non-melancholic (N-MEL) depression results from subjective assessment of psychomotor disturbance, which obscures their accurate diagnosis. CORE instrument assigned participants with severe or refractory depression to MEL or N-MEL group. Participants underwent 7 days of actigraphy. Data was fitted to a cosinusoidal curve corresponding to a 24-hour rhythm. Nocturnal activity was significantly higher in N-MEL. ROC curve shows that average night activity discriminate participants with 71% sensitivity and 100% specificity (area under the curve = 0.84). Actigraphy contribute to the objective differentiation of depression subtypes, and have implications for research on their neurobiology and clinical management.

Keywords: actigraphy; chronobiology; melancholy; depression; clinical markers.

1. Introduction

Current diagnostic criteria suggest that major depressive disorder (MDD) is a unitary construct whose presentation changes in terms of severity or course. (APA, 2013). The binary vision of depression opposes melancholic (MEL) to non-melancholic (N-MEL) depression. MEL is characterized by non-reactivity of mood to circumstances, anhedonia, terminal insomnia, hypercortisolemia, reduced concentration and working memory, and psychomotor retardation (Parker et al., 2010). Substantial evidence so far demonstrate that MEL and N-MEL are markedly different, especially in terms of psychomotor disturbance (Quinn et al., 2012, Caldieraro et at., 2013). Currently, clinical and research differentiation of both subtypes results from subjective assessment of such psychomotor impairment, which includes a questionnaire named CORE Assessment of Psychomotor Change.

Overall, depressed patients show a distinct pattern of physical movement, motor reaction time and motor activity (Hori et al., 2016). Moreover, a recent theory point to the importance of circadian effects on MDD symptoms (Bhattacharjee, 2007). The suprachiasmatic nuclei (SCN) of the hypothalamus coordinate mammals' rhythmic biology by dictating a circadian pace in many physiological and psychological functions. Changes in SCN-dependent rhythms have been observed in patients with mood disorders (Ávila Moraes et al., 2013). Actigraphy is suitable objective technique to assess rhythmic parameters of depressed patients (Smagula, 2016). It is based on the use of body-worn accelerometers to assess daytime or sleep activity, light exposure and other features related to biological rhythms. Recent studies have shown singular patterns in the actigraphy of depressed subjects (Burton et al., 2013; Fasmer et al., 2016).

The long-standing controversy regarding the nature and definition of MEL gained

strength during the groundwork of the DSM-5 (Gili et al., 2012). The clinical and research differentiation of both subtypes results from subjective assessments of psychomotor disturbances. Our goal was to establish a biomarker that could work as an objective mark of psychomotor agitation or retardation. We hypothesize that patterns of motor activity rhythm and light exposure objectively measured by actigraphy would differ individuals with MEL from those with N-MEL, therefore contributing to highlight the importance in distinguishing these two subtypes of MDD.

2. Methods

2.1. Subjects

This preliminary cross-sectional study included 13 women and 2 men (n = 15), aged between 28 and 57 years (46 years ± 8.73), from the Mood Disorders Program of the Porto Alegre Clinical Hospital, an outpatient tertiary care service. All participants used either SSRI or tricyclic antidepressant medications. To confirm clinical diagnosis of MDD, trained psychiatrists blinded for study objective used the Mini International Neuropsychiatric Interview (M.I.N.I. 5.0), Brazilian Portuguese version. The Hamilton Depression Rating Scale (HAM-D) and the Beck Depression Inventory (BDI) assessed severity of depressive symptoms. The CORE Assessment of Psychomotor Change scale was used to assign participants into two groups: the MEL (n=8) and the N-MEL group (n=7). This instrument defines melancholia according to the presence of psychomotor disturbances, following a “trunk and branch” model. “Trunk” items involve cognitive components such as non-interactivity and impaired concentration; “branch” items evaluate physical components of agitation and retardation. A total score is derived by adding all 18 observed signs using a

three-point Likert scale. According to Parker (2007), a CORE score ≥8 indicates melancholia. This was the cut-off adopted in this study.

The investigation was carried out in accordance with the seventh version of the Declaration of Helsinki as revised 1989, and the Research Ethics Committee of the Porto Alegre Clinical Hospital (HCPA) approved all procedures (protocol number 11-0456 GPPG/HCPA). All participants provided written informed consent after receiving a complete description of the study.

2.2. Procedures

Subjects wore actigraphs (Actiwatch-L®, Mini Mitter Company, USA) on the non-dominant wrist over 7 days. This device contains an accelerometer capable of sensing any motion with a minimal resultant force of 0.01 g, which registers motor activity in units of movement. It also contains a luximeter, which records information of the amount, and duration of ambient illuminance in units of lux. The equipment measured activity and light exposure every 15 minutes. Average nocturnal activity and light exposure were considered for records between 8pm and 6am. Nighttime was separated into initial night (from 8pm to 1am) and late night (from 1am to 6am). These arbitrary windows refer to patients' average social day and night periods.

2.3. Quantification of rest-activity and light exposure rhythms

"El Temps" software (©Antoni Díez Noguera, Barcelona, CA, Spain) was used to analyze circadian rhythms. In order to illustrate activity and light exposure rhythms, double-plotted actograms were created using derived series from mean values of each group (Figure 1). Sokolove and Bushell periodogram and Interdaily Stability (IS, the 24h value from the chi-square periodogram) measurement were used to analyze periods and variability

between days of each observed rhythm. Analyses of wave form provided the time at which participants pass through the mesor in the beginning of the activity phase (up-mesor) and before sleep (down-mesor). Furthermore, data from each patient was fitted to a cosinusoidal curve corresponding to a 24-hour rhythm (Cosinor analysis), allowing for an assessment of the amplitude, mesor (rhythm-adjusted mean), and acrophase of the adjusted rhythm.

2.3. Statistical analysis

Student's *t*-test was used to compare symptom severity measured by HAM-D and BDI. Between-group comparisons were made using Mann-Whitney *U*-tests for continuous nonparametric data. Watson-Williams test was used to compare acrophase, up-mesor and down-mesor values. A Receiver Operating Characteristic curve (ROC curve) analysis was performed in order to identify which value of motor activity could differentiate MEL and N-MEL patients with higher accuracy. For all analyses, statistical significance was set at two-tailed $p<0.05$. Statistical tests were performed using the SPSS software, version 23.0.

3. Results

Sokolove and Bushell periodogram analysis show significant 24-hour rhythms, and no between-group differences were found in IS of activity. Wave form analyses show a delay of 37 minutes in the beginning of activity phase (up-mesor) of N-MEL ($F = 0.367$, $p = 0.556$) and a 118 minute difference in the end of activity phase, before sleep phase (down-mesor), in the same group ($F = 2.5$, $p = 0.138$).

Cosinor analysis of the 24h rhythms in 7 days showed no between-group statistical difference in mesor, acrophase and amplitude of total activity or light exposure rhythm.

However, activity mesor revealed a tendency (U = 12, p = 0.64, r = 0.245). When medians for diurnal and nocturnal motor activity were compared between groups, nocturnal activity was found to be significantly higher in the N-MEL than in the MEL group (p = 0.037, r = 0.31), and this difference was more substantial when data for each group was divided in initial and late night periods (both p = 0.028, r = 0.345). Diurnal activity, diurnal and nocturnal light exposure did not differ significantly between groups. The results of actigraphy comparative analyses are shown in Table 1.

HAM-D and BDI scores were 26.38 ± 5.07 and 38.5 ± 8.83 for MEL and 23 ± 4.4 and 40.43 ± 8.12 for N-MEL, respectively. MEL and N-MEL participants did not differ in severity of depressive symptoms evaluated by HAM-D (t = -1.381, df = 13, p=0.191) and BDI (t = 0.416, df = 12.972, p = 0.684) scores.

ROC curve analysis shows cutoffs of late night average activity with positive values indicating N-MEL patients. An average value of 362.9 (sensitivity = 71%; specificity = 100%) is the best value to discriminate between MEL and N-MEL patients. The area under the curve is 0.84 (p = 0.028).

4. Discussion

These preliminary findings suggest that level of nocturnal activity differentiates MEL from N-MEL in patients with severe or refractory MDD. ROC curve analysis based on average late night (from 1am to 6am) activity could discriminate MEL subtype with high specificity. Thus, late night activity could objectively differ these two conditions. Higher levels of nocturnal motor activity seen in the N-MEL group are consistent with the idea that psychomotor agitation is more characteristic of N-MEL depression, also indicating a

possibility of sleep fragmentation in this group (see Figure 1). Comparisons of nocturnal activity show medium effect sizes (Table 1). No statistical significance could be found for total or diurnal activity, although a trend was observed in the activity mesor, which was higher in the N-MEL group. However, MEL patients present lower activity levels also on daytime. In addition, it is possible to see a delay in both beginning and end of the activity phase of N-MEL compared to MEL in the wave form analyses, even though no statistical significance was found. This could be a result of our small preliminary sample.

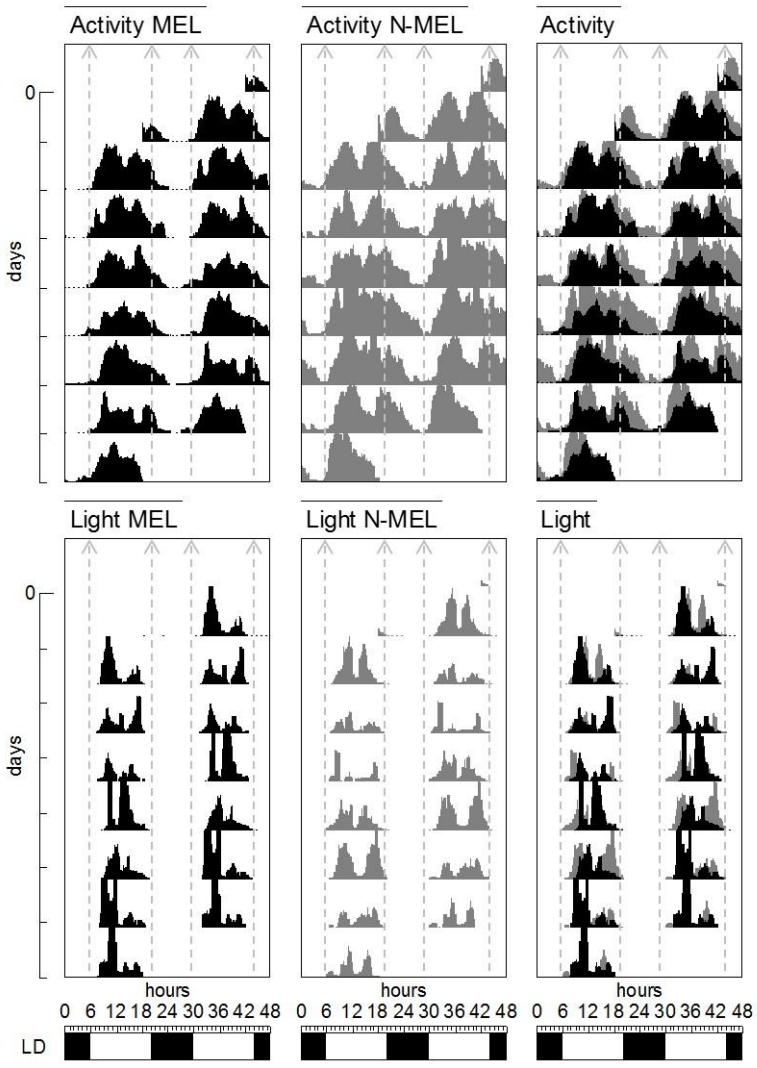

Figure 1. Double-plotted actograms of derived series of activity (top) and light exposure (bottom) rhythms for study groups. Each graph line represents a period of 48 hours. Rightmost graphs represent the overlap of both groups. Daily activity values range from 0 to 2500 units of movement, and daily light exposure values range from 0 to 3000 lux. MEL: melancholic group; N-MEL: non-melancholic group. LD: light-dark cycle.

Table 1. Descriptive statistics and between-group comparisons of average activity and light exposure

Activity (units of movement)						
	MEL (*n* = 8)		**N-MEL (*n* = 7)**			
	Median	IQA	Median	IQA	*p* value	*r* value
Total (24h)	826.00	799.35	1476.64	1279.44	**0.064**	**0.245**
Day (6am-8pm)	1260.08	1096.97	1691.08	1612.62	0.203	0.016
Night						
Total (8pm-6am)	283.98	373.70	775.06	1151.10	**0,037***	**0.310**
Initial (8pm-1am)	331.15	705.24	1152.97	1576.48	**0,028***	**0.345**
Late (1am-6am)	141.55	263.38	505.83	682.91	**0,028***	**0.345**
Mesor	531.28	619.58	1115.88	980.33	**0.064**	**0.245**
Amplitude	485.53	728.01	675.18	1004.58	0.487	0.034
Acrophase †	791.55	-	832.24	-	0.200	-
Light Exposure (lux)						
	MEL (*n* = 8)		**N-MEL (*n* = 7)**			
	Median	IQA	Median	IQA	*p* value	*r* value
Total (24h)	357.78	1576.14	575.19	755.18	0.728	0.009
Day (6am-8pm)	615.88	2408.87	995.40	1296.17	0.817	0.004
Night						
Total (8pm-6am)	6.21	9.72	10.03	11.97	0.247	0.096
Initial (8pm-1am)	12.06	13.38	15.49	11.19	0.105	0.188
Late (1am-6am)	0.77	1.22	0.83	3.39	0.418	0.047
Mesor	273.76	737.67	444.81	587.46	0.643	0.015
Amplitude	464.40	1059.76	726.21	626.32	0.817	0.004
Acrophase †	743.90	-	768.68	-	0.379	-

*p<0.05, Mann-Whitney *U*test for non-parametric independent samples. † acrophase values represent minutes of a period of 1440 minutes; compared acrophase *p* value results from Watson-Williams test. MEL: Melancholic; N-MEL: Non-Melancholic; IQA: Inter-quartile amplitude; *r:*effect sizes

Future studies should address some of the limitations of this study, including more participants, considering sex and age differences, and applying this methodology in different clinical samples. Our data was collected each 15 minutes for 7 days. This chosen

interval could compromise the comparison with other studies. However, we highlight that the methodology of actigraphy-based measures yet have to be discussed so that studies can find consensus in several aspects (Ancoli-Israel et al., 2015). The most important are probably the amount of days of record, and which parameters are more related to the clinical affection of interest. Moreover, the interval of data acquisition of usual actigraphs may vary from one second to 24 hours (e.g. ActTrust, Condor Instruments Ltda, Brazil).

Furthermore, literature suggests that individuals suffering from MEL depression could be more severe. Previous studies also indicate that excessive exposure to light at night is associated with depressive symptoms (Chepesiuk, 2009). Therefore, we hypothesized that participants with MEL might be more exposed to artificial light at nighttime. Perhaps our small sample could not identify such difference.

A substantial amount of studies support that MEL should be reinstated as a distinct disorder (Lin et al., 2016), also emphasizing limitations of symptom-based criteria for MDD (Zaninotto et al., 2016). Hence, the use of actigraphy, which assesses diurnal and nocturnal activity could contribute to the objective identification of MDD subtypes, also having important implications for research on the neurobiology, genetics and treatment of depression.

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CAPÍTULO 6 – PADRÕES DE SONO E ATIVIDADE MOTORA EM ADOLESCENTES DEPRIMIDOS

Title 1: Sleep Disturbances, Circadian Activity and Nocturnal Light Exposure Characterize Current Episode and High Risk of Major Depressive Disorder in Adolescence

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ABSTRACT

Objective: Major Depressive Disorder (MDD) in adolescence is related to irregularities in circadian rhythms and sleep. The characterization of such impairment is critical to design effective interventions aiming to improve the approach of depressive symptoms even in adolescents that are not currently facing a depressive episode but show higher risk of developing the disorder. The aim of this study was to uncover self-reported and actimetry-based circadian rhythms and sleep-wake behavior of adolescents that are associated with current episode of MDD and a higher risk for the disorder.

Method: 96 adolescents aged 14-16 years were recruited from a community sample to form three age and gender matched groups following the IDEA-RS risk criteria for MDD and a score in the Patient Health Questionnaire (PHQ-A). Low risk (LR) showed IDEA-RS<20th percentile and PHQ-A≤6; high-risk (HR) an IDEA-RS>90th percentile and PHQ-A≤6; and MDD an IDEA-RS>90th percentile and PHQ-A≥10. We collected subjective data on insomnia, chronotype, sleep schedule, sleep hygiene as well as objective data on sleep parameters, rest-activity and light exposure rhythms using actimetry for 10 consecutive days.

Results: Adolescents with MDD have more severe symptoms of insomnia, later sleep onset, shorter sleep duration, higher social jetlag (SJL), lower relative amplitude (RA) of activity and higher exposure to artificial light at night (ALAN) compared to the other groups. They also present poorer sleep hygiene compared to the LR group. The HR group also presents higher insomnia, lower RA, higher exposure to ALAN and higher SJL compared to the LR.

Conclusion: The HR group share sleep and rhythm alterations with the MDD group, that may be early signs of a depressive episode, and for which preventive strategies related to sleep hygiene can be designed in future investigations. Furthermore, in addition to subjective assessment of sleep hygiene, we highlight that the actimetry-based parameters of motor activity – particularly the RA – and of light exposure are promising measures to be explored as clinical tools for diagnosis, risk stratification and treatment follow-up of depression in adolescence.

Keywords: Depression, Mood, Actimetry, Actigraphy, Biological Rhythms, Sleep Hygiene, Chronobiology, Psychiatry

1. INTRODUCTION

Adolescence is a critical transitional period characterized by social, psychological, and biological changes. Noteworthy, mental illness is a growing concern in this population, with the prevalence rates of major depressive disorder (MDD) increasing yearly[1,2]. Of potential factors contributing to negative outcomes on mental health in adolescents, the emergence of sleep difficulties and exposure to artificial light at night (ALAN) are under growing attention. Overall, studies in adolescents report high occurrences of irregular sleep wake patterns, insomnia, sleepiness and difficulty staying asleep[3–5]. Particularly, studies have highlighted that many adolescents do not sleep enough at night, and nearly one fourth reports sleeping 6 hours or less per night[6–8].

Moreover, situations that challenge the temporal organization of sleep and biological rhythms, such as exposure to ALAN, sleep deprivation and irregularity of sleep schedules have been recently implicated as a trigger factor or contributor in the pathophysiology of depression and other psychiatric disorders[9]. The use of self-reported data is useful in identifying sleep schedules, chronotype, and related behaviors (i.e., sleep hygiene), as well as symptoms of sleep disorders (like insomnia), all of which have been reported to be altered in individuals with depression[10,11]. In addition, some studies have recently pointed out the clinical applications of actigraphy to evaluate the effectiveness of treatments for sleep disorders, to detect patterns of activity in mood disorders and to distinguish some psychopathologies[12–14]. For example, several reports describe that depressed individuals are more active during the rest period, and sometimes less active during the day, reflecting a lower amplitude of the rhythms of motor activity throughout the day[15–17].

Identifying irregularity in circadian rhythms and sleep as early as possible could be critical to perform effective interventions aiming to improve mental health even in adolescents that are not currently facing a depressive episode. Good sleep hygiene is beneficial for adolescents' sleep health and overall quality of life[18], whereas a negative home environment and evening light are potential risk factors for sleep disturbance[19]. Sleep extension and unstructured sleep hygiene advice can improve sleep quality, insomnia and depressive symptoms even in adolescents not facing a current depressive episode[20,21]. Moreover, cognitive behavioral therapy for insomnia, which is effective to treat insomnia comorbid with depression[22], and improves depressive symptomatology even without a depression-focused treatment component[23]. Hence, the aim of this study was to assess self-reported and actimetry-based circadian rhythms and sleep-wake behavior of adolescents facing a current episode of Major Depressive Disorder (MDD) compared to adolescents at risk for MDD. We hypothesized that sleep disturbances, poor sleep hygiene, irregular rest-activity rhythms and inadequate light exposure characterize adolescents with MDD and possibly relate to a high risk of developing MDD.

2. METHODS

2.1. Sample selection, group assignment and study design

This study was approved by the Research Ethics Committee of the Hospital de Clínicas de Porto Alegre (protocol number 2018-0489) and all the procedures were conducted in accordance with the ethical principles of the Declaration of Helsinki.

Participants were recruited and the data were collected in the context of the project "Identifying Depression in Early Adolescence with a Stratified Risk Cohort" (IDEA RiSCo,

CAAE 50473015.9.0000.5327), which included 150 adolescents aged 14 to 16 years selected from public schools in Porto Alegre, Rio Grande do Sul, Brazil. The current study included 96 adolescents with complete records of subjective questionnaires and actimetry data. In the initial cross-sectional stage of the IDEA-RiSco study, the adolescents were screened both for low- and high-risk factors associated with Major Depressive Disorder (MDD), and for currently untreated MDD episode, allowing for two-by-two comparisons between groups[24]. The risk groups were stratified using a multivariable prognostic model developed and validated by IDEA-RS (**Supplementary File 4**)[24]. The risk strata for determining the three risk groups were: IDEA-RS <20th percentile and PHQ-A≤6 for low-risk adolescents (LR), IDEA-RS >90th percentile, PHQ-A≤6 for high-risk adolescents (HR) and IDEA-RS > 90th percentile and PHQ-A≥10 for adolescents with Major Depressive Disorder (MDD) in the current episode.

Upon acceptance of participating in this study and prior to the study protocol, adolescents and their accountable kin signed a written informed consent. In the presence of a researcher, the adolescent filled the study instruments and was given the actimeter for use in the following 14 days.

2.2. Subjective assessment

2.2.1. Depressive symptoms

For the assessment of depressive symptoms, we used three instruments. The Mood and Feelings Questionnaire (MFQ)[25] and the Patient Health Questionnaire (PHQ)[26] are both self-reported measurements and the Children's Depression Rating Scale (CDRS) is part of a

clinical evaluation[27]. The correlations between risk scores and depressive symptoms are depicted in **Supplementary Figure 1**.

2.2.2. Sleep schedule

The Munich ChronoType Questionnaire (MCTQ) is a self-reported structured questionnaire that assesses information related to sleep-wake behavior on workdays and work-free days separately[28]. In this sample, the workdays correspond to weekdays (school days), and the work-free days correspond to weekends. A Brazilian-Portuguese version of the MCTQ was used (http://www.euclock.org/). The information obtained from the MCTQ are described in **Figure 1**.

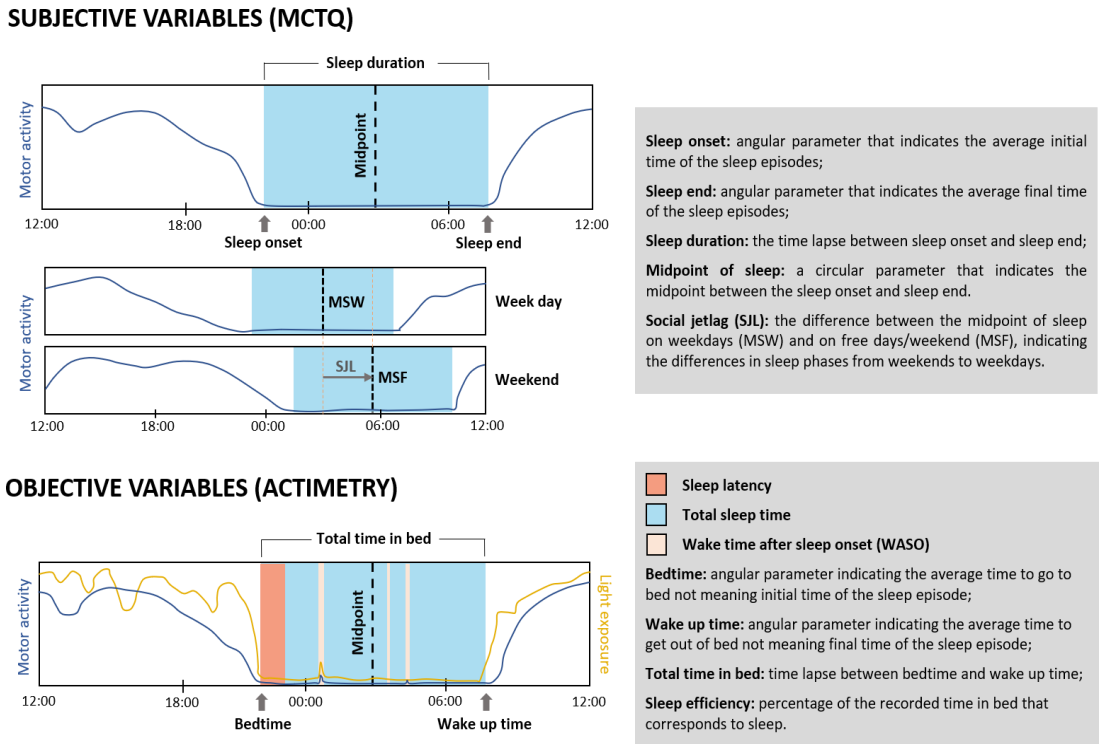


Figure 1. Subjective and objective sleep assessments. MCTQ: Munich ChronoType Questionnaire

2.2.3. Chronotype

The Puberty and Phase Preference Scale (PPPS)[29] is a self-reported questionnaire used to measure chronotype through questions about schedule preferences. This scale also

measures individual preferences to perform different activities, such as scholar practices, resting, and physical activities during daytime. Scores range from a minimum of 10 (minimal morning preference) to a maximum of 43 (maximum morning preference).

2.2.4. Sleep Hygiene

The Sleep Hygiene Index (SHI)[30] is a self-reported instrument designed to assess behaviors and habits related to maladaptive sleep-related practices. The global assessment is based on the total sum of all questions. Therefore, higher scores (ranging from 13 to 65) in the SHI indicate poor sleep hygiene.

2.2.5. Insomnia

The Athens Insomnia Scale (AIS) is a self-administered tool designed to evaluate sleep difficulty based on the ICD-10 criteria. Ranging from 0 to 24, higher scores indicate more severe insomnia symptoms. The Brazilian Portuguese version used in this study is available at http://www.sleepontario.com/.

2.3. Actimetry and assessment of light exposure

Actimetry is a method of assessment of rest-activity profiles using a wrist accelerometer. In this study we used the commercial device from ActTrust Condor™, which is also equipped with a luximeter allowing for the assessment of environmental light exposure. We have collected actimetry data of all adolescents for 14 consecutive days, starting at midnight for all series. To record motor activity and light exposure, data were sampled in 1-minute epochs (i.e., interval between samples) of activity levels (Proportion Integration Mode; PIM) and light exposure (lux), respectively. To ensure the quality and homogeneity of the collected data, only series with at least 10 consecutive days with satisfactory records were considered, totaling 96 records. The ActStudio software

(Condor™, Brazil, v. 1.0.16) was used to export the data from the devices into data frames. Missing data detection was based on off-wrist episodes. To detect these episodes, we used a two steps method: 1) manual inspection of the actograms and 2) the usage of an algorithm developed by our team to modify “zero” values (i.e., the default) to “NA'' if more than 55 in 60 consecutive data points were zeros, and if the difference between wrist temperature and environment temperature was lower than 0.5ºC.

The ActStudio software (Condor™, Brazil, v. 1.0.16) provides an output of sleep parameters based on the manufacturer’s algorithms (**Figure 1**). They are total sleep time (TST), bedtime, wake up time, total time in bed, sleep latency, sleep efficiency (i.e., the percentage of the recorded time in bed that corresponds to sleep), wake time after sleep onset (WASO) and number of awakening episodes after sleep onset. To generate this report, we manually selected the main sleep episodes corresponding to the 10 consecutive days of record and extracted the mean value of these parameters. We have also excluded weekend sleep episodes to obtain only weekday data.

We performed additional three procedures to analyze the time series: 1) Cosinor analysis, 2) regularity analyses, and 3) amplitude measurements. Parameters derived from Cosinor analysis were **acrophase** - a circular measure indicating the moment when the peak of a rhythm occurs; and **MESOR** (i.e., Midline Estimating Statistic Of Rhythm), the rhythm-adjusted mean of a series. Parameters derived from regularity analyses were the **IV** (intracycle variability) - a measure of rhythm fragmentation which reflects the frequency of transitions between higher and lower values; and **IS** (interdaily stability) - which estimates how stable the rhythm is from one day to another. Parameters derived from amplitude measurements were the **M10** (i.e., the average value of the continuous 10 hours of the day with highest values of activity), the **L5** (i.e., the average of the continuous 5 hours of the

day with lowest values of activity) and **RA** (i.e., relative amplitude; the difference between M10 and L5). The actimetry parameters were calculated using *R* software version 4.0.4 and El Temps (™Antoni Díez-Noguera).

2.4. Data analysis

All analyses were performed in *R* software version 4.0.4. Study variables are described as mean ± standard deviation, median [interquartile range] or n (%), as appropriate. Univariate analyses of circular parameters were performed using the Watson-Wheeler test for homogeneity of angles. Univariate analyses of linear parameters were performed using multinomial logistic regression, with the three-level factor "Study group" as grouping variable. A spline interpolation was added to each univariate model, in order to test for linearity of the association with the grouping variable[31]. The spline interpolation was created using three knots representing the quantiles 0.1, 0.5 and 0.9 and a cubic polynomial was chosen to fit the data between two consecutive knots. The univariate models that showed significant differences among groups for the spline interpolation, but not for the linear function, are likely to show a non-linear relationship with the grouping variable. In addition, a plot of probabilities was generated to test the likelihood of belonging to a given study group that each possible value of the independent variable has. These plots served as the basis for categorization of independent variables according to the quartiles of its distribution. The categorized independent variable was then submitted to a univariate analysis in a multinomial logistic regression.

The establishment of the multivariate model to fit subjective and objective predictors followed four steps: 1) selection of variables that showed statistically significant differences in univariate analyses (including actimetry-based variables of activity rhythm,

light exposure and sleep, demographic and subjective data); 2) addition of sex, considering the known influence of this variable on depression; 3) splitting into three distinct models, each including one of the following variables: sleep duration on weekdays, sleep duration on weekends (both derived from the MCTQ), or total sleep time derived from actimetry; and 4) exclusion of variables according to multicollinearity, using a variance inflation factor (VIF) higher than 5 as reference. Of note, only linear parameters were eligible to enter the multivariate model.

3. RESULTS

Table 1 displays the descriptive statistics and univariate analyses of demographic data, school routines, and questionnaire scores. **Table 2** shows the comparisons of sleep hygiene practices measured by the Sleep Hygiene Index (SHI) among groups.

Table 1. Descriptive statistics and univariate analyses.

	Total (n=96)	LR (n=26)	HR (n=31)	MDD (n=39)	p (LRxHR)	p (LRxMDD)	p (HRxMDD)
Age	15.8±0.8	15.4±0.8	15.8±0.9	16.1±0.7	0.083	0.003	0.170
Sex (M)	54 (56.2%)	16 (61.5%)	19 (61.3%)	19 (48.7%)	0.980	0.310	0.300
School shift (morning)	60 (63.8%)	18 (69.2%)	17 (56.7%)	25 (65.8%)	0.330	0.770	0.440
Chronotype (PPPS)	25.3±5.8	26.5±6	27.2±4.8	23.1±5.7	0.634	0.020	0.004
Insomnia (AIS)	6.9±5	2.8±1.5	4.9±3.2	11.5±4	0.007	<0.001	<0.001
Sleep Hygiene (SHI)	31.1±6.2	27.3±4.8	28.9±2.9	35.3±6.5	0.128	<0.001	<0.001
Depressive symptoms (PHQ)	5 [3.0, 17.5]	2 [2, 3]	4 [3, 5]	19 [16.0, 22.8]	0.006	<0.001	<0.001
Depressive symptoms (MFQ)	13[6.0, 38.2]	5.5[3.0, 8.8]	11[6.0, 14.5]	42[34.0, 49.5]	0.011	<0.001	<0.001
Depressive	25 [19,	18.5 [17.0,	21 [18,	49 [43.0,	0.014	0.000	0.002

symptoms (CDRS)	44]	19.8]	25]	61.5]

Descriptive statistics are presented in mean ± standard deviation, median [interquartile range] or n (%). Univariate analyses were performed by multinomial logistic regression. AIS: Athens Insomnia Scale; HR: High risk; LR: Low risk; MDD: Major depressive disorder; MFQ: Mood and Feelings Questionnaire; PPPS: Puberty and Phase Preference Scale; SHI: Sleep Hygiene Index.

Table 2. Comparisons of the Sleep Hygiene Index questions among groups.

	HR x LR		**MDD x HR**		**MDD x LR**	
	OR	***P***	**OR**	***P***	**OR**	***P***
1. Naps	1.39	0.22	1.17	0.48	1.62	0.06
2. Bedtime irregularity	1.65	0.09	1.94*	0.01	3.19*	< 0.001
3. Wakeup time irregularity	1.89*	0.02	0.92	0.7	1.74*	0.03
4. Physical activity close to bedtime	1.22	0.48	0.88	0.59	1.07	0.81
5. Prolonged time in bed	1.99*	0.02	1.54	0.08	3.05*	< 0.01
6. Use of stimulants close to bedtime	0.48	0.16	2.55	0.06	1.23	0.45
7. Arousing activities close to bedtime	0.62	0.04	1.32	0.15	0.81	0.38
8. Subjective stress at bedtime	1.27	0.53	6.8*	< 0.01	8.6*	< 0.001
9. Arousing activities in bed	1.26	0.25	0.94	0.74	1.18	0.37
10. Uncomfortable bed	0.89	0.78	1.64	0.16	1.46	0.26
11. Uncomfortable bedroom	1.78	0.21	1.36	0.15	2.42*	0.04
12. Dealing with important tasks close to bedtime	0.6	0.08	2.13*	< 0.01	1.28	0.32
13. Thinking, planning, worrying while in bed	0.95	0.84	2.8*	< 0.001	2.71*	< 0.001

Univariate analyses were performed by multinomial logistic regression, and significant odds ratio (OR) are marked with *. HR: High risk; LR: Low risk; MDD: Major depressive disorder.

Figure 2 describes the actimetry and light exposure parameters, as well as the actimetry or MCTQ-derived sleep timing and the sleep duration on weekdays and weekends. **Supplementary table 1** shows a detailed description of the descriptive statistics of linear parameters. Regarding motor activity parameters derived from actimetry, the HR presents a significantly higher MESOR and M10 compared to the other groups, and the LR group has a higher M10 compared to the MDD group. The MDD group shows significantly higher L5, and lower RA compared to both the LR and HR groups. Of the light exposure parameters, the only significant association was found for the L5, which was higher in the MDD group compared to the low risk. The IV of light exposure showed a non-linear relationship with the study groups. This variable was dichotomized with the percentile 75 as cutoff (i.e., IV > 0.41), but it did not show significant differences among groups. None of

the sleep parameters calculated from actimetry (all sleep episodes or weekday episodes only) showed statistical differences among groups in univariate analyses using continuous scores (**Supplementary table 1**). However, when the total sleep time (TST) measured by actimetry was categorized following the probabilistic indications (**Figure 2**), the first quartile of TST (i.e., TST < 6h) was significantly more prevalent in the MDD group compared to the LR group (**Supplementary table 3**). This association was no longer significant when weekend sleep episodes were excluded from the TST data (**Supplementary table 3**).

The Watson-Wheeler test for homogeneity of angles showed that the average subjective sleep onset of the MDD group was significantly later than those of the other two groups, for both weekdays and weekends (**Supplementary table 2**). Sleep end or midpoint of sleep were not different between study groups. The sleep duration on weekdays of adolescents with MDD was found to be of 5.8 ± 2.1 hours on average, which is significantly shorter than the average of 7.5 ± 1.8 hours of the LR group and the 7.6 ± 1.7 hours of the HR group. On weekends, the average sleep duration of the MDD group (8 ± 1.9 hours) was significantly shorter than the one of the HR group (9.1 ± 1.3 hours), but not different from the LR group (8.5 ± 1.5). Social jetlag was considerably high in all three groups (LR: 2.4 ± 1.2, HR: 2.4 ± 1.4, MDD: 2.8 ± 1.6), but no differences were found comparing the continuous SJL score among them. The probability plot of SJL indicates that the probability of belonging to the MDD group increases with the increase of hours of SJL. Thus, we chose the percentile 75 as cutoff (i.e., SJL > 3.4h) to dichotomize this variable (**Supplementary table 3**). The prevalence of adolescents with MDD that show a SJL in the fourth quartile is significantly higher compared to the LR groups.

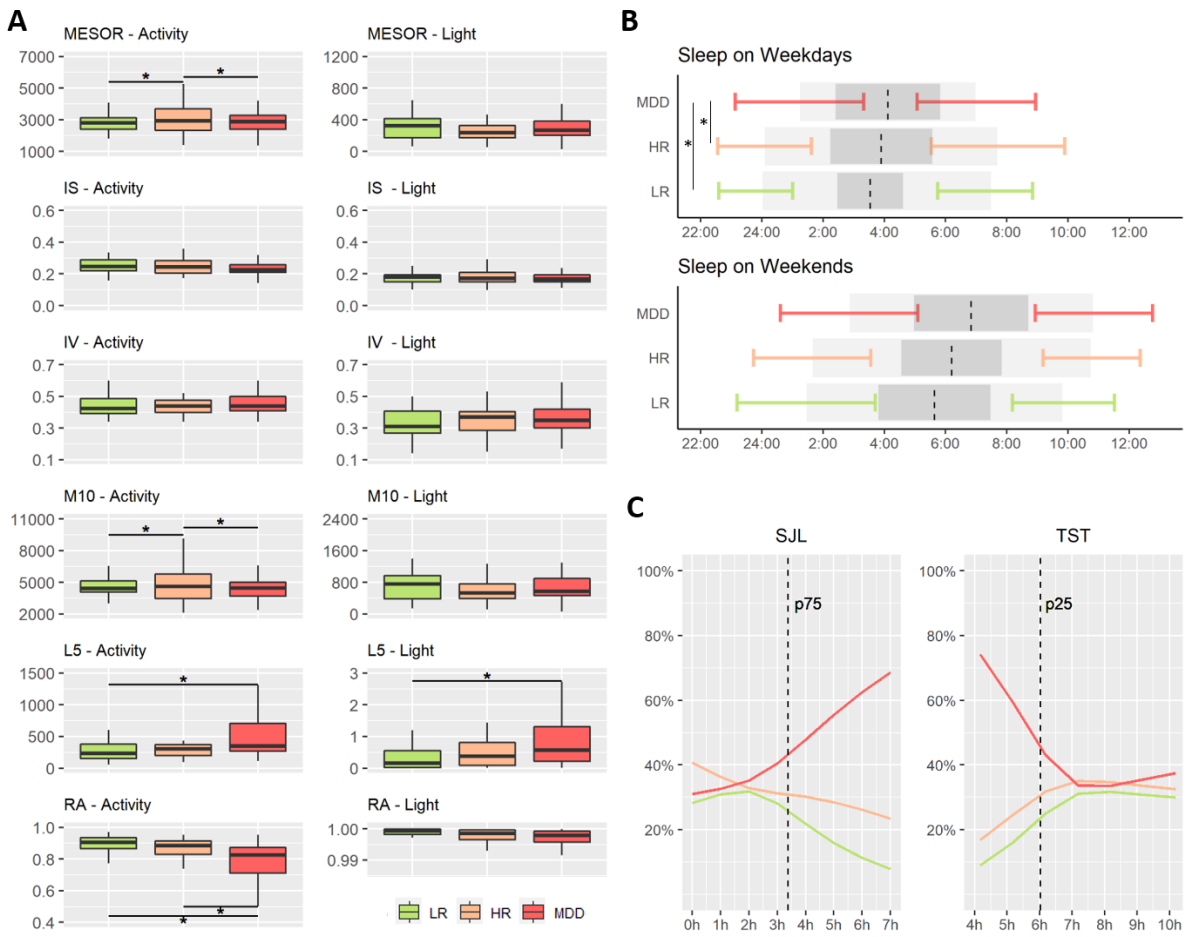


Figure 2. Panel (A) shows the distributions of actimetry-assessed parameters of motor activity and light exposure. Group distributions are compared using multinomial logistic regression, and pairwise comparisons showing $p < 0.05$ are marked with "*". Panel (B) shows the sleep parameters on weekdays or weekends assessed by the Munich ChronoType Questionnaire for each group. Horizontal coloured intervals are the 95% confidence intervals of sleep onset and sleep end. The light grey bars represent the average sleep duration (i.e., the distance from the average sleep onset to the average sleep end). The dashed black lines and the dark grey rectangles indicate the average and the 95% confidence interval of the midpoint of sleep, respectively. Sleep onset, end and midpoint were compared with the Watson-Wheeler test for homogeneity of angles, and the only significant pairwise comparisons was observed for the sleep onset on weekdays ("*" indicating $p < 0.05$). Panel (C) shows probability plots for social jetlag (SJL) and total sleep time (TST). The "y" axis indicates the probability of belonging to each group at a given value of the "x" axis. Dashed black lines indicate the percentile chosen for dichotomization of the parameter.

The multivariate models including actimetry-based TST, subjective sleep duration on weekdays or weekends are depicted in **Figure 3**. The MESOR of activity manifests multicollinearity with the M10 of activity, and the L5 of activity with the RA of activity. M10 and RA were chosen for the multivariate models. A full description of the multivariate model parameters is described in **Supplementary table 4**.

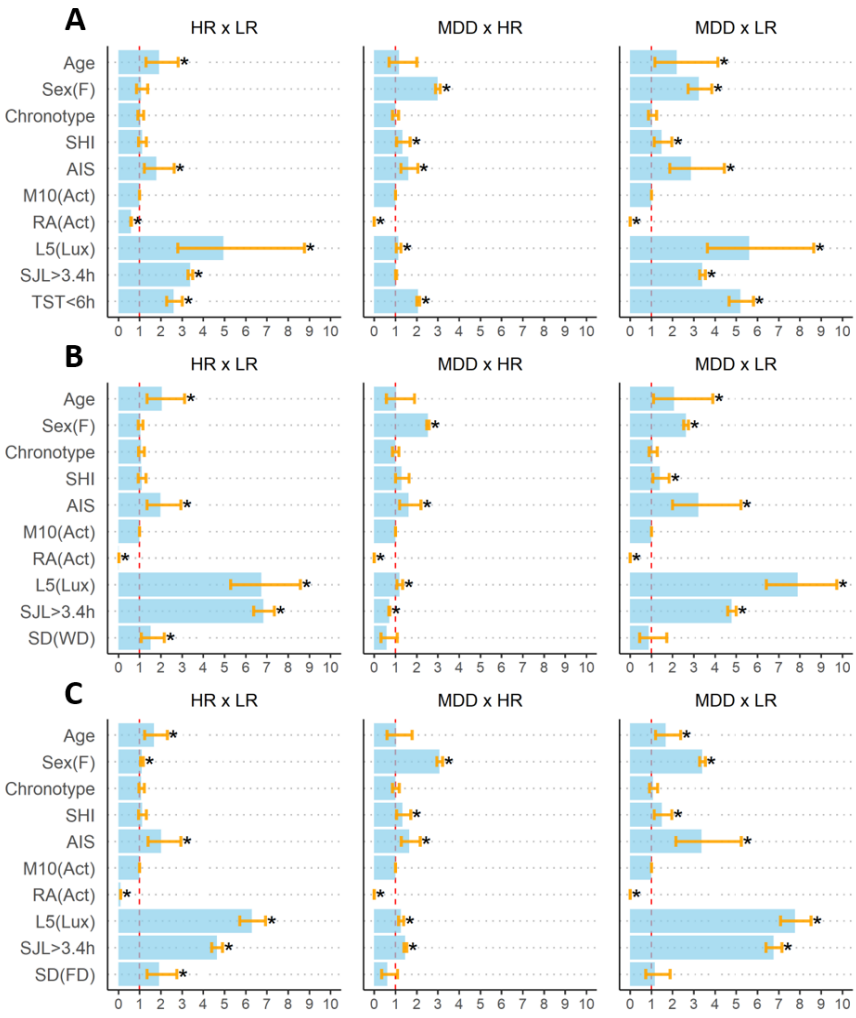


Figure 3. Odds ratio (x-axis) of the multivariate models of the significant linear parameters in the univariate analyses, adjusted for sex and total sleep time (TST) measured by actimetry (**A**), sleep duration on weekdays (**B**), or sleep duration on weekends (**C**) measured by the Munich ChronoType Questionnaire. Each panel represents the first named group in comparison to the second (reference), i.e., the high risk (HR) compared to the low risk (LR), the Major Depressive Disorder (MDD) compared to the HR, and the MDD compared to the LR. Bars represent the odds ratio with a 95% confidence interval of each parameter in the multinomial logistic regression. The dashed vertical line indicates the null value (i.e., 1). Chronotype was measured using the Puberty and Phase Preference Scale. AIS: Athens Insomnia Scale; L5(Lux): Mean light exposure of the lowest 5 hours of light exposure; M10(Act): mean of activity of the highest 10 hours of activity; RA(Act): the difference between the M10 and the L5 of activity; SD(FD): Sleep duration on free days (weekends); SD(WD): Sleep duration on weekdays; SHI: Sleep Hygiene Index; SJL>3.4h: social jetlag greater than 3.4h (representing the fourth quartile compared to the others).

4. DISCUSSION

This study provides novel evidence on the characteristics of self-reported and actimetry-based circadian rhythms and sleep-wake behavior related to depression in adolescence, some of which are also shared with high-risk individuals. This is a pioneering report of discoveries that are in line with the recent discoveries in adults. Moreover, our sample was collected in a developing country with alarming trends of growth in adolescent depression, where preventive strategies are urgent[1]. A recent systematic review reported rates of up to 28% for significant symptoms of depression or anxiety among youth in low-income and middle-income countries (LMIC)[32].

Sleep progressively changes towards the end of adolescence. Adolescents tend to sleep later and also wake up later, characterizing an evening preference representative of late chronotypes (or night owls)[33]. As in this phase of the life cycle, many individuals wake up early to follow the school schedule, this natural sleep phase delay brings several hindrances to a healthy sleep routine[34]. For many adolescents (especially the late types), an early wakening means a significant reduction in total sleep time, as they are unable to adjust their bedtime accordingly[35,36]. A consistent body of evidence associates depressive symptomatology to eveningness in young age[37,38]. However, recent and growing evidence documents that the adverse outcomes related to mental health in evening types are not intrinsic to this chronotype, but a reflection of a physiological incongruence of the individual's own biological rhythmicity with social time[39].

We observed that adolescents in the MDD group presented higher scores of insomnia severity compared to those in the HR or LR groups. This may be related to the delayed sleep onset and shorter sleep duration seen in the MDD group. Corroborating our

hypotheses, we observed that depressed adolescents sleep later, but wake up at a time like the other groups, thus resulting in a shorter sleep duration. Sleep duration is significantly shorter on weekdays and on weekends, which indicates that they are probably not able to compensate for the sleep debt accumulated over the school week. Even though adolescents of the MDD group show the shortest sleep duration and highest insomnia HR adolescents show an intermediate clinically significant grade of insomnia severity and an increased probability of sleeping less than 6h compared to the LR group. Insomnia in a adolescence is a risk factor for mood or psychotic disorder[40], early adult depression, substance abuse[41] and suicide ideation[42]. Given the known impact of sleep deprivation on mental health[43,44], we argue that the binomial insomnia-sleep deprivation is one major contributor to adverse outcomes in the depressed group, and might represent early signs of impairment in the HR group.

It is worth mentioning that we observed differences between the duration of sleep recorded by the actimetry of that recorded by the MCTQ, and the continuous score of total sleep time measured by the actimetry was not different between the groups. On the one hand, depressed adolescents tend to underestimate the total sleep time[45,46]. On the other hand, it is known that actimetry has a remarkably high sensitivity for detecting sleep, which frequently makes it identify periods of immobility as sleep[47]. Considering the context of the depressed adolescents in this study – who are more prone to report eveningness, show later sleep time and higher sleep latency, in addition to having more complaints of initial insomnia – they are likely to stay in bed for any reason other than sleeping. Thus, actimetry may erroneously detect periods of immobility as sleep. Anyway, when we analyzed the data of the objective scores of total sleep time less than 6h measured by actimetry, we observed a clear prevalence of sleep duration lower than 6h among depressed adolescents.

Adolescence is marked with a progressive shift towards eveningness, which often has no corresponding adjustments in social routines (i.e., early school start times in the morning). The adolescents' sleep timing in this study is, on average, 2h later on weekends compared to weekdays. We call this difference in sleep phases the social jetlag (SJL), which is associated with long-term health consequences like mood disorders and is related to depressive symptoms[48]. Although SJL levels are high in the entire sample, the group of depressed adolescents is the one who reports the highest rates of discrepancy in sleep timing. We observed that having a SJL above 3.4h considerably increases the chance that the analyzed individual belongs to the MDD group, and it also raises the probability of being a HR as opposed to a LR adolescent.

In our study, the amplitude of rest-activity rhythms of depressed adolescents measured by actimetry is significantly lower than that of the other adolescents in the sample. These adolescents show higher levels of motor activity at night, but do not appear to have a significant change in activity during the day. When interpreting these results, one should consider the way in which these parameters are calculated: 1) individual average daily profiles are initially computed; 2) the 10 most and 5 least active hours in that profile are identified; 3) average activity of those periods is computed (i.e., M10 and L5); and 4) RA is computed based on these values. We then have two plausible explanations for this result. The first is that these adolescents have greater irregularity in their periods of activity and rest, significantly increasing the average of activity corresponding to certain periods of the night. This first hypothesis is quite plausible vis-à-vis the results of increased SJL in this group and the report of greater irregularity in both bedtime and wakeup time. The second is that depressed adolescents are more active in their rest period, as current depressive

symptoms might lead to psychomotor reduction during waking periods and more fragmented sleep at night, both of which are common in mood disorders[16].

This pattern of flattening the rest-activity rhythm curve is thought to indicate weaker entrainment to external temporal cues. Once light is a prominent temporal cue that entrains biological rhythms to a circadian pattern, little variations in light exposure throughout the day - such as lack of exposure to sunlight and ALAN - weakens this temporal signal and can contribute to dampen activity amplitude[49]. Indeed, the MDD group presented increased light exposure and activity at night, as per the observed in L5 of light and L5 of activity. However, no difference was observed in terms of light exposure throughout the day. Beyond central modulation of circadian rhythms and sleep, light also plays an important role in the regulation of mood. Currently, literature points to distinct pathways that could explain the influence of light on mood. The indirect pathway consists of irregular light exposure, such as ALAN, leading to rhythms and sleep disturbances that eventually affect mood. In the case of the direct pathway, abnormal light can primarily impact mood independent of circadian rhythms and sleep alterations[50]. A recent study by Paksarian and colleagues showed that higher area-level outdoor ALAN was associated with increased mood and anxiety disorders, bipolar disorder, specific phobias, and major depressive disorder among adolescents[51]. Our findings suggest that higher light exposure in the hours of lowest daytime light exposure, measured by the L5, significantly increased the odds of belonging to MDD compared to HR and LR groups, and of belonging to HR as opposed to the LR. This indicates that a possible irregular pattern of light exposure could be related to depressive phenotypes.

Current evidence does not allow for the establishment of a unidirectional relationship between ALAN and delayed sleep timing. Although we already have sufficient

experimental evidence on the impact of ALAN on sleep, it is possible for individuals to expose themselves to artificial light because they are late-types and are unable to sleep at an earlier time. Thus, these factors most likely overlap and co-influence each othe. Still, there are obvious benefits of targeted interventions aiming to minimize ALAN. These interventions are part of the concept of sleep hygiene, a series of beneficial behavioral and environmental attitudes towards a better sleep. When compared to both HR and LR groups, adolescents in the MDD group reported worse overall sleep hygiene mainly because of factors like bedtime irregularity, and subjective stress at bedtime. However, compared to the LR groups, the HR shows significantly greater wake up time irregularity, prolonged time in bed and performance of arousing activities close to bedtime. Zhang and colleagues (2017) found that poor sleep hygiene (more specifically, having non-regular sleep schedules and poor emotional condition at bedtime) were independently associated with mental health problems among adolescents[52]. Moreover, a myriad of self-reported symptoms of psychopathology have been recently linked with worse sleep hygiene[53,54].

With several self-reported instruments, we collected data that allows for a multidimensional view of the adolescents' sleep. In addition, we have gathered data of 10 consecutive days of rest-activity and light exposure rhythms using actimetry, a cost-efficient methodology with growing use in Psychiatry. Besides sleep, this technique provides additional parameters that estimate the magnitude, stability, and regularity of activity rhythms. Actimetry is also a promising tool for its objective and non-invasive nature, being independent of patient, parents, and physician subjective impressions. Even so, these results must be analyzed in view of some limitations. For example, because of our sample size, we might not have enough power to uncover significant differences among groups when comparing some parameters with greater variability (e.g., the M10 and the

MESOR). Moreover, the homogeneity of our sample guarantees internal validity, but might hinder generalizability of our results to a community sample. We cannot assume the reproducibility of these results in different age groups, especially younger adolescents. Finally, as this is a cross-sectional report, longitudinal studies are needed to understand whether sleep and biological rhythm alterations in LR or HR individuals guard any causal relationship with MDD.

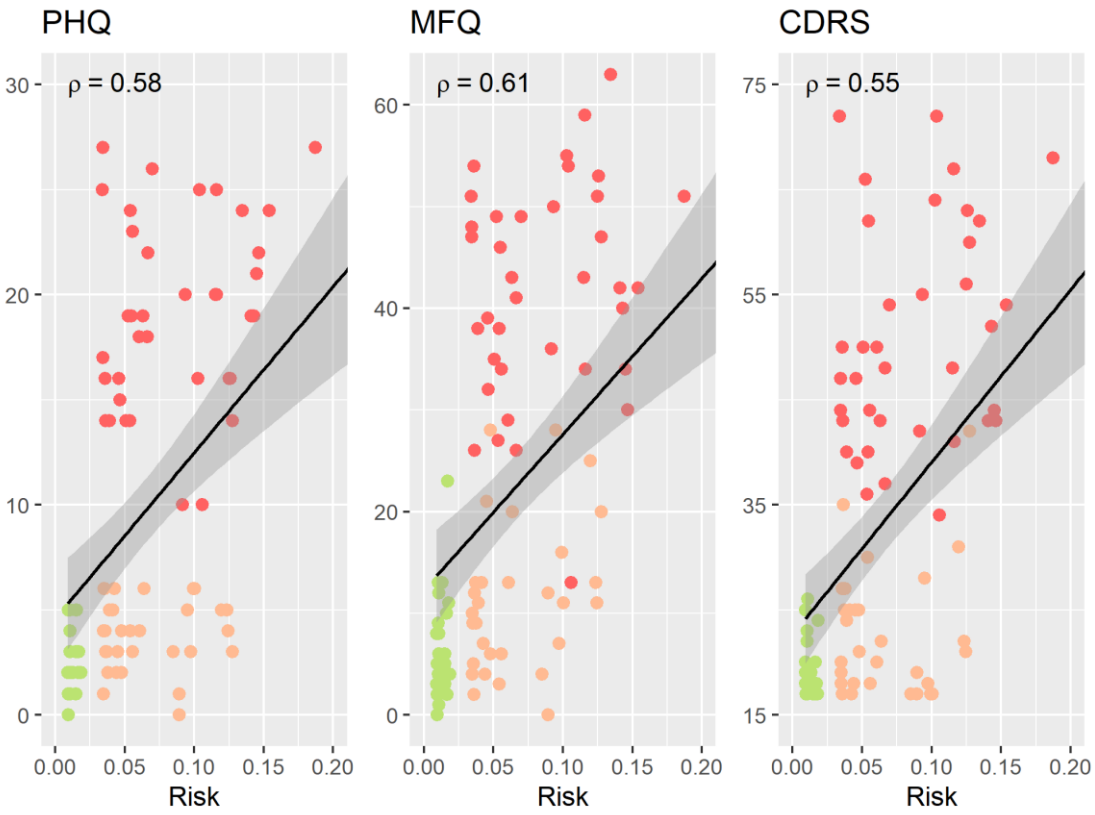
5. CONCLUSION

In sum, the adolescents in a current depressive episode express severe symptoms of insomnia, and alteration of sleep routines, more specifically a late sleep onset, a shorter sleep duration and important differences between the sleep phases of the work or school days compared to the free days - determining a higher SJL. In addition, they report poor sleep hygiene practices (particularly, irregular sleeping and waking times in addition to exposure to artificial light at night) which build upon the current body of evidence on the topic. Changes in sleep routines and practices are also accompanied by a decrease in the amplitude of motor activity rhythms, which may indicate a change in the range of activity throughout the day or reflect the irregularity of routines. Therefore, this evidence is compatible with the characteristic of greater eveningness found in depressed individuals, which is accompanied by other outcomes possibly related to circadian disruption and misalignment (e.g., social jetlag, irregular sleep-wake schedules, and ALAN).

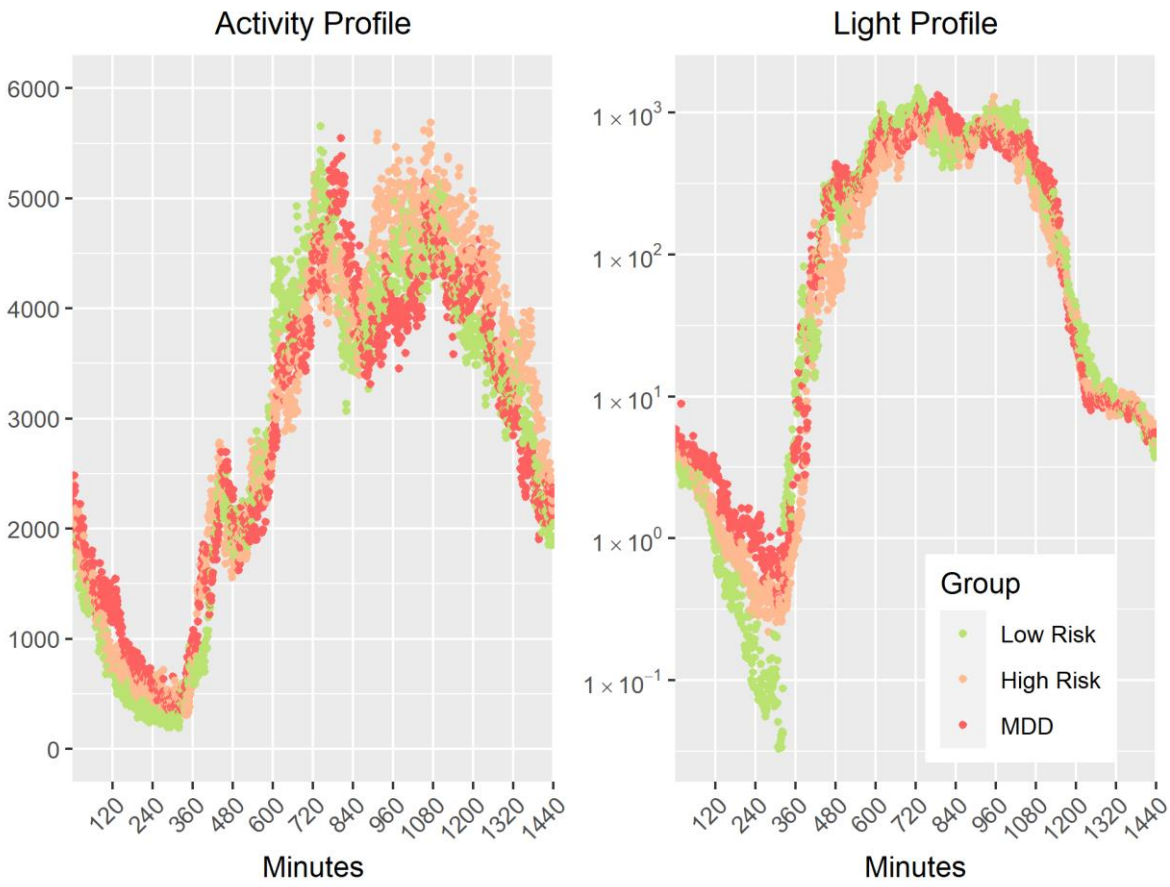
Compared to the LR, the HR group also presents higher insomnia, lower RA, higher exposure to ALAN and higher SJL. These differences could be associated with the spectrum of depressive symptoms shown in this group but also suggest that some changes of sleep

and biological rhythms that are characteristic of MDD are already present in individuals at risk for the disorder. However, they sleep more in free days compared to adolescents with MDD, which indicates that they might be able to compensate for sleep debt on weekends. This compensation could be associated with protective factors such as helping in the modulation of emotional experiences[55]. There is great clinical interest in exploring protective factors related to sleep and biological rhythms, which can impact the future of these adolescents[56]. Still, our results concerning sleep hygiene indicate that it is possible to educate adolescents and their family to identify actions that they can perform in their daily lives, even in the absence of a formal intervention for insomnia or depression.

Even though the risk score is composed of non-modifiable factors, here we present evidence of some potentially modifiable factors that are known to negatively impact mental health. Insomnia precedes the onset and recurrence of a depressive episode in up to 40% of the cases[57]. Thus, we hypothesize that the other sleep and rhythm alterations associated with the HR in this group – like bedtime irregularity, SJL and exposure to ALAN - could also precede a depressive episode, for which preventive strategies related to sleep hygiene can be designed in future investigations. Furthermore, in addition to subjective assessment of sleep hygiene, we highlight that the actimetry-based parameters of motor activity – particularly the RA – and of light exposure are promising measures to be explored as clinical tools for diagnosis, risk stratification and treatment follow-up of depression in adolescence.



Supplementary figure 1. Correlations between risk scores and depressive symptoms measured by the Patient Health Questionnaire (PHQ), the Mood and Feelings Questionnaire (MFQ) and the Children's Depression Rating Scale (CDRS). All three Spearman's ρ indicated in the top of the panels show p levels < 0.001. Green dots represent adolescents from the Low Risk group, while orange represent adolescents from the High Risk group and red represent adolescents from the Major Depressive Disorder group.



Supplementary figure 2. Profiles of rest-activity and light exposure of study groups. Each dot represents the daily average of activity for each individual (10 days). MDD: Major Depressive Disorder

Supplementary Table 1. Descriptive statistics and univariate analyses of linear parameters.							
	Total (n=96)	**LR (n=26)**	**HR (n=31)**	**MDD (n=39)**	**p(LRxHR)**	**p(LRxMDD)**	**p (HR vs MDD)**
Actimetry - activity							
MESOR	2,874.8 (800.5)	2,816.3 (575.8)	2,990.8 (1,013.1)	2,821.6 (746.6)	0.002	0.920	0.001
IS	24.2 (4.8)	25.1 (4.8)	24.7 (4.9)	23.1 (4.5)	0.748	0.092	0.152
IV	0.4 (0.1)	0.4 (0.1)	0.4 (0.1)	0.5 (0.1)	0.880	0.710	0.570
M10	4,546.7 (1,275.7)	4,560.6 (904.7)	4,780.9 (1,678.1)	4,351.2 (1,105.6)	0.017	0.010	0.000
L5	310.9 [195.1, 468.3]	234.1 [153.4, 374.7]	305.6 [195.5, 368.4]	353.7 [268.0, 705.6]	0.227	0.009	0.061
RA	0.87 [0.79, 0.91]	0.91 [0.86, 0.94]	0.89 [0.83, 0.91]	0.83 [0.71, 0.87]	0.297	0.003	0.018
Actimetry - light							
MESOR	294.5 (169.8)	320.2 (200.3)	253 (123.6)	310.2 (177.5)	0.126	0.825	0.149
IS	0.2 (0)	0.2 (0)	0.2 (0.1)	0.2 (0)	0.350	0.820	0.200
IV	0.3 (0.1)	0.3 (0.1)	0.3 (0.1)	0.4 (0.1)	0.510	0.158	0.430
M10	675.5 (382.5)	743.2 (474.5)	591.9 (286.8)	697 (378.6)	0.133	0.647	0.234
L5	0.6 (0.8)	0.3 (0.4)	0.6 (0.6)	0.9 (1)	0.100	0.008	0.100
RA	0.99 (0.03)	0.99 (0.02)	0.99 (0.03)	0.99 (0.03)	0.093	0.054	0.977
Actimetry - sleep							
TST (total, h)	6.9 (1.3)	7.1 (1.2)	6.9 (1.2)	6.7 (1.4)	0.741	0.130	0.220
TST (WD, h)	6.9 (1.3)	7.1 (1.2)	6.9 (1.2)	6.7 (1.3)	0.610	0.190	0.400
Time in bed (total, h)	7.8 (1.3)	7.9 (1.3)	7.9 (1.2)	7.5 (1.5)	0.948	0.250	0.200
Time in bed (WD, h)	7.7 (1.4)	7.9 (1.3)	7.9 (1.3)	7.5 (1.6)	0.940	0.300	0.310
Latency (total, min)	2.6 (2.3)	2.8 (2)	2.8 (2.3)	2.4 (2.5)	0.939	0.448	0.480
Latency (WD, min)	2.6 (2.9)	2.5 (1.9)	2.8 (3.5)	2.5 (3)	0.665	0.960	0.592
Efficiency (total, %)	88.9 (4.4)	90.1 (3.9)	88.3 (4.8)	88.7 (4.4)	0.137	0.213	0.692
Efficiency (WD, %)	89.2 (4.3)	90.2 (3.8)	88.1 (4.7)	89.3 (4.2)	0.081	0.468	0.276
WASO (total, h)	0.8 (0.4)	0.7 (0.4)	0.8 (0.4)	0.8 (0.4)	0.190	0.330	0.670
WASO (WD, h)	0.8 (0.4)	0.7 (0.3)	0.8 (0.4)	0.8 (0.4)	0.190	0.480	0.470
Sleep timing (MCTQ)							
Sleep Duration WD	6.9 (2.1)	7.5 (1.8)	7.6 (1.7)	5.8 (2.1)	0.784	0.002	0.001
Sleep Duration FD	8.5 (1.7)	8.4 (1.5)	9.1 (1.3)	8 (1.9)	0.076	0.340	0.007
Social jet lag	2.6 (1.5)	2.4 (1.2)	2.4 (1.4)	2.8 (1.6)	0.979	0.260	0.250
FD: weekends (free days); HR: High Risk; LR: Low Risk; MDD: Major Depressive Disorder; TST: total sleep time; WASO: wake after sleep onset; WD: weekdays. P values indicate between group comparisons using multinomial logistic regression.							

Supplementary Table 2. Descriptive statistics and univariate analyses of circular parameters.

	Total (n=96)	LR (n=26)	HR (n=31)	MDD (n=39)	W	p
Actimetry - activity						
Acrophase	15:55 [15:00, 17:25]	15:30 [14:45, 17:15]	16:06 [15:06, 17:40]	16:15 [14:50, 17:20]	0.68	0.953
Actimetry - light						
Acrophase	13:45 [12:30, 14:30]	13.30 [12:30, 14:40]	13.55 [12:30, 14:30]	13:40 [12:40, 14:30]	0.78	0.942
Actimetry - sleep						
Bed time (total)	1:05 [00:00, 2:10]	1:00 [23:55, 2:00]	00:55 [00:05, 1:45]	1:20 [00:15, 2:30]	1.32	0.858
Bed time (WD)	00:30 [23:40, 2:00]	00:40 [23:55, 1:45]	00:45 [00:00, 2:00]	00:45 [23:55, 2:00]	0.40	0.983
Wake up time (total)	8:50 [7:40, 10:05]	8:55 [7:50, 9:55]	8:50 [8:00, 10:20]	8:45 [7:20, 10:10]	2.85	0.583
Wake up time (WD)	8.7 (1.9)	8:30 [7:40, 9:55]	8:30 [7:30, 9:55]	8:30 [7:00, 10:05]	3.56	0.469
Sleep timing (MCTQ)						
Sleep Onset WD	00:10 [23:25, 1:30]	00:05 [23:00, 00:40]	00:00 [23:10, 00:30]	1:00 [23:50, 2:05]	9.93	0.042
Sleep End WD	6:30 [6:00, 8:00]	6:40 [6:30, 7:50]	6:45 [6:00, 9:30]	6:00 [6:00, 7:00]	5.78	0.216
Sleep Onset FD	2:00 [00:30, 3:10]	1:40 [00:10, 2:50]	1:10 [00:30, 2:30]	2:50 [00:55, 4:30]	5.55	0.236
Sleep End FD	11:00 [9:00, 12:00]	10:10 [8:05, 11:00]	11:00 [10:00, 12:00]	11:00 [10:00, 12:00]	1.11	0.893
MSW	3:30 [2:55, 4:30]	3:30 [2:55, 4:15]	3:20 [2:55, 4:40]	3:50 [2:50, 4:10]	2.76	0.599
MSF	5.8 (1.8)	5:20 [4:05, 6:55]	5:55 [4:05, 6:55]	6:40 [4:30, 7:10]	3.56	0.469

Data is expressed as median and interquartile range. Groups were compared using the Watson-Wheeler test for homogeneity of angles. FD: weekends (free days); HR: High Risk; LR: Low Risk; MDD: Major Depressive Disorder; MSF: midpoint of sleep on free-days (weekends); MSW: midpoint of sleep on weekdays; WD: weekdays.

Supplementary Table 3. Descriptive statistics and univariate analyses of categorized variables.

	LR (n=26)	HR (n=31)	MDD (n=39)	p (LRxHR)	p (LRxMDD)	p (HRxMDD)
IV (Lux) >0.41 (4thQ)	6 (23.1%)	7 (22.6%)	11 (28.2%)	0.965	0.650	0.590
TST (total) <6h (1stQ)	3 (11.5%)	6 (19.4%)	16 (41.0%)	0.420	0.016	0.057
TST (WD) <6h (1stQ)	4 (15.4%)	8 (25.8%)	14 (35.9%)	0.34	0.078	0.37
SJL >3.4h (4thQ)	3 (11.5%)	8 (25.8%)	13 (35.1%)	0.18	0.043	0.41

P values indicate between group comparisons using multinomial logistic regression. HR: High Risk; LR: Low Risk; MDD: Major Depressive Disorder; TST: total sleep time; WD: weekdays.

Supplementary table 4. Multivariate models.

Model adjusted for TST measured by actimetry

	High Risk vs Low Risk				MDD vs Low Risk				MDD vs High Risk			
	OR	**95% CI**		**p-value**	**OR**	**95% CI**		**p-value**	**OR**	**95% CI**		**p-value**
Age	1.91	1.30	2.81	0.001	2.20	1.17	4.13	0.015	1.18	0.69	2.01	0.550
Sex(F)	1.08	0.85	1.37	0.547	3.20	2.72	3.85	0.000	3.00	2.89	3.11	0.000
Chronotype (PPPS)	1.04	0.92	1.19	0.523	1.04	0.86	1.25	0.712	0.99	0.86	1.15	0.921
Sleep Hygiene (SHI)	1.12	0.95	1.32	0.180	1.49	1.13	1.96	0.005	1.33	1.05	1.68	0.017
Insomnia (AIS)	1.79	1.22	2.62	0.003	2.90	1.86	4.43	0.000	1.61	1.26	2.05	0.000
M10 (activity)	1.00	1.00	1.00	0.206	1.00	1.00	1.00	0.558	1.00	1.00	1.00	0.790
RA (activity)	0.60	0.58	0.61	0.000	0.00	0.00	0.00	0.000	0.00	0.00	0.00	0.000
L5 (light)	5.00	2.80	8.76	0.000	5.60	3.64	8.65	0.000	1.14	1.03	1.26	0.010
SJL >3.4h	1.79	3.28	3.49	0.000	3.41	3.27	3.55	0.000	1.02	1.00	1.04	0.129
TST <6h	1.12	2.27	3.01	0.000	5.20	4.66	5.80	0.000	2.07	2.01	2.13	0.000

Model adjusted for sleep duration on WD measured by MCTQ

	High Risk vs Low Risk				MDD vs Low Risk				MDD vs High Risk			
	OR	**95% CI**		**p-value**	**OR**	**95% CI**		**p-value**	**OR**	**95% CI**		**p-value**
Age	2.05	1.35	3.12	0.001	2.07	1.10	3.89	0.024	1.04	0.57	1.90	0.902
Sex(F)	1.04	0.93	1.15	0.496	2.63	2.52	2.74	0.000	2.53	2.48	2.59	0.000
Chronotype (PPPS)	1.07	0.94	1.22	0.286	1.07	0.89	1.28	0.485	1.00	0.86	1.16	0.963
Sleep Hygiene (SHI)	1.10	0.93	1.30	0.257	1.41	1.08	1.84	0.013	1.28	1.00	1.64	0.051
Insomnia (AIS)	1.99	1.34	2.95	0.001	3.20	2.00	5.21	0.000	1.62	1.20	2.19	0.002
M10 (activity)	1.00	1.00	1.00	0.078	1.00	1.00	1.00	0.516	1.00	1.00	1.00	0.616
RA (activity)	0.02	0.02	0.02	0.000	0.00	0.00	0.00	0.000	0.00	0.00	0.00	0.000
L5 (light)	6.70	5.29	8.58	0.000	7.90	6.41	9.73	0.000	1.19	1.06	1.34	0.003
SJL >3.4h	6.84	6.37	7.35	0.000	4.79	4.59	4.99	0.000	0.70	0.68	0.73	0.000
SD (WD)	1.52	1.07	2.16	0.010	0.88	0.45	1.73	0.720	0.58	0.31	1.08	0.087

Model adjusted for sleep duration on FD measured by MCTQ

	High Risk vs Low Risk				MDD vs Low Risk				MDD vs High Risk			
	OR	**95% CI**		**p-value**	**OR**	**95% CI**		**p-value**	**OR**	**95% CI**		**p-value**
Age	1.69	1.24	2.30	0.001	1.68	1.19	2.37	0.003	1.03	0.60	1.79	0.909
Sex(F)	1.11	1.05	1.17	0.000	3.40	3.27	3.54	0.00	3.08	2.94	3.22	0.000
Chronotype (PPPS)	1.08	0.96	1.22	0.217	1.08	0.91	1.29	0.38	1.00	0.86	1.17	0.974
Sleep Hygiene (SHI)	1.12	0.95	1.31	0.176	1.50	1.14	1.96	0.00	1.34	1.05	1.71	0.020
Insomnia (AIS)	2.02	1.39	2.95	0.000	3.40	2.15	5.24	0.00	1.66	1.27	2.16	0.000
M10 (activity)	1.00	1.00	1.00	0.073	1.00	1.00	1.00	0.49	1.00	1.00	1.00	0.543
RA (activity)	0.10	0.10	0.10	0.000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.000
L5 (light)	6.30	5.71	6.94	0.000	7.78	7.09	8.53	0.00	1.30	1.15	1.39	0.000
SJL >3.4h	4.65	4.40	4.91	0.000	6.76	6.40	7.14	0.00	1.50	1.40	1.53	0.000
SD (FD)	1.93	1.35	2.75	0.000	1.17	0.73	1.88	0.520	0.61	0.34	1.09	0.096

P values indicate between group comparisons using multinomial logistic regression. AIS: Athens Insomnia Scale; L5(light): Mean light exposure of the lowest 5 hours of light exposure; M10(activity): mean of activity of the highest 10 hours of activity; MDD: Major Depressive Disorder; PPPS: Puberty and Phase Preference Scale; RA(activity): the difference between the M10 and the L5 of activity; SD(FD): Sleep duration on free days (weekends); SD(WD): Sleep duration on weekdays; SHI: Sleep Hygiene Index; SJL>3.4h: social jetlag greater than 3.4h (representing the fourth quartile compared to the others).

SUPPLEMENTARY FILE 1. The Identifying Depression Early in Adolescence Risk Score.

Administration of the IDEA-RS questionnaire in the schools was performed using a coded, unidentified form distributed to students. Questions were selected to match *ipsis litteris* the original phrasing used in the Pelotas 1993 Birth Cohort study. On average, less than 15 minutes were required for administration of the IDEA-RS.

Sex:	Male/Female
Your skin color or race is:*	White/Yellow/Brown/ Black/Indigenous
Do you meet your friends often to talk, play or do anything else?	No/yes
Have you ever failed a school grade?	No/yes
Have you ever run away from home?	No/yes
Have you ever tried cigarettes?**	No/yes
Have you ever tried alcohol?**	No/yes
Have you ever tried sniffing glue?**	No/yes
Have you ever tried sniffing solvents or ethyl chloride (EC)?**	No/yes
Have you ever tried marijuana?**	No/yes
Have you ever tried cocaine or crack?**	No/yes
Have you ever tried LSD or acid?**	No/yes
Have you ever tried ecstasy or molly?**	No/yes
Have you ever used weight loss pills?**	No/yes
Have you ever used tranquilizers or sleeping pills?**	No/yes
Have you ever used any drug? **	No/yes
In the last year, did you get into any fight in which somebody got hurt?	No/yes
Would you say your relationship with your father is:	Great/Very good/ Good/Regular/Bad
Would you say your relationship with your mother is:	
Would you say the relationship between your father and mother is:	
Have you ever been separated from your parents so that you had to stay with someone else?***	No/yes
At home, have you witnessed fights with physical aggression between adults, or has any adult assaulted a child or teenager?***	No/yes
Have you experienced not having enough food at home, or have you had to wear dirty or torn clothes because you had no other?***	No/yes
Have you ever thought or felt that your parents wished you were never born?***	No/yes
Have you ever thought or felt that someone in your family hated you?***	No/yes
Have you ever been beaten by an adult in your family or by someone who was taking care hard enough to leave marks or hurt you?***	No/yes
Has anyone ever tried to touch you in a sexual way, or tried to make you touch them against your will, threatening you or hurting you?***	No/yes

* Self assigned skin color following Brazilian official census categories. For analyses, two categories (white vs. non-white) were formed.

** Questions about any lifetime use of alcohol, tobacco, cannabis, cocaine and inhalants were combined into one variable using the OR rule, generating a binary variable for analyses.

*** Responses to seven dichotomous questions regarding lifetime psychological, physical and sexual abuse and/or neglect were combined into three categories: zero positive answers=none, 1 positive=probable, 2 or more=severe.

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CAPÍTULO 7 – DISCUSSÃO E CONCLUSÃO

1. CONTRIBUIÇÕES METODOLÓGICAS DESTA TESE

A versão traduzida e validada para o português brasileiro do instrumento *Sleep Higyene Index* (Índice de Higiene do Sono, SHI-BR, Anexo 3) mostrou ser um instrumento de fácil aplicação e compreensão que pode ser útil no planejamento do tratamento e no manejo de práticas de higiene do sono. A coleta de dados para o estudo em uma população de jovens alistados para o serviço militar (Capítulo 2) foi desenvolvida antes deste processo de adaptação da SHI-BR. Sendo assim, o estudo foi capaz de observar que alguns parâmetros relacionados a higiene do sono parecem ser mais importantes para indivíduos jovens deprimidos. No entanto, a utilização da escala SHI-BR poderia trazer informações mais completas e que descrevessem possíveis estratégias de ação para melhora dos padrões de sono e quiçá dos sintomas depressivos nesta população.

Esta escala foi utilizada na avaliação de adolescentes no capítulo 6, onde observamos uma maior prevalência de má higiene do sono nos adolescentes deprimidos comparados aos não-deprimidos. Dentro deste constructo, observamos uma maior exposição à luz artifical à noite neste grupo, além de maior irregularidade de horários de dormir e de acordar. A identificação destas questões é útil para um diagnóstico do comportamento basal relacionado ao sono, além de permitir o desenho de intervenções comprovadamente eficazes através da terapia cognitivo comportamental.

Nosso estudo acerca do impacto de dados faltantes (*missings*) em séries de atividade motora medidos pela actimetria demonstramos os efeitos do manuseio comum destes dados faltantes que simulam perídos de não-uso (“off-wrist”) do aparelho. Com base nos resultados, podemos afirmar que a manutenão dos “zeros” detectados automaticamente pelos *softwares* de actimetria – e que é um procedimento comum em estudos da área –

deve ser evitada em todos os casos. Sendo assim, é imperativa a identificação dos períodos de imobilidade que possam representar não-uso do aparelho, e subjstituição dos valores nulos por dados faltantes (“missing”, “NA”). Se por algum motivo os parâmetros desejados não puderem ser calculados na presença de “NA”, recomendamos o uso da média semanal dos pontos de tempo correspondentes, pois foi a medida que mais se aproximou do cálculo derivado da série original. Entendemos que estes estudos fornecem oportunidades complementares de avaliação subjetiva e objetiva do comportamento do sono tanto em protocolos de pesquisa, quanto em abientes clínicos.

2. IMPLICAÇÕES DOS PRINCIPAIS RESULTADOS DOS ESTUDOS CLÍNICOS

O estudo de variáveis dentro do paradigma da Cronobiologia (ou mesmo da Cronomedicina) necessita de protocolos com amostragens ou intervenções em diferentes momentos do dia, já que a variável “tempo” ou “momento” sempre será objeto de discussão. A fundamentação para tal procedimento metodológico é simples e já foi extensamente revisada ao longo deste texto: uma variável que apresenta ritmo circadiano se mostra diferente ao longo das 24 horas do dia. A Cronomedicina é uma disciplina já difundida na Cardiologia, na medida em que é bem descrito o descenso na pressão arterial à noite e, por isso, foram desenvolvidos protocolos de tratamento que consideram a hora de administração de anti-hipertensivos(183,184). Também a Endocrinologia leva em conta os padrões oscilatórios de hormônios para realização, por exemplo, de testes de supressão com dexametasona ou de resposta de cortisol ao despertar. Afinal, também já é óbvia a ciclicidade em período infradiano (i.e., com período maior do que 24h) do ciclo menstrual ovulatório; há estratégias terapêuticas e recomendações de dosagens hormonais particulares a determinados períodos do ciclo(185,186). Os estudos desenvolvidos nesta

tese reforçam a ainda incipiente – porém promissora – disciplina da Cronomedicina no campo da Psiquiatria.

Os resultados aqui apresentados documentam que a síndrome depressiva pode existir num contexto de vespertinidade associada a alteração de rotinas de sono, mais especificamente um início de sono tardio, uma menor duração de sono e diferenças importantes entre as fases de sono dos dias de trabalho ou escola comparados aos dias livres – determinando um maior JLS. As descrições de más práticas de higiene do sono associadas a sintomas depressivos, com destaque para horários irregulares de dormir e de acordar além da exposição à luz artificial no período da noite incorporam-se ao crescente corpo de evidência da área. Além das mudanças no comportamento de sono, observamos algumas alterações de padrões rítmicos de atividade motora.

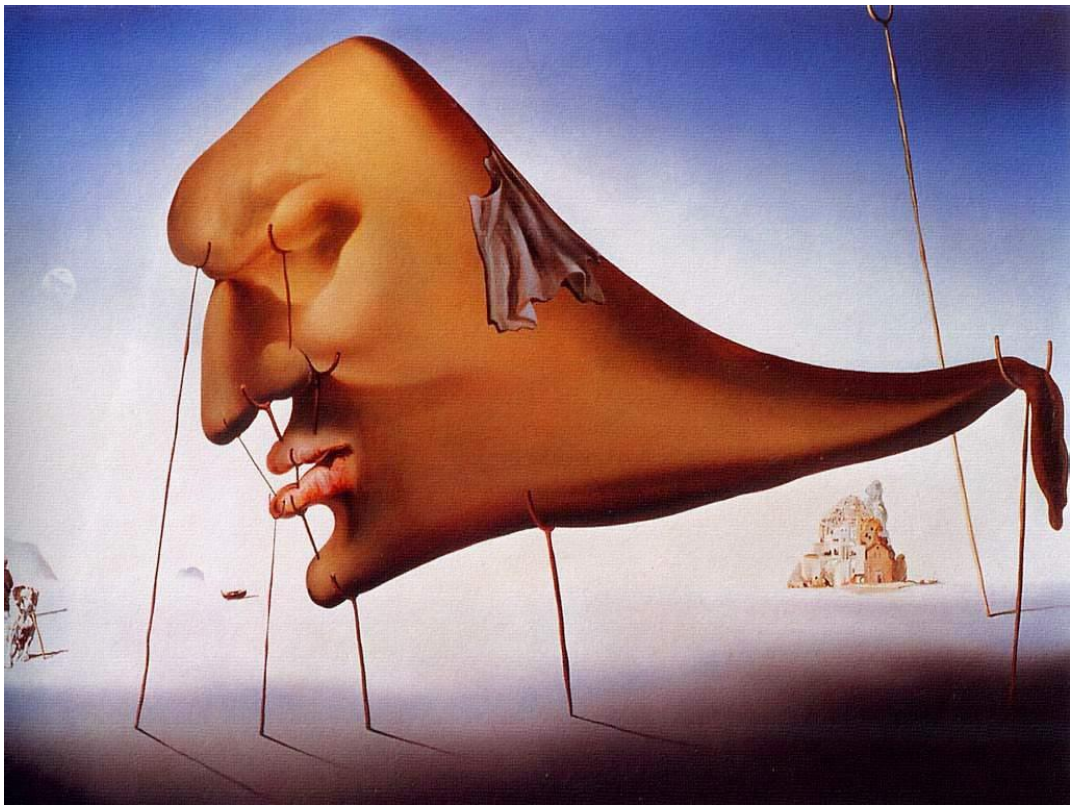
Alterações possíveis da ritmicidade de uma variável são 1) a mudança de fase, como é o caso do estudo de protocolos experimentais de manipulação de iluminação(187), 2) a perda do padrão oscilatório, tendo como exemplo quadros de sepse em pacientes internados para unidades de cuidados intensivos (188,189), e 3) a mudança na magnitude do ritmo em algum momento do dia, resultando na redução da amplitude deste ritmo, que foi observada em estudos desta tese. Uma redução dos níveis de cortisol matinal dos jovens em alistamento militar se correlaciona com sintomas depressivos. É importante reforçar, no entanto, que é plausível também assumir que estes jovens tenham um atraso de fase no pico de cortisol matinal já que são mais vespertinos. Além disso, os níveis de atividade motora noturna aumentados são características de indivíduos com depressão não-melancólica. Também, a amplitude dos ritmos de atividade-repouso dos adolescentes deprimidos é menor às custas de maior atividade no período da noite. Ao obtermos as médias da atividade motora correspondente a todos os dias de registro, entendemos que,

tanto a menor atividade à noite em deprimidos melancólicos, quanto a amplitude de atividade diminuída em adolescentes deprimidos, podem também ser explicados pela maior irregularidade nos seus períodos de atividade e repouso ao longo dos dias.

A partir dos nossos resultados, apontamos para a possibilidade de que aconselhamento de higiene do sono pode ser uma eficiente intervenção comportamental para melhorar a saúde do sono e sintomas depressivos em populações jovens(138). Este aconselhamento é parte da terapia comportamental para insônia, uma estratégia comprovadamente eficaz para melhora de padrões de sono em indivíduos que sofrem de depressão, mas também para melhora global dos sintomas depressivos e de insônia em indivíduos sem o diagnóstico.

Com o desenvolvimento da SHI-BR como parte desta tese, abrimos a possibilidade de estudo destes fatores potencialmente modificáveis que possam traçar diretrizes de intervenção clínica. A higiene do sono é um exemplo relevante de estratégia de Cronomedicina que pode ser adotada para Psiquiatria. Dependendo da hora do dia, espera-se um efeito diferente dos diversos componentes da higiene do sono. Por exemplo, nitidamente a hora de consumo de café e de outros estimulantes é importante para que se classifique um comportamento inadequado relacionado ao sono. Além disso, já se sabe que a prática regular de atividades físicas melhora padrões de sono como aumento do tempo total e da eficiência de sono e melhora da qualidade percebida(190,191), mas também se propõe que estes exercícios, se possível, não sejam feitos perto do horário de dormir(133), embora ainda sejam necessárias mais evidências para provar que atividade física à noite pode piorar padrões de sono(192). Além do mais, a redução da ELAN é uma das principais recomendações dadas a pacientes com dificuldade de sono, pela sabida influência da luz no sistema temporizador e na regulação do sono.

3. CONCLUSÃO



“Le Sommeil (Sleep)” de Salvador Dalí. 1937. Oléo sobre tela. 51 x 78 cm.

Atualmente compreendemos diversos mecanismos de regulação do sono e dos ritmos biológicos, mas ainda não chegamos perto da resolução da questão elementar: “Por que dormimos?”. Ainda assim, com base no corpo sólido de evidências construído até hoje, somos capazes de afirmar que, se não dormimos, enfrentamos consequências para nossa saúde física e mental. Além do mais, os modelos estudados que afetam questões basilares da ritmicidade circadiana (e.g., ELAN, trabalho em turno noturno) consistentemente se associam com problemas de saúde física e mental a longo prazo.

Com a progressão da adolescência até os primeiros anos da idade adulta, o início do sono se torna mais atrasado(193), o que pode ser explicado pela também progressiva tendência à vespertinidade nesta faixa etária(64). No entanto, uma parte destes indivíduos não é capaz de também atrasar o sono em função de compromissos sociais. Sendo assim, um percentual significativo dos adolescentes e adultos jovens não consegue dormir a

quantidade de horas de sono recomendadas por noite nos dias de estudo ou de trabalho(193–195). Mesmo sendo uma característica desta faixa etária, a vespertinidade é constantemente associada a depressão em estudos clínicos e epidemiológicos. Os estudos desenvolvidos nesta tese complementam estes achados. As evidências aqui mostradas caracterizam uma *síndrome clínica de cronorruptura*, que definimos como uma consequência da associação entre vespertinidade e a necessidade de seguir uma rotina social, resultando em irregularidades dos horários de sono, insônia, má qualidade de sono, sinais de desalinhamento circadiano (e.g., jetlag social e ELAN) e sintomas depressivos. Argumentamos que a depressão não necessariamente é consequência da cronorruptura, mas pode fazer parte deste contexto sindrômico, para os quais identificamos fatores de risco modificáveis.

Estes estudos fazem parte de uma linha de pesquisa do Laboratório de Cronobiologia e Sono HCPA/UFRGS, que investiga parâmetros do comportamento do sono e dos ritmos de atividade-repouso na depressão, já tendo mostrado resultados semelhantes em amostras em adultos. Assim, as manifestações comportamentais descritas que formam a síndrome de cronorruptura sedimentam o papel da Cronomedicina no estudo da depressão. Além de trazer contribuições metodológicas que sedimentam um terreno para o desenho de estudos clínicos futuros, esta tese agrega conhecimento acerca de fatores relacionados às rotinas de sono e aos padrões de atividade que auxiliam na busca pelos processos etiopatológicos de subtipos do transtorno depressivos, na estratificação de risco e acompanhamento do tratamento clínico, além de mostrar alvos potenciais de ações preventivas e/ou terapêuticas.

4. PERSPECTIVAS

Abaixo, serão descritos os três pilares que pretendo solidificar nos próximos anos como pesquisador independente que contribui para o crescimento de um laboratório de pesquisa.

4.1. Projetos Acadêmicos a Serem Desenvolvidos no Pós-doutoramento

As produções científicas desta tese são parte de projetos em desenvolvimento pelo Laboratório de Cronobiologia e Sono HCPA/UFRGS. O trabalho dos impactos de dados faltantes em actimetria está incluído em uma série de outras iniciativas de documentação de questões metodológicas desta tecnologia. Nos últimos anos, outros instrumentos além do Índice de Higiene do Sono (SHI-BR) foram desenvolvidos com o objetivo de se avaliar quantitativamente aspectos ainda inexplorados do sono e dos ritmos biológicos em estudos clínicos. Ao mesmo tempo, a partir de uma linha de pesquisa de avaliação objetiva dos ambientes internos em colaboração com o Laboratório de Engenharia Biomédica do HCPA, desenhou-se um sensor compacto para monitoramento de luz, temperatura e umidade ambiental.

Em conjunto, a adaptação do questionário de higiene do sono, o aperfeiçoamento da metodologia de manejo dos dados actimetria e o desenvolvimento dos sensores supracitados servem de base para um estudo iniciado no Hospital de Clínicas de Porto Alegre (HCPA) que fará parte do meu projeto de pós-doutorado. Este trabalho, aprovado pelo Comitê de Ética em Pesquisa do HCPA (nº 2020-0272), tem como objetivos: 1) compreender a qualidade do espaço físico de trabalho a partir da percepção e satisfação do trabalhador, e a partir de dados objetivos coletados tanto pela actimetria como pelos sensores; e 2) entender o impacto deste ambiente no sono e em questões de saúde mental.

Além disso, incorporaram-se ao corpo desta tese os dados do estudo transversal com adolescentes, que serão reavaliados em um estudo prospectivo para acompanhar a evolução de aspectos de sono e ritmo biológico e sua relação com a depressão

4.2. Projetos Acadêmicos com Foco no Impacto Social

As condições atuais para se fazer ciência através de estudos clínicos e epidemiológicos passaram por profundas transformações com a pandemia devido ao COVID-19. A situação de distanciamento social e as novas políticas de isolamento – tão necessárias para o controle adequado da disseminação da doença – impedem a continuação de alguns estudos clínicos ao mesmo tempo em que representam um chamado aos cientistas para que usem sua expertise para contribuir com a sociedade. Nesse contexto, entendemos que indivíduos em isolamento social podem estar sujeito a menor exposição a iluminação natural e a pistas temporais sociais, além de poderem apresentar maiores irregularidades nos horários de sono, possivelmente manifestando a *síndrome clínica de cronorruptura*.

A partir desta hipótese, descrevemos o estado da arte do isolamento social em desfechos de saúde mental (revisão sistemática a submetida para publicação no periódico *Current Psychiatry Reports*, anexo 2), e delineamos um projeto com objetivo de estudar em detalhes as rotinas de sono e os padrões de atividade diárias de sujeitos em isolamento. Desse modo, utilizando conhecimentos adquiridos nesta tese, buscamos compreender as vulnerabilidades do indivíduo no isolamento, possibilitando o desenho de intervenções que auxiliem as pessoas a cumprirem com medidas de isolamento necessárias nesse período.

4.3. Projetos de Extensão Universitária

Participo como tutor em um 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 Guilherme Hidalgo Caumo, Luana Lima Aniola e Andressa Martins e Silva. Alguns resultados dos projetos foram apresentados em eventos científicos (Semana Científica HCPA, Salão UFRGS Jovem e MOSTATEC) e publicação de artigos científicos, e um dos trabalhos foi publicado em uma revista internacional[1].

Este projeto é renovado anualmente com uma nova turma de alunos, onde seguirei meu processo de tutoria de novos alunos. Meu crescimento como pesquisador independente nas próximas etapas de titulação acadêmica necessariamente estará associado à formação destes alunos IC Jr., além de alunos de graduação e pós-graduação.

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APÊNDICE 1 – OUTRAS PRODUÇÕES E PARTICIPAÇÕES

Outros Artigos Publicados Durante o Período de Doutoramento

1. Carvalho FG, Cunha AMD, Tonon AC, Pereira FDS, Matte U, Callegari-Jacques SM, Hidalgo MP. *Poor sleep quality associates with self-reported psychiatric and cardiometabolic symptoms independently of sleep timing patterns in a large sample of rural and urban workers.* 2020.
DOI: 10.1111/jsr.12969.
Revista: Journal of Sleep Research

2. Caumo GH, Spritzer D, Carissimi A, Tonon AC. *Time of exposure of electronic devices and sleep quality in adolescents: a matter of type, duration and timing.* 2020.
DOI: 10.1016/j.sleh.2019.12.004.
Revista: Sleep Health

3. Dunleavy G, Bajpai R, Tonon AC, Chua AP, Cheung KL, Thach TQ, Rykov Y, Soh CK, Vries H, Car J, Christopoulos G. *Prevalence of psychological distress and its association with perceived indoor environmental quality and workplace factors in under and aboveground workplaces.* 2020.
DOI: 10.1016/j.buildenv.2020.106799
Revista: Building and Environment

4. Jury Freitas J, Bertuol Xavier N, Comiran Tonon A, Carissimi A, Timm Pizutti L, Vieira Ilgenfritz CA, Pekelmann Markus R, Paz Hidalgo M. *6-sulfatoxymelatonin predicts treatment response to fluoxetine in major depressive disorder.* 2019.
DOI: 10.1177/2045125319881927
Revista: Therapeutic Advances in Psychopharmacology

5. Dunleavy G, Bajpai R, Tonon AC, Chua AP, Cheung KL, Soh CK, Christopoulos G, Vries H, Car J. *Examining the Factor Structure of the Pittsburgh Sleep Quality Index in a Multi-Ethnic Working Population in Singapore*. 2019.
DOI: 10.3390/ijerph16234590.
Revista: Int J Environ Res Public Health

6. Dunleavy G, Tonon AC, Chua AP, Zhang Y, Cheung KL, Thach TQ, Rykov Y, Soh CK, Christopoulos G, de Vries H, Car J. *A Multifactorial Approach to Sleep and Its Association with Health-Related Quality of Life in a Multiethnic Asian Working Population: A Cross-Sectional Analysis*. 2019.
DOI: 10.3390/ijerph16214147.
Revista: Int J Environ Res Public Health

Resumos Publicados em Anais de Eventos Científicos

1. Tonon AC, Quiles CL, Oliveira MAB, Beauvalet JC, Hidalgo MP. *A luz artificial modifica os ritmos biológicos e o metabolismo de ratos Wistar.*
Evento: 37ª Semana Científica do Hospital de Clínicas de Porto Alegre – 2017
URL: http://hdl.handle.net/10183/179368

2. Amorim FA, Santos DP, Carissimi A, Oliveira MAB, Beauvalet JC, Tonon AC, Ilgenfritz CAV, Pilz LK, Hidalgo MP. *Seu corpo, seu tempo: uma experiência de difusão na internet*
Evento: 37ª Semana Científica do Hospital de Clínicas de Porto Alegre – 2017
URL: http://hdl.handle.net/10183/170846

3. Medeiros MS, Tonon AC, Fuchs DFP, Gomes WB, Levandovski RM, Fleck MPA, Alencastro LS, Hidalgo MP. *Atividade motora e exposição à luz noturnas: marcadores objetivos de depressão melancólica e não melancólica medidos por Actigrafia.*
Evento: 37ª Semana Científica do Hospital de Clínicas de Porto Alegre – 2017
URL: http://hdl.handle.net/10183/173645

4. Tonon AC, Fleck MP, De Alencastro L, Pizutti L, Freitas J, Ilgenfritz CA, Carissimi A, Markus R, Hidalgo MP. Objective chronobiology-based markers of depression: perspectives in diagnosis and treatment of mood disorders
Evento: XIV Latin American Symposium on Chronobiology (LASC) – 2017
URL: http://cinv.uv.cl/lasc2017/

5. Amando GR, Tonon AC, Carissimi A, Schimitt RL, Lima LS, Hidalgo MP. *Como o estresse, a qualidade de sono e os ritmos biológicos associam-se a sintomas clínicos depressivos clinicamente significativos*?
Evento: 38ª Semana Científica do Hospital de Clínicas de Porto Alegre – 2018
URL: http://hdl.handle.net/10183/210324

6. Constantino DB, Pilz LK, Xavier NB, Levandovski RM, Oliveira MAB, Tonon AC, Roenneberg T, Hidalgo MP. *Cronobiologia e depressão: um estudo da associação entre alterações de ritmos e sintomas depressivos em comunidades quilombolas*.
Evento: 38ª Semana Científica do Hospital de Clínicas de Porto Alegre – 2018
URL: http://hdl.handle.net/10183/210326

7. Andrade GPB, Tonon AC, Beauvalet JC, Xavier NB, Chiamenti P, Freitas JJ, Caumo GH, Gawlinski L, Hidalgo MP. *Tradução e validação da escala "Sleep Hygiene Index" e relação de higiene do sono com problemas de sono e sintomas psiquiátricos em trabalhadores de turnos.*
Evento: 38ª Semana Científica do HCPA – 2018
URL: http://hdl.handle.net/10183/210360

8. Caumo GH, Spritzer DT, Carissimi A, Tonon AC. *O uso de eletrônicos e qualidade de sono em adolescentes: uma questão de tipo, duração e hora.*
Evento: 39ª Semana Científica do Hospital de Clínicas de Porto Alegre – 2019
URL: http://hdl.handle.net/10183/213307

9. Tonon AC, Caumo GH, Aniola LL, Spritzer DT, Hidalgo MP, Caumo W. *A relação do comportamento aditivo a jogos com BDNF, sintomas depressivos e qualidade de sono em adolescentes.*
Evento: 39ª Semana Científica do Hospital de Clínicas de Porto Alegre – 2019
URL: http://hdl.handle.net/10183/213313

10. Schmalfuss TO, Silva RK, Tonon AC, Hidalgo MP. *Percepções, influências e efeitos da sexualidade e da exposição à pornografia no adolescente.*
Evento: 39ª Semana Científica do Hospital de Clínicas de Porto Alegre - 2019
URL: http://hdl.handle.net/10183/213242

11. 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

12. 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

13. Cunha AD, Carvalho FG, Tonon AC, de Souza CM, Pereira FS, Matte US, Callegari-Jacques SM, Hidalgo MP. *Relação entre qualidade do sono e polimorfismos dos genes do relógio.*
Evento: 39ª Semana Científica do Hospital de Clínicas de Porto Alegre - 2019
URL: http://hdl.handle.net/10183/213253

Outras Apresentações de Trabalhos em Eventos Científicos

1. World Congress on Brain, Behavior and Emotions 2017 – pôster “ARTIFICIAL LIGHT MODIFIES BIOLOGICAL RHYTHMS AND METABOLISM OF WISTAR RATS”. 17 de junho de 2017, Centro de Eventos FIERGS em Porto Alegre/RS.

2. 69ª Reunião Anual da SBPC – pôster “EFEITOS DA ILUMINAÇÃO ARTIFICIAL NOS RITMOS BIOLÓGICOS E METABOLISMO EM RATOS WISTAR”. 16 a 22 de julho de 2017. Universidade Federal de Minas Gerais - UFMG, Belo Horizonte – MG

3. XXXII Reunião Anual da FeSBE – pôster “ARTIFICIAL LIGHT MODIFIES BIOLOGICAL RHYTHMS AND METABOLISM OF WISTAR RATS”. 03 de setembro de 2017 a 06 de setembro de 2017, Campos do Jordão Convention Center em Campos do Jordão – SP.

4. XIV Latin American Symposium on Chronobiology – LASC 2017 – pôster “Objective chronobiology-based markers of depression: perspectives in diagnosis and treatment of mood disorders”. 14 a 18/11/2017. Valparaíso, Chile.

5. XXXIV Reunião Anual da FeSBE – pôster “Development and testing of a mechanized apparatus for rodent models of chronic sleep deprivation”. 09 a 13 de setembro de 2019, Campos do Jordão Convention Center em Campos do Jordão – SP. Trabalho premiado.

Apresentações de Trabalhos Relacionados à Difusão da Ciência

1. XXIII Semana Acadêmica de Medicina da UFRGS – palestrante do minicurso “FATORES ASSOCIADOS À RESTRIÇÃO DE SONO: O QUE FAZER?”. Carga horária: 1h30min (13/11/2017). Faculdade de Medicina – UFRGS.

2. PAS 8th Annual Science Fair – palestrante. 26/05/2018. Pan American School of Porto Alegre, Porto Alegre – RS.

3. LIGA DE CRONOBIOLOGIA E MEDICINA DO SONO 2018 – palestrante no evento “Cronobiologia e Estresse”. Carga horária: 2h (13/06/2018). Coordenadora Profa Maria Paz Hidalgo, Faculdade de Medicina – UFRGS.

4. LIGA DE CRONOBIOLOGIA E MEDICINA DO SONO 2019 – palestrante no evento “Insônia”. Carga horária: 1h (12/06/2019). Coordenadora Profa Maria Paz Hidalgo, Faculdade de Medicina – UFRGS.

5. VIII SEMANA DO CÉREBRO - palestrante. Carga horária: 1h (15/03/2019). Coordenadora Profa Renata Menezes Rosat, Departamento de Fisiologia, ICBS – UFRGS

APÊNDICE 2 – A CONSTRUÇÃO DE MODELO EXPERIMENTAL DE PRIVAÇÃO DE SONO

Outra contribuição metodológica desenvolvida durante o período de doutoramento diz respeito à conceitualização de um aparato de privação de sono para ratos Wisar. Como demonstrado nos estudos clínicos, a privação/restrição de sono é um problema com crescente prevalência e que acarreta mudanças substanciais na fisiologia humana. Ainda há controvérsias relacionadas aos mecanismos principais que conectam a privação de sono com problemas de saúde.

Há diversas limitações metodológicas em estudos com seres humanos. Estudos feitos fora do laboratório não são capazes de isolar uma série de variáveis, incluindo a exposição à luz e as rotinas sociais. Um modelo animal permite um ambiente de luz e dieta controlados, fatores muito difíceis de se isolar em estudos em seres humanos, além de permitir o prolongamento de dias de privação. Infelizmente, os modelos animais atuamente propostos em laboratórios são focados em privação de sono aguda devido ao alto custo e dificuldade de logística de se realizar protocolos crônicos.

Os principais modelos descritos na literatura são a manipulação gentil durante o sono, a atividade forçada em tambores rotatórios e a colocação dos animais em plataformas sobre um aquário de água. Apesar de as metodologias diferirem e de existir uma série de efeitos não específicos dependendo do protocolo estudado, via de regra, os efeitos fisiológicos destes protocolos parecem consistentes ente os estudos(196).

Ainda assim, há efeitos paradoxais descritos, como efeito ansiolítico(197) e perda de peso(198), principalmente relacionados aos modelos de tambores giratórios e de plataformas acima da água(197). Estes efeitos não-congruentes com estudos em seres humanos são um reflexo de que os modelos animais atualmente utilizados ainda têm importantes limitações metodológicas. Além disso, um importante fator complicador dos protocolos é a necessidade de ter um pesquisador sempre presente para monitorar os animais:

- No protocolo de tambores rotatórios, para garantir que os animais não caiam dos tambores;
- No protocolo de plataformas sobre a água, para garantir que os animais possam retornar às plataformas quando caem;
- No protocolo de manipulação gentil, para efetuar a manipulação.

Propusemos um projeto de desenvolvimento (aprovado pela CEUA HCPA, #2018-0504) com o objetivo de construção de um aparato de privação de sono automático, semelhante a outros já descritos(199), em colaboração com a Unidade de Experimentação Animal e com o Serviço de Engenharia Biomédica do HCPA. Neste protocolo, a privação de sono é estimulada através de uma caixa especial que contém uma barra metálica que se desloca lentamente logo acima do chão da caixa com um período determinado (no estudo citado,

a cada 2 minutos), e que força os animais a se esquivarem (e portanto, despertarem) durante o período desejado de atividade forçada.

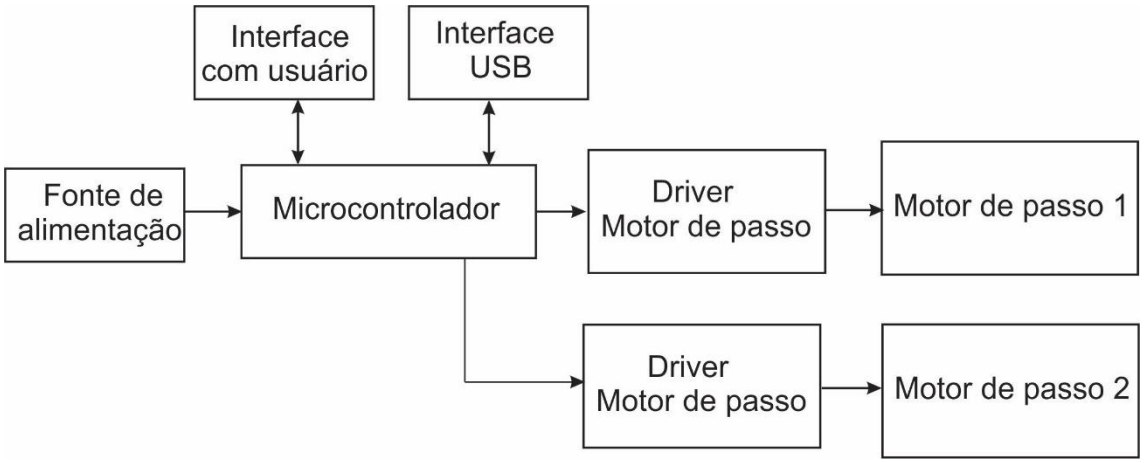

Figura A. Diagrama de blocos do circuito.

O sistema mostrado na figura A consiste em um microcontrolador que executa o controle de 2 motores de passo responsáveis pelo movimento de barras no interior de uma caixa para acomodação de ratos. A programação dos parâmetros de funcionamento será por meio de interface USB sendo possível ajustar, conforme necessidade do protocolo:

- Horário de início e término da movimentação das barras. Para a testagem, o aparato será ligado apenas no momento de observação dos animais.
- Velocidade de movimento das barras. Para a testagem, o aparato será programado em 2 velocidades: 2 movimentos por minuto e 1 movimento por minuto, com tempo de rotação de 30 segundos.
- Acionamento simultâneo ou alternado dos motores.

Dois animais foram testados em intervalos de 2 horas a cada 2 dias durante 2 semanas durante o período de repouso (entre a 6ª e a 8ª hora da fase de luz). As hastes giravam uma vez a cada dois minutos. A eficácia do equipamento foi determinada por 1) necessidade de evasão do animal das hastes, com tempo de descanso não superior a dois minutos e 2) operação ininterrupta do equipamento por 2 horas em 4 horários diferentes, sem quaisquer modificações ou ajustes no equipamento. As sessões de teste foram filmadas diretamente observadas por também filmadas e analisadas posteriormente. Como resultados obtidos, observamos que todos os animais permaneceram ativos durante o período de repouso. O repouso mais longo registrado foi de 2 minutos (o tempo de uma volta inteira da haste). Ao longo das observações, todos os animais ainda eram capazes de se alimentar e beber água, bem como interagir uns com os outros. Nenhum animal foi ferido ou mortalmente preso pelas hastes durante os experimentos.

O trabalho de desenvolvimento e teste deste aparato foi apresentado e premiado com destaque na XXXIV Reunião Anual da FeSBE de 2019 (Anexo 4).

Figura B. Aparato para protocolo de privação de sono em ratos Wistar. O sistema consiste em um microcontrolador que executa o controle de 2 motores de passo responsáveis pela movimentação de duas hastes em forma de L. Essas hastes de metal giram em uma velocidade programável. Suas movimentações descrevem circunferências adjacentes que cobrem quase todo o alojamento dos animais. A comida e a bebida são acopladas pelos lados da caixa, de modo a permitir alimentação e água *ad libitum* mesmo durante a privação de sono. Os animais podem permanecer na caixa mesmo durante o repouso, não sendo necessária a troca de ambiente. Recurso audiovisual disponível em "Tonon AC et al. FeSBE 2019 - Demonstration of sleep deprivation model in Wistar rats", pelo link: https://www.youtube.com/watch?v=o39yWw7Uw5g.

APÊNDICE 3 – OUTROS PROJETOS E COLABORAÇÕES DESENVOLVIDAS

Participação em outros projetos

1. Desenho de um ensaio clínico randomizado sobre o uso de aparelhos de proteção ocular na recuperação clínica de neonatos prematuros (CEP-HCPA 2017-0208)
2. Criação de sensores de ambiente e caracterização da iluminação da Unidade de Internação Psiquiátrica do HCPA (CEP-HCPA 2017-0425)
3. Co-orientação do trabalho de conclusão de curso em Biomedicina (UFRGS) da aluna Débora Barroggi Constantino, que desenvolveu um trabalho 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).
4. Avaliação da variação circadiana do microbioma intestinal de ratos Wistar macho através do método de cultura (CEP-HCPA 2019-0413).

Colaborações internacionais desenvolvidas durante o período de doutoramento

Desde meu período de intercâmbio na University of Toronto (2014-2015), sigo em colaboração com a professora Andrea Charise, professora do Department of Psychiatry e do Graduate Department of English. Juntos, desenvolvemos um projeto de análise da terapia da fala através dos monólogos teatrais que foi premiado com o "Mary Seeman Award for Achievement in the Area of Psychiatry and the Humanities" pela University of Toronto em 2015. Os projetos em andamento podem ser acessados pela pagina do *SCOPE: The Health Humanities Learning Lab* em www.scopelab.ca.

Em 2017 desenvolvi uma colaboração com as professoras uruguaias Ana Silva e Bettina Tassino da Facultad de Ciencias da Universidad de la Republica (UDELAR) em um projeto de avaliação dos ritmos biológicos de pesquisadores uruguaios me missão na Antártida. Esta colaboração possibilitou o intercâmbio de uma de suas alunas, Julieta Castillo, para viagem de trabalho ao Brasil em outubro de 2018 para o aprendizado das técnicas utilizadas aqui, além da análise dos dados coletados.

Em 2018, iniciei a colaboração com um grupo da Nanyang Technological University através do aluno de doutorado Gerard Dunleavy e seu orientador Josip Car. Este grupo conduziu um grande estudo de coorte em trabalhadores de ambientes de subsolo e acima do solo, onde foi coletada acimetria, dosada 6-sulfatoximelatonina urinária, além de uma avaliação de sono e bem-estar. Através deste contato, direcionei as análises da actimeria e auxiliei na discussão das evidências relacionadas ao sono e a bem-estar físico e mental. Fui incluído na autoria de artigos já descritos no apêndice 1.

LISTA DE ANEXOS