Universidade Federal do Rio Grande do Sul
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MALES DO MUNDO CONTEMPORÂNEO: EFEITOS DE PERTURBAÇÕES DE RITMICIDADE CIRCADIANA E DE ESTRESSE CRÔNICO SOBRE SAÚDE E COMPORTAMENTO

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Orientadora: Professora Doutora Maria Paz Loayza Hidalgo
Coorientadora: Professora Doutora Elaine Elisabetsky
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“The truth. It is a beautiful and terrible thing, and should therefore be treated with caution.”

J.K. Rowling em *Harry Potter and the Sorcerer’s Stone*

RESUMO

Objetivo: Identificar, através de revisão sistemática, o efeito de perturbações da ritmicidade circadiana sobre a saúde humana e, através de experimento animal, o efeito sobre a vulnerabilidade a eventos estressantes. **Métodos:** Estudo 1: Foi conduzida uma revisão sistemática da literatura nas bases de dados eletrônicas PubMed, Scopus, Embase e LILACS utilizando os termos "social AND (jet lag OR jetlag)". A busca foi finalizada em 22 de agosto de 2016, resultando em 26 artigos incluídos na revisão. Estudo 2: Camundongos Balb/c foram submetidos a um protocolo de estresse crônico moderado (CMS), sozinho ou precedido de 4 ciclos claro-escuro de período encurtado (10h claro, 10h escuro), e foram comparados a animais submetidos apenas ao encurtamento do ciclo claro-escuro ou a nenhuma intervenção. Em um primeiro experimento, os animais dos grupos estressados foram submetidos a 3 semanas de CMS e foram avaliados parâmetros dos ritmos de atividade-repouso e temperatura central e o ganho de peso corporal. Em um segundo experimento, o período de CMS foi reduzido para 2 semanas e foram analisados parâmetros metabólicos séricos, teste de preferência por solução de sacarose (para aferir comportamento tipo-depressivo) e teste claro-escuro (para aferir comportamento tipo-ansioso). **Resultados:** Estudo 1: Os desfechos de saúde e comportamento associados ao jetlag social são diversos (epilepsia, sintomas psiquiátricos menores, agressão e problemas de conduta, transtornos de humor, prejuízo cognitivo, uso de substâncias, risco cardiometabólico e perfil endócrino adverso), mas há grande variabilidade de metodologias e populações e grande risco de viés dos estudos analisados. Estudo 2: A exposição ao CMS precedida de ciclos claro-escuro encurtados resultou em aumento na amplitude do ritmo de temperatura, mantido mesmo após o término do protocolo de estresse, e redução do peso corporal no período do CMS, havendo uma clara associação entre estes desfechos. Não foram observadas alterações significativas no comportamento, possivelmente devido a problemas metodológicos, nem nos parâmetros metabólicos avaliados. **Conclusões:** Este trabalho contribui para o conhecimento sobre o papel da cronorruptura na vulnerabilidade ao desenvolvimento de patologias através de revisão das evidências associadas a um modelo de cronorruptura em humanos (jetlag social) e de demonstração de evidências em um modelo animal (encurtamento do ciclo claro-escuro). As evidências relacionadas ao jetlag social devem ser avaliadas com cautela devido à heterogeneidade metodológica e alto risco de viés, sendo necessários estudos longitudinais e com metodologia padronizada para estabelecer associações mais confiáveis. Em animais, a vulnerabilidade ao estresse parece ser aumentada pelo encurtamento do ciclo claro-escuro no

que se refere a ritmos circadianos e metabolismo, mas resta determinar o efeito sobre comportamentos tipo-depressivo e tipo-ansioso. **Apoio:** FIPE/HCPA, CNPq e CAPES.

Palavras-chave: Cronobiologia; Metabolismo; Estresse Fisiológico; Vulnerabilidade.

ABSTRACT

Objective: Identify through a systematic review the effect of circadian disturbances on human health and, through animal experimentation, the effect on vulnerability to stress**.** **Methods:** Study 1: A systematic review of the literature was conducted on PubMed, Scopus, Embase and LILACS electronic databases using the terms "social AND (jet lag OR jetlag)". The search was finalized on August 22, 2016, resulting in 26 research articles included in the review. Study 2: Balb/c mice were exposed to a chronic mild stress (CMS) protocol alone or preceded by 4 shortened light-dark cycles (10h light, 10h dark) and compared to animals exposed only to the shortened light-dark cycles or to no intervention. In one experiment, animals of stressed groups were exposed to 3 weeks of CMS for evaluation of rest-activity and core body temperature rhythms and body weight gain. In a second experiment, CMS was shortened to 2 weeks and serum metabolic parameters, sucrose preference test (to assess depressive-like behavior) and black and white test (to assess anxiety-like behavior) were evaluated. **Results:** Study 1: The health and behavioral outcomes associated to social jetlag are diverse (epilepsy, minor psychiatric symptoms, aggression and conduct problems, mood disorders, cognitive impairment, substance use, cardiometabolic risk and adverse endocrine profile), but there is great variation of methodologies and populations as well as high risk of bias in analyzed studies. Study 2: Exposure to CMS preceded by shortened light-dark cycles resulted in increased amplitude of core body temperature rhythm, sustained even after the end of CMS, and reduced body weight during CMS, with a clear association between these two outcomes. No significant alterations were observed either in behavior, likely due to methodological issues, or metabolic parameters assessed. **Conclusions:** This work contributes to the knowledge on the role of chronodisruption on the vulnerability to development of pathologies through a review of evidences associated with a model of chronodisruption in humans (social jetlag) and demonstration of evidences from an animal model of chonodisruption (shortened light-dark cycles). The evidence regarding social jetlag must be analyzed with caution due to methodological heterogeneity and high risk of bias of articles reviewed and longitudinal studies with standardized methodology are needed to establish reliable associations. In animals, it seems that vulnerability to stress is increased by the light-dark cycle shortening in what refers to circadian rhythms and metabolism, but the effect on depressive-like and anxiety-like behaviors remains to be determined. **Financial support:** FIPE/HCPA, CNPq and CAPES.

Keywords: Chronobiology; Metabolism; Physiological Stress; Vulnerability.

LISTA DE ILUSTRAÇÕES DA REVISÃO DA LITERATURA

LISTA DE ABREVIATURAS EM PORTUGUÊS DA REVISÃO DA LITERATURA

subNPV - Núcleo sub-paraventricular

HDM - Hipotálamo dorsomedial

HPA - Hipotálamo-pituitária-adrenal

lx - lux

K - Kelvin

NPV - Núcleo paraventricular

NSQ - Núcleo supraquiasmático

AVP - Arginina vasopressina

GLU - Glutamato

LISTA DE ABREVIATURAS EM INGLÊS DA REVISÃO DA LITERATURA

ipRGCs - Intrinsically photosensitive retinal ganglion cells

PACAP - Pituitary adenylate cyclase-activating polypeptide

CLOCK - Circadian Locomotor Ouptup Cycles Kaput

BMAL - Brain and muscle ARNT-like protein

E-box - Enhancer box

PER - Period protein

CRY - Cryptochrome protein

FBXL3 - F-box/LRR-repeat protein

ROR - RAR-orphan receptor protein

HIOMT - Hydroxyindol-O-metiltransferase

AANAT - Aralkylamine N-acetyltransferase

SCN - Suprachiasmatic nucleus

PVN - Paraventricular nucleus

SubPVN - Subparaventricular nucleus

DMH - Dorsomedial hypothalamus

CRH - Corticotropin-releasing hormone

GABA - Gamma-aminobutyric acid

GREs - Glucocorticoids response elements

ACTH - Adrenocorticotropic hormone

AMPK1 - AMP-activated protein kinase 1

GSK3β - Glycogen synthase kinase 3 beta

CK1δ/ε - Casein kinase 1δ/ε

βTrCP - Beta-transducin repeats-containing protein

SUMÁRIO

1 INTRODUÇÃO

O desenvolvimento científico e tecnológico acelerado que vivenciamos atualmente representa benefícios claros à sociedade, mas também riscos potenciais que não são percebidos ou recebem pouca atenção. O advento da luz elétrica, seguido da produção em massa de lâmpadas e aparelhos eletrônicos emissores de luz, ocasionou mudanças radicais no estilo de vida dos seres humanos. Construímos estruturas com cada vez menos acessos para luz solar, iluminadas quase estritamente por luz elétrica, nas quais passamos a maior parte das 24 horas do dia (1). Possuindo autonomia para determinar nosso fotoperíodo (o período de exposição à luz) e buscando atender às necessidades crescentes de produtividade, nos submetemos a jornadas de trabalho cada vez mais longas, reservando apenas poucas horas de escuridão para o descanso (2), e a diferentes horários de descanso e atividade ao longo dos dias da semana (3). Em condições de luz natural, nosso organismo é sincronizado ao período de 24 horas do dia pelo sistema circadiano, estabelecendo os chamados ritmos circadianos (de duração de cerca de um dia) de diversas funções fisiológicas (4). Sob rotinas de trabalho que impõem horários sociais em desalinho com nosso ritmo endógeno, a sincronização do relógio biológico e dos ritmos circadianos é afetada e pode ocasionar diversas doenças como, por exemplo, transtornos psiquiátricos e síndrome metabólica/obesidade (5).

Porém, a iluminação não é o único fator capaz de afetar o funcionamento do sistema circadiano e potencialmente desencadear patologias. Dentre os agentes potencialmente prejudiciais aos ritmos biológicos e com associação a transtornos de humor, voltamos nosso interesse ao estresse, presente cada vez mais no nosso dia-a-dia, e definido como qualquer situação capaz de perturbar a homeostase fisiológica ou psicológica (6). O estilo de vida atual da sociedade, com demandas de produtividade que não respeitam as necessidades fisiológicas dos indivíduos sendo impostas continuamente e acompanhadas de insegurança perante a violência crescente, impõe uma condição de estresse crônico muitas vezes inevitável sobre os indivíduos (7). Estudos já demonstraram que exposição ao estresse, agudo ou crônico, leva ao desenvolvimento de transtorno depressivo, com alterações no apetite e no peso, anedonia, distúrbios de sono e tendências suicidas (8). Porém, nem todos os indivíduos expostos ao estresse desenvolvem transtornos psiquiátricos, sendo resilientes a estes efeitos do estresse (9). Assim, tem sido sugerida a existência de um mecanismo de modulação, através do qual fatores atuariam tornando indivíduos mais resistentes ou mais vulneráveis ao estresse. Neste

sentido, vem sendo atribuído um papel importante ao sistema circadiano na modulação da resposta ao estresse (10), pela forte relação existente entre estes dois sistemas.

A exposição à iluminação, predominantemente artificial, e as rotinas inconstantes com horários de dormir e acordar variáveis ao longo dos dias da semana é algo difundido pelo mundo, ao qual estão expostas todas as classes sociais e faixas etárias. Além disso, vivemos em um momento na história em que a violência e a cobrança são constantes, mantendo-nos em condição de estresse crônico com episódios de estresse agudo imprevisíveis. No contexto de uma virtual inevitabilidade de passar por situações de estresse, torna-se relevante discutir o quanto as condições de luz artificial a que submetemos nosso sistema circadiano desde os primeiros dias de vida podem estar tornando-nos vulneráveis aos efeitos destes eventos estressantes e mais suscetíveis ao desenvolvimento de doenças e transtornos psiquiátricos. Deste modo, destaca-se a importância de entender como esses fatores, de forma independente e associada, afetam a saúde e o comportamento, de modo a auxiliar na busca por alternativas que minimizem a perturbação da sincronização do sistema circadiano em nosso dia-a-dia.

2 REVISÃO DA LITERATURA

2.1. SISTEMA CIRCADIANO E A REGULAÇÃO DOS RITMOS BIOLÓGICOS

A rotação do planeta Terra em seu próprio eixo, de período de aproximadamente 24 horas, é um dos principais fenômenos responsáveis pela alteração cíclica das condições do ambiente externo. Desde o princípio da vida na Terra, os organismos que nela se desenvolveram passaram por pressões evolutivas que favoreceram o desenvolvimento de mecanismos de organização temporal das funções fisiológicas e sua sincronização ao ambiente externo (11). Esta sincronia representou uma vantagem evolutiva às espécies por proporcionar uma noção temporal capaz de preparar o organismo para desempenhar funções adequadas a cada período do dia (p. ex., para animais diurnos, dormir à noite e buscar alimento durante o dia), otimizando o consumo energético e aumentando as chances de sobrevivência e manutenção da espécie (12). Dessa forma, os seres humanos e a maioria das espécies de seres vivos apresentam um sistema chamado circadiano (do latim: *circa* = cerca de; *diem* = dia), responsável pela ritmicidade das funções do organismo e sua sincronização ao ritmo de aproximadamente 24 horas do dia terrestre (13). As variações entre períodos de iluminação pela luz do Sol (dia) e períodos de escuridão (noite) são o principal sinalizador de alterações ambientais previsíveis (p.ex. alterações de temperatura). Acredita-se que por isso a luz tenha sido mantida como o principal sinal sincronizador da fisiologia e comportamento (14).

As funções fisiológicas sob regulação do sistema circadiano que apresentam variações ao longo do período de um dia são os chamados ritmos circadianos (15). Alternativamente, algumas funções podem apresentar períodos de duração maior ou menor do que um dia, sendo chamadas de ritmos infradianos (p. ex., ciclo menstrual) e ultradianos (p.ex., batimentos cardíacos), respectivamente (16). Diversos comportamentos e funções essenciais à vida humana apresentam ritmicidade circadiana, com variações facilmente identificáveis: o ciclo sono-vigília e o ritmo de atividade-repouso, por exemplo, variam de alto nível de vigília e atividade durante o dia e períodos de sono e repouso durante a noite em seres humanos (e demais animais diurnos), influenciando o equilíbrio entre consumo e gasto energético (17,18), entre reparo e desgaste tecidual (19) e consolidação de memória (20); o ritmo de temperatura corporal, além do ritmo de atividade-repouso, é um dos mais estudados, atingindo valores mais baixos no início da manhã e níveis mais altos ao final da tarde, interferindo com o metabolismo e o controle de reservas lipídicas (21); vários hormônios também apresentam

secreção com ritmicidade circadiana, como o cortisol, com pico de liberação no início do período de atividade, e a melatonina, produzida e secretada no período de escuridão (22).

A sincronização destes ritmos ao ambiente externo pode ser regulada por diversos fatores indicadores de temporalidade, de maior ou menor influência sobre o sistema circadiano. Um destes fatores é o ritmo social, representado pelas rotinas diárias estabelecidas pelas relações sociais, tais como horários de início de rotinas de trabalho, por exemplo (23). Porém, tem sido discutido que, em realidade, a influência das rotinas e do ritmo social sobre a sincronização circadiana se dá através de sua influência sobre a exposição a outros fatores sincronizadores mais fortes, tais como pistas alimentares (p.ex., horários de refeições) e luminosas (p.ex., início da rotina de trabalho influenciando exposição à luz artificial e/ou solar) (24). A sincronização por pistas alimentares se dá principalmente pela regulação de ritmicidade dos chamados osciladores periféricos, presentes em tecidos periféricos como fígado, pâncreas, músculo esquelético, intestino e tecido adiposo (25). As pistas alimentares, supostamente através de sinalização por metabólitos e hormônios desencadeada pelos alimentos consumidos, promovem a sincronização dos tecidos periféricos e de suas funções sem efeitos significativos conhecidos sobre a sincronização do oscilador central do sistema circadiano, o núcleo supraquiasmático (NSQ) (26). Por outro lado, o apetite e consequentemente os horários de alimentação são regulados pelo NSQ e contribuem, portanto, para a transmissão da sincronização central para os tecidos periféricos (27) **(Figura 1)**.

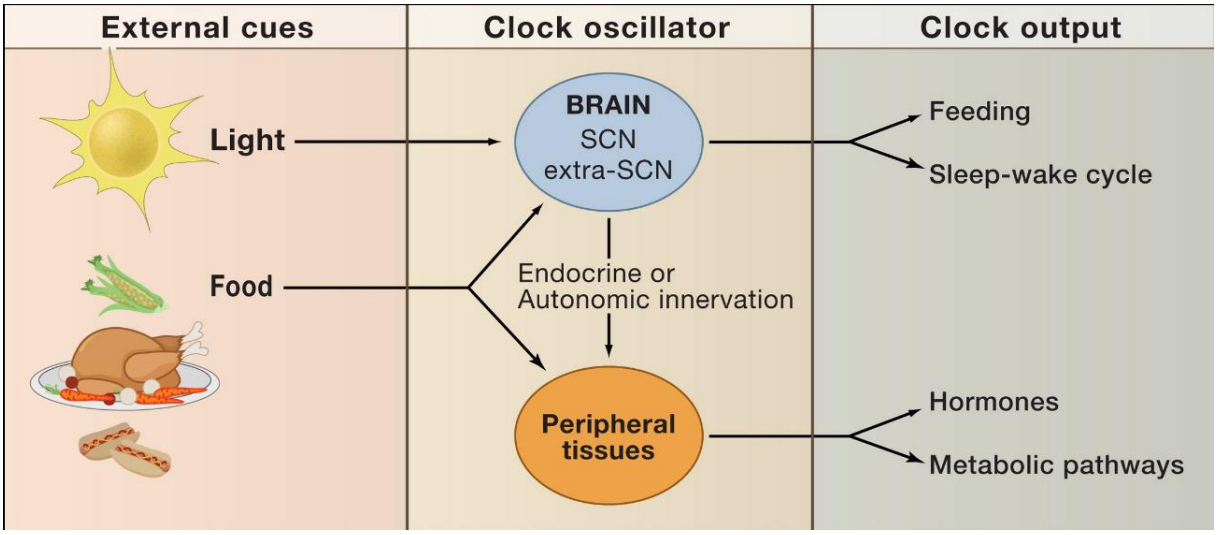

Figura 1. Pistas ambientais e regulação circadiana das funções fisiológicas. As principais pistas externas responsáveis pela sincronização dos osciladores centrais e tecidos periféricos são a luz e a alimentação, respectivamente. A sincronização do núcleo supraquiasmático (*suprachiasmatic nucleus, SCN*) e outras estruturas encefálicas pela luz regula a ritmicidade do ciclo sono-vigília, da inervação endócrina e autônoma e dos horários de alimentação, influenciando a sincronização dos osciladores periféricos. Porém, a alimentação é capaz de sincronizar os tecidos periféricos de forma independente da sinalização luminosa, influenciando a produção hormonal e o metabolismo. Adaptada de Green, Takahashi e Bass, 2008 (28).

O principal fator responsável pela sincronização do sistema circadiano ou *zeitgeber* (do alemão: *zeit* = tempo; *geber* = doador), atuando sobre os osciladores centrais do sistema circadiano, é a luz (29). A adaptação dos seres humanos ao longo da evolução à sinalização temporal determinada pela luz do sol resultou no atual sistema de transdução de sinais fóticos ao NSQ (30). A retina humana apresenta, além dos fotorreceptores responsáveis pela visão (cones e bastonetes), as chamadas células ganglionares intrinsecamente fotossensíveis (*intrinsically photosensitive retinal ganglion cells*, ipRGCs). Essas células apresentam o fotopigmento melanopsina e, ao contrário dos cones e bastonetes, sofrem despolarização quando há detecção de luz e aumentam sua taxa de disparo (31). A sinalização da informação fótica detectada pelas ipRGCs segue por seus axônios via nervo óptico, pelo trato retinohipotalâmico, diretamente ao NSQ onde estabelecem sinapses dependentes de glutamato e polipeptídeo hipofisário ativador de adenilato ciclase (*Pituitary adenylate cyclase-activating polypeptide*, PACAP) (32). As células do NSQ, assim como dos osciladores periféricos, apresentam oscilações endógenas na expressão gênica dos chamados *clock genes* (genes do relógio) de cerca de 24 horas (33). Porém, a sinalização aferente das ipRGCs é responsável

por sincronizar esta oscilação ao ritmo de 24 horas do ambiente, de modo a manter o organismo em fase com o que acontece ao seu redor (34).

A sincronização da ritmicidade circadiana do NSQ a nível celular se dá através de *feedback loops* (alças de retroalimentação) de transcrição e tradução dos *clock genes*, que são reiniciados ao início do período de luz pela estimulação proveniente das ipRGCs (35) **(Figura 2)**. Em resumo, em mamíferos, os ativadores de transcrição CLOCK (*Circadian Locomotor Ouptup Cycles Kaput)* e BMAL1 (*Brain and Muscle ARNT-Like protein-1*) heterodimerizam e se ligam a regiões E-box (*enhancer box*) do DNA, elementos responsivos presentes na região promotora dos genes *Per1* (*Period 1*), *Per2*, *Cry1* (*Cryptochome 1*) e *Cry2* (36). A ligação do heterodímero CLOCK:BMAL1 promove a transcrição destes genes, resultando na tradução das proteínas PER1/2 e CRY1/2. As proteínas PER1/2 e CRY1/2, então, dimerizam e translocam para o núcleo, onde suprimem a atividade promotora de transcrição realizada por CLOCK:BMAL1, consequentemente regulando e reduzindo sua transcrição (33). Outros elementos são responsáveis pelo controle da degradação das proteínas PER1/2 e CRY1/2, como a CK1δε (*casein kinase 1δ/ε*), que promove a fosforilação, ubiquitinação de PER1/2 por βTrCP (*beta-transducin repeats-containing protein*) e degradação pelo proteossomo 26S; e a FBXL3 (*F-box/LRR-repeatprotein 3*), que promove a ubiquitinação das proteínas CRY1/2 após sua fosforilação por AMPK1 (*AMP-activated protein kinase 1*) ou GSK3β (*glycogen synthase kinase 3 beta*) (35). Além de PER1/2 e CRY1/2, CLOCK:BMAL1 também promove a transcrição dos genes precursores das proteínas REV-ERB-α/β e ROR-α/β/γ (*RAR-orphan receptor α/β/γ*). As proteínas REV-ERB atuam no núcleo suprimindo a transcrição do gene *Bmal1*, enquanto as proteínas ROR têm ação contrária, promovendo a transcrição de *Bmal1* (27). Esse sistema de *feedback loops* reiniciado pela sinalização fótica mantém um ritmo diário de expressão dos *clock genes* e a sincronização do NSQ ao ritmo de 24 horas do ambiente.

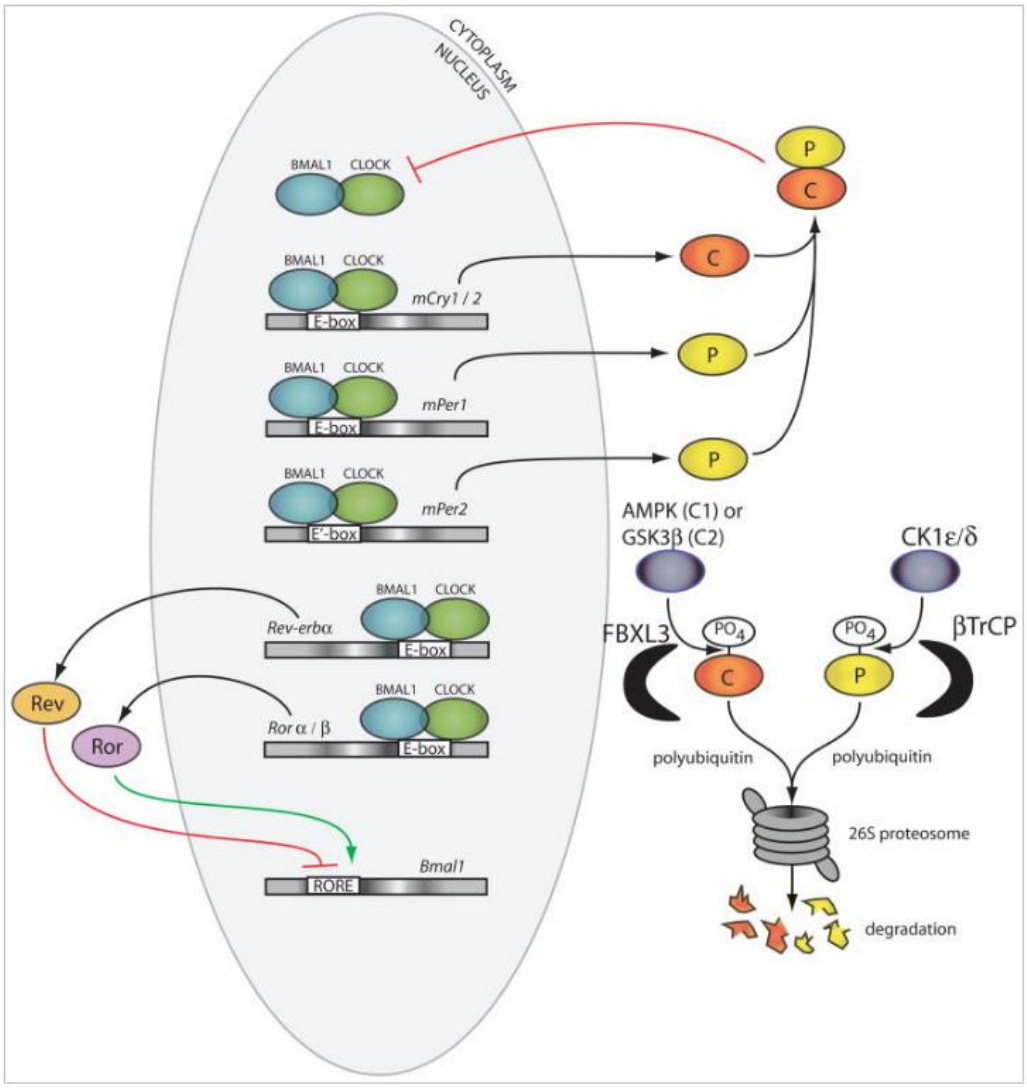

Figura 2. Regulação da ritmicidade circadiana a nível molecular, pelos *feedback loops* de transcrição e tradução de *clock genes* e pela degradação das proteínas PER e CRY. PER: *Period*. CRY: *Cryptochrome.* Adaptada de Buhr e Takahashi, 2013 (35).

Uma vez sincronizado pela luz, o NSQ, marca-passo central do sistema circadiano, sincroniza outras regiões centrais e periféricas (37) através de sinalização autônoma, humoral e variação da temperatura corporal (38). Um importante sinalizador responsável pela transmissão da informação fótica recebida pelo NSQ ao organismo é o hormônio melatonina, sintetizado pela glândula pineal (39). O NSQ envia projeções para o núcleo paraventricular do hipotálamo que, por sua vez, transmite as informações recebidas através de vias que seguem até a medula espinhal e, retornando através do gânglio cervical superior, chegam à glândula pineal (34,40). Na ausência de luz, a sinalização decorrente da inatividade do NSQ resulta em sinalização noradrenérgica nos pinealócitos (41). A ação da noradrenalina promove a ativação

da enzima *aralkylamine N-acetyltransferase* (AANAT), responsável pela conversão de serotonina em n-acetil-serotonina que é então convertida pela enzima *hydroxyindol-O-metiltransferase* (HIOMT) em melatonina (42,43). A melatonina produzida no escuro é então liberada na corrente sanguínea em sua totalidade, enviando o sinal temporal do período noturno de forma sistêmica pelo organismo. Como, na presença de luz, a produção de melatonina é imediatamente bloqueada e toda a melatonina produzida é metabolizada no fígado e excretada na urina, a regulação da síntese pineal de melatonina pelo NSQ permite uma sinalização difusa e de duração restrita do ritmo ambiental (43) **(Figura 3)**.

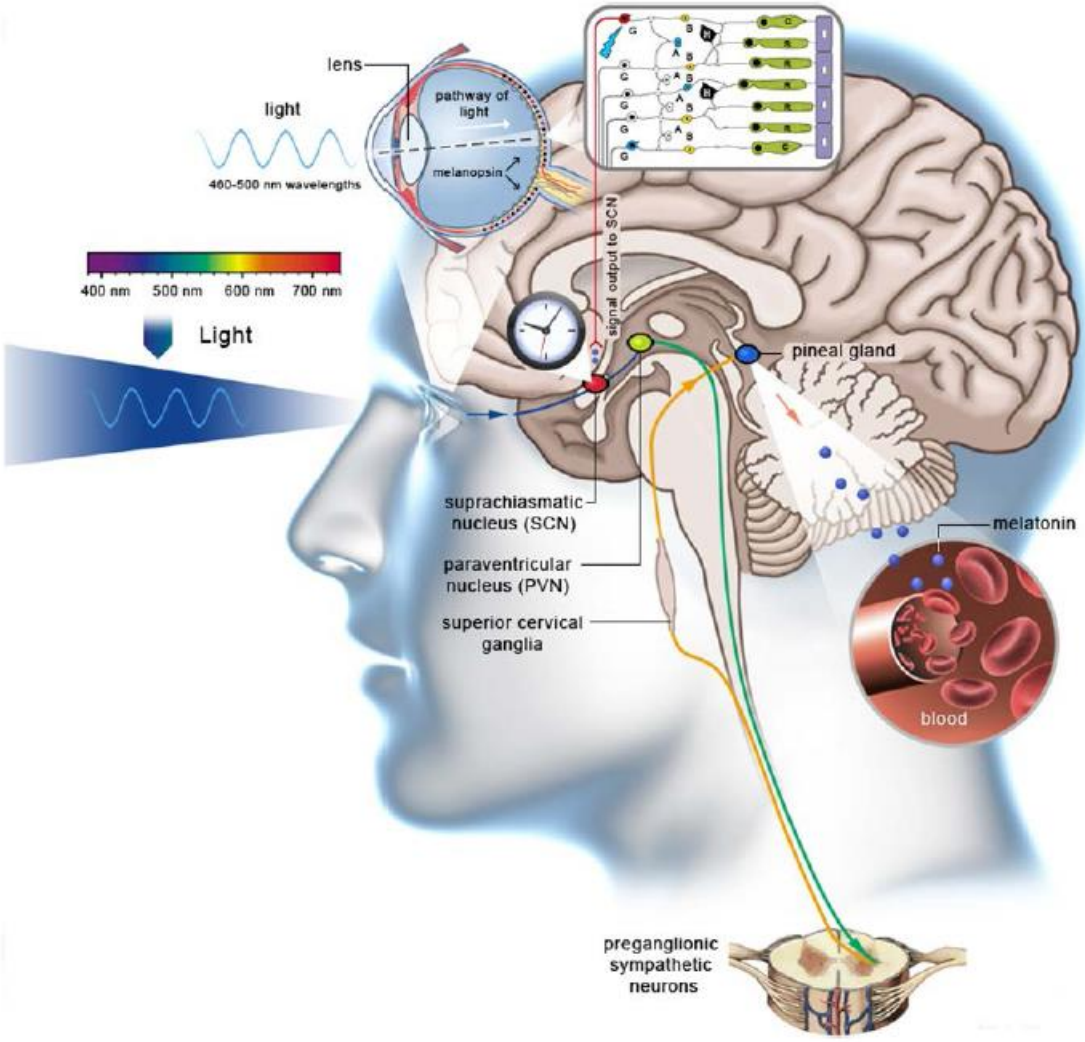

Figura 3. Mecanismo de sincronização do sistema circadiano pela luz. *Suprachiasmatic nucleus*: núcleo supraquiasmático. *Paraventricular nucleus*: núcleo paraventricular. *Superior cervival ganglia*: gânglio cervical superior. *Preganglionic sympathetic neurons*: neurônios preganglionares do sistema simpático. *Pineal gland:* glândula pineal. *Melatonin*: melatonina. Adaptada de < https://iristech.co/color-temperature-and-blue-light/> (44).

2.2. SOCIEDADE CONTEMPORÂNEA: ILUMINAÇÃO E CRONORRUPTURA

O sistema circadiano nos mamíferos se desenvolveu de modo a criar uma organização temporal nos organismos e sincronizá-los aos ciclos diários de luz solar (11). As diferenças individuais que surgem deste processo de sincronização estão refletidas não apenas em diferentes horários de dormir e acordar, mas em praticamente todas as variáveis fisiológicas sob regulação do sistema circadiano.

Nossos ancestrais organizavam suas rotinas predominantemente utilizando o nascer e o pôr do sol como referência. A domesticação do fogo, não apenas uma fonte de calor, mas também uma fonte de luz, permitiu que nossos ancestrais estendessem a duração de seus dias um pouco além do início da noite. Porém, desde o desenvolvimento da luz elétrica comercializável em 1880 e do processo de industrialização difundido pelo globo, o ser humano mudou drasticamente seu regime de exposição à luz, sendo o padrão atual completamente distinto do vivenciado por nossos antepassados (45,46). A construção de ambientes fechados, com poucas janelas e excessivamente iluminados por lâmpadas elétricas ocasionou o fenômeno atual chamado *light pollution* (poluição luminosa), no qual a iluminação elétrica excedente é tão intensa que pode ser vista de naves em órbita no espaço como "ilhas de luz" à noite nas regiões mais desenvolvidas do planeta (47). Também vivemos uma rotina predominantemente determinada pelo trabalho e pela necessidade de produtividade cada vez mais restrita a ambientes com pouco ou nenhum acesso à luz natural e nos deslocamos em veículos fechados, passando apenas poucos minutos durante o dia expostos, de fato, à luz do sol (48). Durante a noite, muitos indivíduos exercem profissões como trabalhadores noturnos, permanecendo acordados e expostos à iluminação artificial quando deveriam estar expostos à escuridão, invertendo seu ciclo de sono-vigília em relação ao que seria ditado pelo ritmo natural do ambiente (49).

Como em termos de evolução estas alterações na relação do homem com a luz são muito recentes, nosso organismo (e nosso sistema circadiano) ainda não está adaptado (46). A luz solar apresenta uma ampla distribuição de poder espectral, com temperaturas de cor que variam de 5000 a 10000 Kelvin (K) e intensidade de luz que chega a 100000 lux (lx). A iluminação artificial típica, porém, apresenta menor distribuição de poder espectral, sem variação de temperatura de cor ao longo do dia, e intensidade de luz muito menor, de 200-500 lx (50). A combinação de luz insuficiente durante o dia e excesso de exposição durante a noite enfraquece a amplitude da luz, o *zeitgeber* mais importante para a sincronização de ritmos biológicos (51). Essa condição faz com que as diferenças individuais nos horários de dormir e

acordar e de outros ritmos se tornem ainda mais pronunciadas devido à ausência de pistas luminosas fortes para sincronizar nosso relógio interno ao tempo externo (52) **(Figura 4)**.

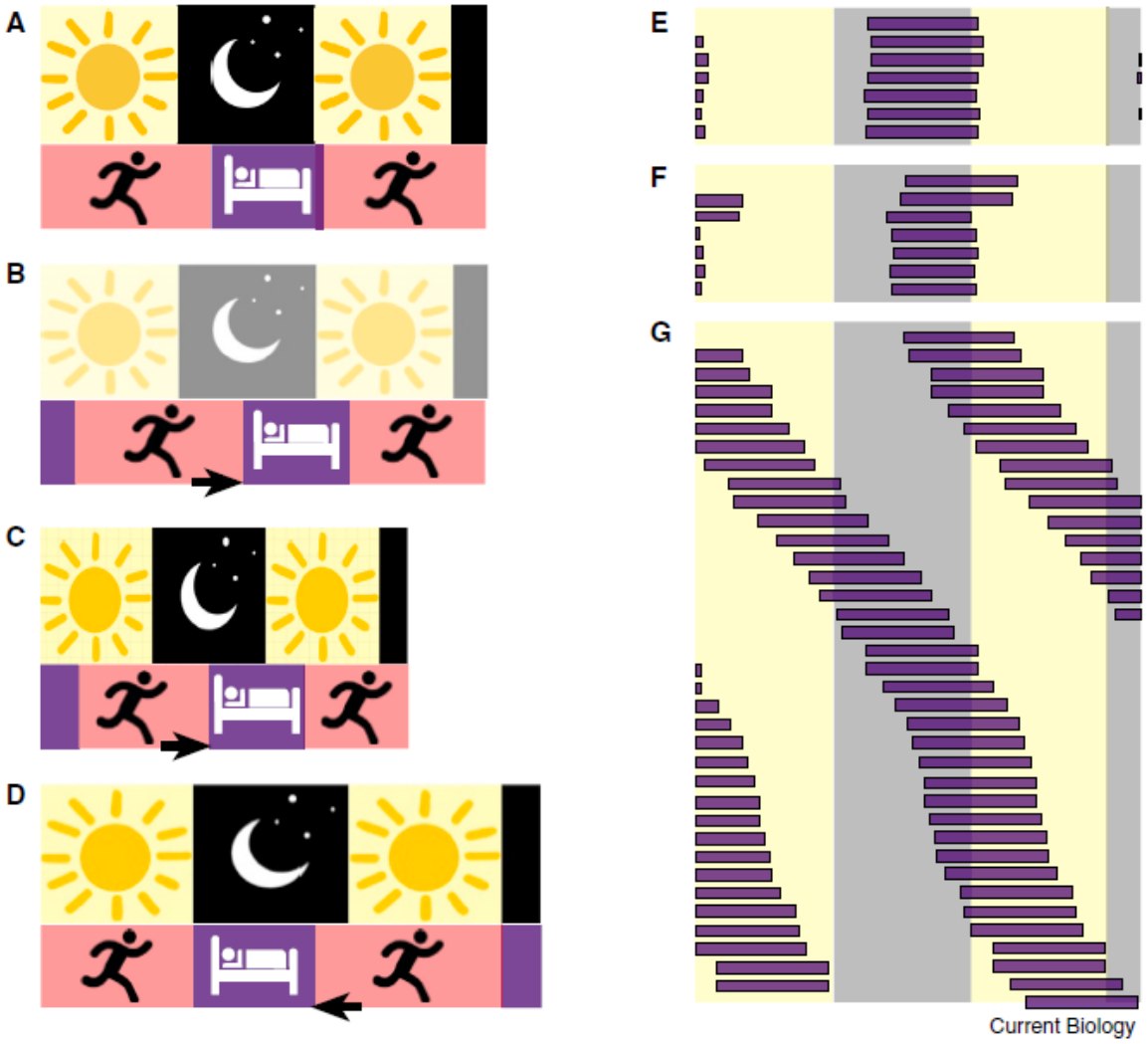

Figura 4. Relação entre a variação diária de iluminação e o ciclo sono-vigília humano. Em A e E, o padrão de sono em condições de luz natural, com exposição à luz solar durante o dia e escuridão durante a noite. Em B e F, o enfraquecimento das pistas luminosas pela permanência em ambientes fechados e iluminação artificial à noite, exacerbando a diferença entre os horários de dormir e acordar nos dias de trabalho e dias livres em indivíduos vespertinos. Em C, D e G, os extremos de sincronização do sistema circadiano a ciclos de claro-escuro mais curtos (C) e mais longos (D) e o consequente atraso ou avanço de fase respectivo (G). Adaptada de Roenneberg e Merrow, 2016 (52).

A alteração de fase entre os ritmos internos - como sono-vigília, atividade-repouso e temperatura corporal - e o ritmo externo, do ambiente, é uma condição chamada de cronorruptura (53). Outras formas de desalinhamento da ritmicidade circadiana podem ocorrer

quando osciladores centrais e periféricos não estão sincronizados, ou quando outros ritmos, como o de alimentação, estão em desacordo com o ritmo ambiental (5). A estrutura social atual exige que as atividades escolares e de trabalho se iniciem nas primeiras horas da manhã, resultando em duração de sono e horários de dormir significativamente diferentes entre os dias de trabalho (submetidos às convenções sociais) e os dias livres (oportunidades de respeitar o ritmo biológico interno) em grande parte da população (52,54) **(Figura 4)**. A necessidade do uso de despertadores nos dias de semana, identificada em cerca de 80% da população de uma das maiores bases de dados sobre características do sono, evidencia o quanto nosso sono durante os dias de trabalho não corresponde a um sono natural, regulado pelas necessidades fisiológicas de cada indivíduo (14,55) Esta discrepância ente o relógio biológico interno (individual do organismo, manifestado nos dias livres) e o externo (ditado pelo ritmo social, manifestado nos dias de trabalho) é conhecida como jetlag social, por ser resultante de condições impostas pelo ambiente social e por se assemelhar aos efeitos causados por viagens transmeridionais (jetlag) (56). Trabalhadores noturnos são uma população particularmente exposta à cronorruptura devido à inversão dos horários de dormir e acordar e à exposição à iluminação artificial durante todo o período noturno, frequentemente associados ao desalinhamento dos horários de alimentação em relação ao ciclo sono-vigília devido às refeições noturnas no turno de trabalho (57). Porém, mesmo em indivíduos que trabalham em turnos diurnos, a perturbação da ritmicidade circadiana causada pelas diferenças entre os horários de sono dos dias livres para os dias de trabalho (evidenciada pela medida do jetlag social) pode ter grande impacto sobre a saúde e o comportamento a longo prazo, uma vez que se trata de uma condição de cronorruptura crônica (58).

Estas perturbações da ritmicidade circadiana associada aos padrões atuais de exposição à luz artificial implicam em efeitos nocivos sobre nossa fisiologia, levando ao desenvolvimento de patologias. Já foi demonstrada associação entre maior exposição à luz durante a noite, condição a qual trabalhadores de turno estão submetidos, e risco elevado para desenvolvimento de obesidade e síndrome metabólica (59). Em experimentos com seres humanos em laboratório, restrição de sono (oportunidades de no máximo 5, 6h de sono a cada 24h) associada a protocolo de ruptura circadiana (dias prolongados de 28h) por 3 semanas resultou em níveis de glicose pós-prandiais elevados, sendo revertidos após 9 dias de recuperação (60). A relação entre ruptura circadiana e alterações no metabolismo também foi evidenciada em estudos com modelos animais, nos quais camundongos com mutação e perda de função do gene *Clock* apresentam hiperfagia, hiperlipidemia, hiperleptinemia,

hiperglicemia e hipoinsulinemia, características de síndrome metabólica (61). Estudos transversais e longitudinais também demonstraram associação entre menor duração de sono e níveis elevados de pressão sanguínea e hipertensão (62). Além das evidências indicando uma relação entre alterações de ritmicidade circadiana e de sono e risco metabólico e cardiovascular (62–64), evidências sugerem uma associação com maior risco de câncer, principalmente de mama, próstata, cólon e endométrio, em trabalhadores de turno noturno (65). Ainda não foram identificados quais fatores associados ao trabalho noturno – cronorruptura, exposição à luz à noite, privação de sono, estilo de vida inadequado, menores níveis de vitamina D pela menor exposição à luz solar – estão relacionados ao maior risco de câncer (66). Porém, foi demonstrado que camundongos com predisposição ao desenvolvimento de câncer de mama apresentam menor supressão tumoral e maior ganho de peso quando submetidos a um protocolo de perturbação da ritmicidade circadiana (inversão semanal do ciclo claro-escuro), sugerindo que o trabalho de turno está relacionado ao desenvolvimento de câncer e obesidade através de mecanismos relacionados a dessincronização interna dos ritmos biológicos e à privação de sono (67).

Além de efeitos sobre câncer e metabolismo, alterações da ritmicidade circadiana têm sido associadas ao desenvolvimento de transtornos psiquiátricos e alterações de comportamento (23,68,69). A depressão sazonal, por exemplo, é uma condição mais frequente em países nortenhos mais distantes da linha do Equador, que sofrem variações significativas na duração do dia e da noite ao longo das estações devido à inclinação da Terra. Nestes países, durante o inverno, a curta duração dos dias está relacionada ao surgimento de transtorno depressivo (70). Em ratos, exposição à luz constante por 8 semanas levou à cronorruptura dos ritmos de atividade, melatonina e corticosterona (glicocorticoide de roedores, equivalente ao cortisol em humanos) e ao desenvolvimento de comportamentos tipo-depressivo e tipo-ansioso (71). Em camundongos, demonstrou-se que o silenciamento da expressão do gene *Bmal1* no NSQ resulta em atenuação do ritmo molecular dos osciladores centrais e comportamento de desamparo aprendido, tipo-ansioso e tipo-depressivo (72). Apesar de evidências sugerirem causalidade, a relação entre perturbações da sincronização circadiana e o desenvolvimento de sintomas e transtornos psiquiátricos ainda não foi esclarecida, podendo haver causalidade direta, efeito da cronorruptura sobre a severidade e progressão dos transtornos, ou um aumento da suscetibilidade do organismo ao desenvolvimento de patologias em decorrência da ruptura circadiana (68). Por outro lado, a associação entre o sistema circadiano e transtornos como depressão, ansiedade, transtorno de estresse pós-traumático, transtorno

bipolar e esquizofrenia é inegável, tendo em vista a frequente presença de alterações de ritmicidade circadiana nestas patologias em elementos como o ciclo sono-vigília, o ritmo de temperatura corporal e os ritmos de secreção de melatonina e cortisol (5,69).

2.3. SOCIEDADE CONTEMPORÂNEA: ESTRESSE AGUDO E CRÔNICO

As alterações de iluminação e consequente ruptura de ritmo não são o único fator do estilo de vida atual capaz de desencadear patologias. O desenvolvimento de grandes centros urbanos, paralelamente ao aumento radical no uso de iluminação elétrica, promoveu grandes mudanças na estrutura da sociedade. As preocupações que assolavam a humanidade há um século diziam respeito principalmente a questões como a fome, a peste e a guerra. Atualmente, ainda que haja alguma parcela da população submetida à fome, à peste e à guerra, são outras grandes questões que compõem a agenda de preocupações da humanidade (73). Com o surgimento da internet e o desenvolvimento de inteligências artificiais e equipamentos eletrônicos, dispomos atualmente de acesso à informação e a ferramentas avançadas que eram restritos ao universo da ficção científica para as gerações passadas. Contudo, no lugar de uma melhor qualidade de vida generalizada, o que presenciamos atualmente são índices cada vez mais altos de depressão e suicídio (74) **(Figura 5)**. Com o poder que adquirimos, as cobranças por produtividade se tornaram igualmente grandiosas, exigindo menor desempenho físico mas demandando um alto desempenho psicológico por longas horas, muitas vezes se estendendo além do turno de trabalho (75). Além das demandas no ambiente trabalhista, as convenções sociais atuais demandam esforços constantes daqueles que buscam ser considerados indivíduos bem-sucedidos: preocupações com relação a padrões específicos de beleza e comportamento, à aquisição de bens materiais e à criação adequada dos filhos, por exemplo (76,77). Somando-se a isso, as desigualdades sociais e as políticas muitas vezes ineficazes de segurança e aplicação da lei contribuem para os altos níveis de violência urbana, com altas taxas de roubos, assaltos, sequestros e violência sexual (78). Estas condições somam-se na construção de um cenário de estresse crônico para os indivíduos que as vivenciam diariamente e que, frequentemente e de forma imprevisível, são submetidos a eventos de estresse agudo em seu dia-a-dia.

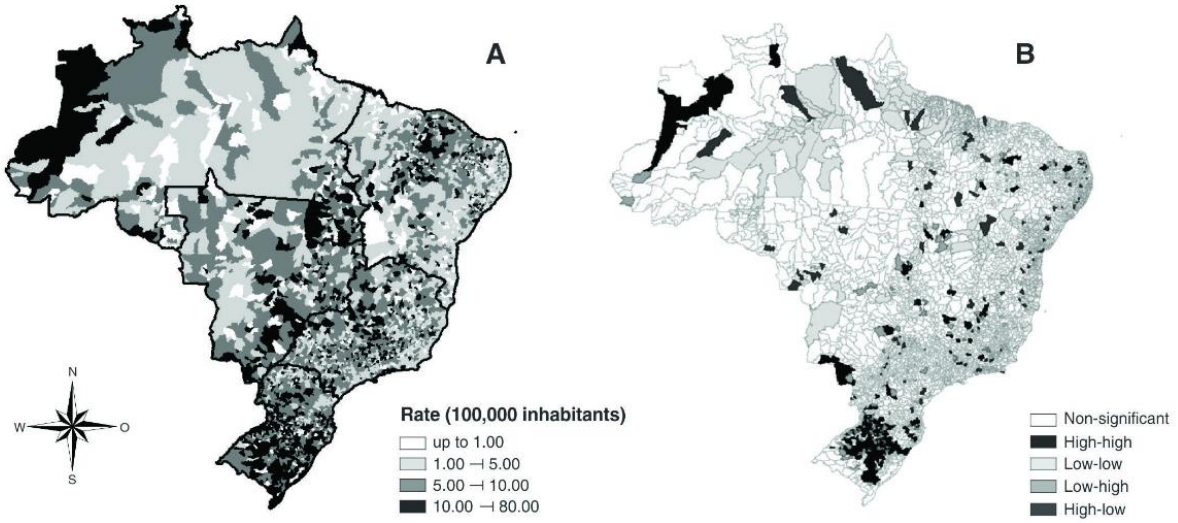

Figura 5. Análise da mortalidade por suicídio no Brasil, avaliada em 2010-2014. Em (A), a distribuição por municípios da mortalidade por suicídios. Em (B), a distribuição da mortalidade por suicídios em clusters de acordo com significativa associação espacial. Adaptada de Dantas et al., 2017 (79).

A condição de estresse, de natureza física (p.ex., lesão por arma de fogo ou atropelamento) ou psicológica (p.ex., relacionamento abusivo ou morte de alguém próximo), é considerada como qualquer situação capaz de perturbar a homeostase (i.e., o equilíbrio) do organismo (80). Em resposta às situações de estresse percebidas, nosso corpo ativa uma série de processos destinados a tentar reestabelecer o equilíbrio perturbado pelo evento estressante, adaptados ao longo da evolução de modo a promover condições que contribuam para a sobrevivência. O principal sistema envolvido na reação a situações estressantes é o eixo hipotálamo-hipófise-adrenal (HPA, de hipotálamo-pituitária-adrenal). A percepção de um evento estressante desencadeia a ativação do NPV do hipotálamo que, em reação, sintetiza e libera o hormônio liberador de corticotrofina (CRH, *corticotropin-releasing hormone*). O CRH liberado, através do sistema porta-hipofisário, chega à hipófise anterior, onde liga-se a seu receptor e induz a síntese e liberação de hormônio adrenocorticotrófico (ACTH, *adrenocorticotropic hormone*) na corrente sanguínea. O ACTH, então, é levado pela corrente sanguínea à glândula adrenal, onde liga-se a seu receptor e estimula a síntese e liberação de glicocorticoides na corrente sanguínea (hormônio cortisol em humanos e corticosterona em roedores). Paralelamente à ativação do eixo HPA ocorre a ativação do sistema nervoso simpático que, através da ativação do locus ceruleus e sua liberação de norepinefrina, estimula a síntese e liberação de epinefrina e norepinefrina pela glândula adrenal (81). Em casos de estresse agudo, em que é necessária uma resposta rápida e de curta duração, este sistema é

rapidamente ativado de modo a permitir a ação de “luta ou fuga” dos glicocorticoides e da ativação do sistema nervoso simpático – p.ex., alerta, vigilância, analgesia, supressão de funções alimentares e reprodutivas, aumento da frequência respiratória e da oxigenação cerebral e muscular -, mas logo tem sua atividade reduzida por *feedback* negativo através de sinalização dos glicocorticoides a nível central, sobre o hipotálamo e a hipófise, regulando sua própria concentração (80) **(Figura 6)**.

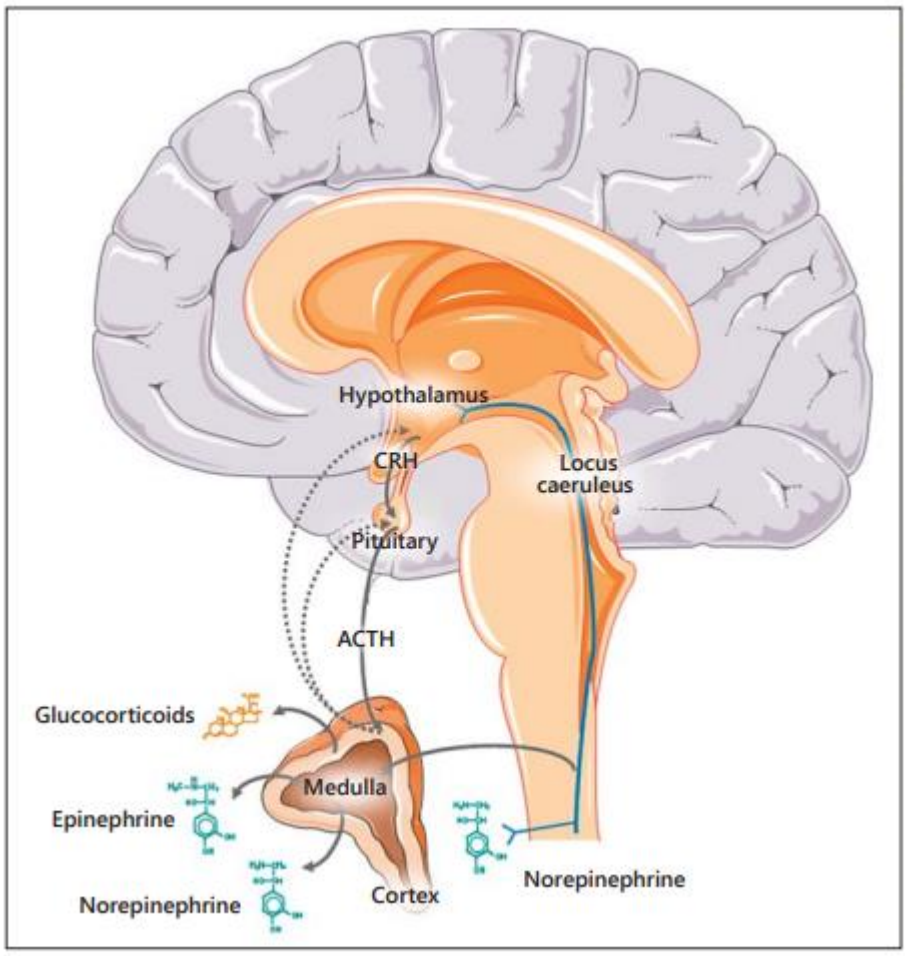

Figura 6. O sistema de resposta ao estresse. *Hypothalamus*: hipotálamo. CRH: *corticotropin-releasing hormone* (hormônio liberador de corticotrofina). *Pituitary*: hipófise. ACTH: *adrenocorticotropic hormone* (hormônio adrenocorticotrófico). *Glucocorticoids*: glicocorticoides. *Epinephrine*: epinefrina/adrenalina. *Norepinephrine*: norepinefrina/noradrenalina. Adaptada de Nicolaides et al., 2015 (81).

Atualmente, porém, vivenciamos predominantemente situações de estresse de caráter crônico, como a cobrança diária no ambiente de trabalho ou o medo constante de sofrer algum tipo de agressão ou assalto em regiões de maiores índices de violência. Condições de exposição repetida a um ou a vários estressores podem gerar respostas de hiporresponsividade

ou de facilitação. Alguns indivíduos, ao serem expostos repetidamente a determinado estressor, apresentam ativação diminuída do eixo HPA e menor liberação de glicocorticoides em resposta às apresentações do estresse. Outros indivíduos, porém, apresentam aumento da sensibilidade da adrenal ao ACTH, resultando em uma resposta de glicocorticoides amplificada quando o organismo é exposto repetidamente ao estresse (82). Essas respostas diferenciadas entre indivíduos podem ser resultantes de perfis de maior resiliência ou de maior suscetibilidade aos efeitos do estresse, que estão associados ao conceito de *coping*. Estratégias de *coping* se referem às maneiras pelas quais um indivíduo lida com uma situação estressante a qual ele é apresentado, podendo ser ativas (buscar soluções para os problemas, enfrentamento do medo) ou passivas (evitar os problemas, negação, supressão de emoções). Estratégias de *coping* ativas estão relacionadas com ressignificação positiva de eventos passados e maior resiliência, enquanto *coping* passivo parece ser mal-adaptativo e não resultar em resiliência a longo prazo (83,84). Quando submetido a um evento estressante, a resposta do organismo promove adaptações em sua fisiologia em um processo chamado de alostase, que busca reestabelecer a homeostase do organismo. Em condições em que o organismo é submetido repetidamente a estresse, as alterações necessárias em resposta a estas situações formam uma carga alostática, que pode ser adaptativa e positiva em indivíduos resilientes, associada a estratégias ativas de *coping* e resultando em um estado mais preparado para responder a um novo desafio. Porém, em indivíduos suscetíveis, as adaptações do organismo são insuficientes para retomar o equilíbrio, por dependerem de estratégias ineficientes de *coping* passivo ou por superarem capacidade de adaptação do organismo, e ocorre a chamada sobrecarga alostática e o desenvolvimento de patologias (85,86).

A investigação sobre mecanismos envolvidos na resiliência ou suscetibilidade aos efeitos do estresse é de grande relevância tendo em vista o crescente número de condições adversas de saúde e comportamento que têm sido identificadas em associação ao estresse. A obesidade e a síndrome metabólica, condições de alta prevalência atualmente, parecem estar relacionadas a condições de estresse em humanos, apesar de a relação não ser tão clara segundo as evidências de estudos com animais (87). Quanto ao consumo de alimentos, observa-se uma preferência pelas chamadas *comfort foods* (alimentos mais prazerosos e palatáveis) em detrimento de alimentos saudáveis durante períodos de estresse, sem alterar a ingestão total de calorias, tanto em humanos quanto em animais (88). Porém, a relação entre as alterações na ingestão de alimentos observada no estresse e os efeitos sobre o peso corporal ainda não estão claras, sendo observada associação de estresse com ganho de peso em

indivíduos previamente acima do peso normal, mas não em indivíduos com peso normal ou abaixo do normal (89). Em ratos e camundongos, porém, estresse agudo e crônico tem consistentemente demonstrado um efeito predominante de redução do peso corporal em animais, sendo necessários estudos voltados a identificar os mecanismos envolvidos no efeito diferenciado do estresse sobre o ganho de peso em humanos e roedores (90). Eventos estressantes também têm sido considerados como possíveis fatores no desenvolvimento de transtorno depressivo maior (91), estimado a se tornar a principal causa de incapacitação mundial em 2030 pela Organização Mundial da Saúde (92). Diversos modelos animais de estresse, desenhados de modo a simular condições estressantes vividas por seres humanos (p.ex., derrota social e estresse crônico), têm demonstrado o desenvolvimento de comportamentos anedonia, evitação social e desesperança após exposição a estresse, sintomas chave do transtorno depressivo em humanos (77). O estresse também tem sido considerado na investigação da etiologia de outros transtornos psiquiátricos, como o transtorno bipolar (93,94), o transtorno de ansiedade (95,96) e o transtorno de estresse pós-traumático (97), ressaltando a importância de estudos voltados a compreender os mecanismos envolvidos na resposta ao estresse e no potencial desenvolvimento de patologias.

2.4. CONEXÕES ENTRE SISTEMA CIRCADIANO E RESPOSTA AO ESTRESSE

Observamos uma crescente prevalência de transtornos psiquiátricos atualmente (92,98) e que essas patologias parecem estar associadas a perturbações de ritmicidade circadiana (71,72) ou a condições de estresse (91). Sabendo disso, tem sido sugerido que a conexão entre o sistema circadiano e o desenvolvimento de transtornos psiquiátricos se dê através de um efeito modulatório da resposta do organismo a eventos estressantes que, por variarem de indivíduo para indivíduo, levariam ao surgimento de diferentes transtornos (p.ex., depressão, ansiedade, esquizofrenia ou transtorno de estresse pós-traumático) dependendo das experiências individuais de cada um (10). Essa hipótese se baseia na existência de diversas conexões entre os sistemas responsáveis pela sincronização dos ritmos circadianos e pela resposta adaptativa ao estresse. Por exemplo, em condições basais, as concentrações de glicocorticoides apresentam flutuação com ritmicidade circadiana, com aumento de sua produção pela adrenal precedendo o despertar, tanto em humanos quanto em outras espécies animais (sendo no início do dia para animais diurnos e no início da noite para animais noturnos), de modo a aumentar a produção de energia e preparar o organismo para seu período de atividade (99). Além disso, o NPV do hipotálamo, principal componente do eixo HPA, é também elemento essencial na via de sinalização do oscilador central do sistema

circadiano, o NSQ, para a glândula pineal. Estudos verificaram projeções do NSQ para os neurônios que expressam CRH no NPV, sugerindo poder existir uma regulação direta do sistema circadiano sobre a ativação do eixo HPA (100). Estruturas intermediárias, como o núcleo sub-paraventricular (subNPV) e o hipotálamo dorsomedial (HDM) – que possuem neurônios glutamatérgicos (excitatórios) e GABAérgicos (inibitórias) –, têm sido implicadas na modulação da sinalização do NSQ para o NPV, de modo a explicar como a sinalização excitatória (por liberação de vasopressina) proveniente do NSQ durante o dia resulta em estimulação da liberação de glicocorticoides em espécies diurnas, mas em inibição dessa liberação em espécies noturnas (99) **(Figura 7)**.

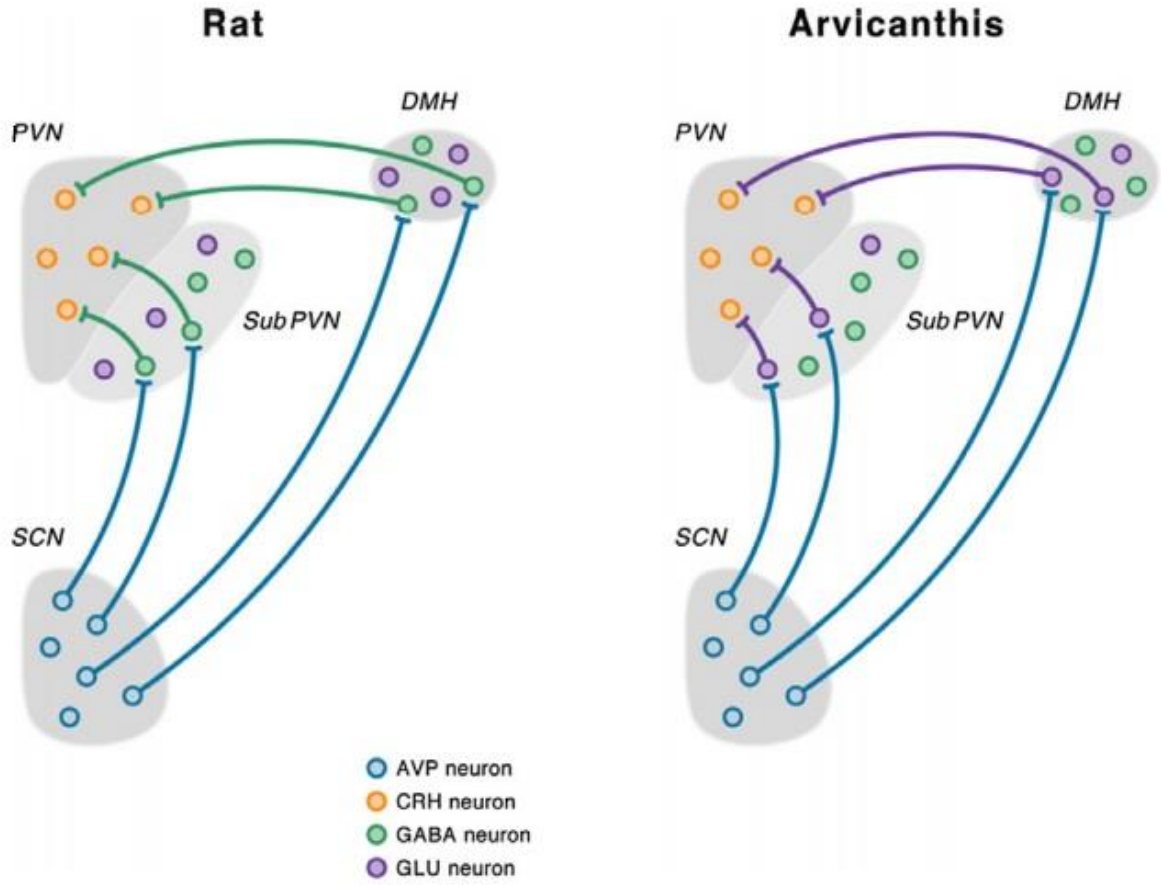

Figura 7. Esquema proposto da sinalização dos núcleos supraquiasmáticos para os núcleos paraventriculares do hipotálamo. SCN: *suprachiasmatic nucleus* (núcleo supraquiasmático). PVN: *paraventricular nucleus* (núcleo paraventricular). SubPVN: *subparaventricular nucleus* (núcleo sub-paraventricular). DMH: *dorsomedial hypothalamus* (hipotálamo dorsomedial). AVP: arginina vasopressina. CRH: *corticotropin-releasing hormone* (hormônio liberador de corticotrofina). GABA: *gamma-aminobutyric acid* (ácido gama-aminobutírico). GLU: glutamato. Adaptada de Kalsbeek et al., 2012 (99).

Além da regulação do sistema circadiano sobre o eixo HPA, o eixo HPA também está envolvido na regulação da ritmicidade circadiana, principalmente do ciclo sono-vigília. Sabe-se que os glicocorticoides são capazes de reconfigurar a ritmicidade dos osciladores periféricos, induzindo a expressão dos *clock genes Per1* e *Per2* através de elementos responsivos a glicocorticoides (GREs, *glucocorticoids response elements*), sem alterar a sincronização do NSQ (101). Portanto, quando o organismo é exposto a um estressor e a resposta do eixo HPA é ativada, a elevação na liberação adrenal de glicocorticoides independente da regulação circadiana pode alterar a ritmicidade dos osciladores periféricos, resultando em ritmos periféricos (p.ex., na adrenal, no fígado ou outros osciladores) dessincronizados com o ambiente e com os osciladores centrais (102). Somado a isso, a exposição a ciclos irregulares de claro-escuro ou a horários irregulares de sono e vigília podem induzir a um estado de cronorruptura a nível central, afetando a sincronização do NSQ e, por consequência, prejudicando a sincronização dos osciladores periféricos e a regulação adequada dos ritmos biológicos (72) **(Figura 8)**. Assim, a regulação inadequada da sincronização das funções fisiológicas - incluindo a atividade do eixo HPA - pelo sistema circadiano submetido à cronorruptura representa uma forma de perturbação da homeostase e induz um estado de carga alostática no organismo, podendo ser tão extrema a ponto de afetar a saúde por si só. Porém, frente a um evento estressante, que pode contribuir para a dessincronização interna entre os osciladores circadianos, a resposta do organismo dificilmente será suficiente, proporcionando um estado de sobrecarga alostática (10).

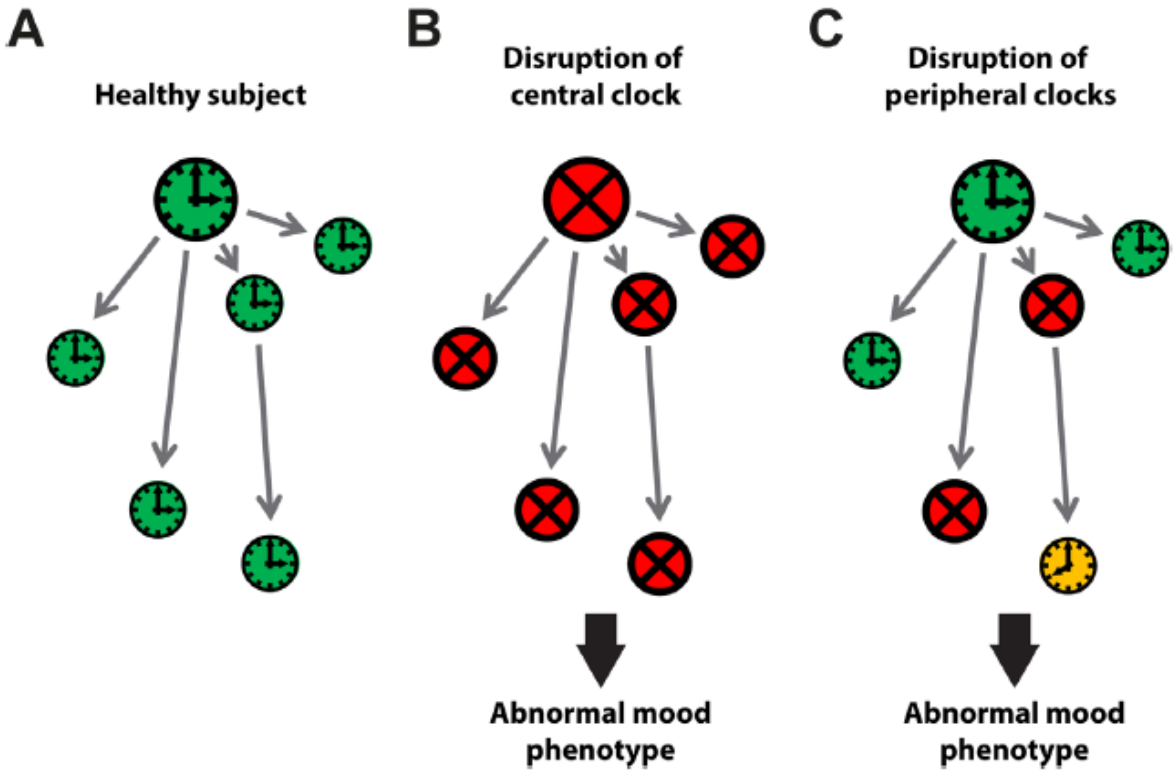

Figura 8. Modelos alternativos de como a cronorruptura leva ao desenvolvimento de fenótipos de humor alterado (*abnormal mood phenotype*). O relógio maior indica os osciladores centrais e os menores, osciladores periféricos. Em verde, osciladores íntegros; em vermelho, osciladores dessincronizados com o ambiente; e em amarelo, osciladores dessincronizados em relação aos demais tecidos. Em (A), o modelo de um organismo saudável (*healthy subject*), sincronizado ao ambiente. Em (B), um organismo em que os osciladores centrais perderam sua sincronização (*disruption of central clock*), levando a uma dessincronização sistêmica e fenótipo de humor alterado. Em (C), um modelo em que a sincronização de alguns osciladores periféricos se mantém, enquanto em outros foi perdida ou está fora de fase em relação aos demais tecidos (*disruption of peripheral clocks*), também resultando em alterações de humor. Adaptada de Landgraf et al., 2016 (72).

Tendo em vista os efeitos de perturbações do sistema circadiano sobre a homeostase e sobre a carga alostática, nosso primeiro estudo busca identificar e avaliar as evidências atuais de prejuízos à saúde e ao comportamento humano de um dos tipos de cronorruptura mais comuns atualmente, o jetlag social. Em seguida, considerando o nível de integridade do sistema circadiano como um potencial modulador da resiliência/suscetibilidade ao desenvolvimento de alterações de humor e desenvolvimento de patologias em resposta ao estresse (85), nosso segundo estudo destina-se a investigar os efeitos de alteração da

sincronização circadiana sobre a vulnerabilidade aos desfechos de estresse crônico imprevisível nos ritmos biológicos (atividade-repouso e temperatura central), no metabolismo (peso corporal e parâmetros metabólicos séricos) e no comportamento (tipo-depressivo e tipo-ansioso) de camundongos Balb/c.

3 JUSTIFICATIVA

O estudo derivado desta dissertação justifica-se com base nos seguintes pontos:

- Tendo em vista as condições atuais de excessiva iluminação artificial, ruptura da ritmicidade circadiana e estresse crônico, seus efeitos sobre a saúde e o comportamento devem ser investigados;

- Apesar de um dos fenômenos de cronorruptura mais comuns na sociedade contemporânea, o jetlag social, já ter sido amplamente estudado, há a necessidade de sumarizar as evidências sobre sua associação com desfechos de saúde e comportamentais de forma sistemática;

- Devido aos altos índices de transtornos de humor e de alterações de metabolismo verificados atualmente (98,103), é necessário investigar fatores relacionados a estas condições (como a cronorruptura e o estresse) que possam estar envolvidos no aumento da prevalência destas patologias;

- Apesar de há alguns anos ter sido sugerida na literatura a hipótese de que perturbações do sistema circadiano podem estar relacionadas a transtornos psiquiátricos através de ação modulatória dos efeitos do estresse (10), não existem estudos experimentais destinados a investigar esta possível associação;

- A determinação dos efeitos de cronorruptura, de forma isolada e como fator de modulação dos efeitos do estresse, é necessária como embasamento para que a organização da sociedade seja revista e potencialmente remodelada de modo a minimizar o impacto da iluminação artificial e das rotinas atuais sobre a ritmicidade circadiana.

Pelas razões apresentadas, justifica-se a realização do presente estudo com o propósito de sumarizar as evidências da associação entre jetlag social e desfechos de saúde e comportamento; e de avaliar a influência de perturbação da ritmicidade circadiana na resposta do organismo a estresse crônico em camundongos, observando os efeitos sobre ritmos de atividade-repouso e temperatura central, peso corporal, parâmetros metabólicos e comportamentos tipo-depressivo e tipo-ansioso.

4 HIPÓTESE

Os padrões de iluminação aos quais os seres humanos se expõem atualmente induzem uma perturbação da ritmicidade circadiana, que tem sido associada ao desenvolvimento de transtornos psiquiátricos e alterações metabólicas. Nossa hipótese é que a cronorruptura possa aumentar a vulnerabilidade ao estresse e/ou prejudicar a resposta do organismo ao estresse, o que torna mais difícil o retorno à homeostase e facilita o aparecimento destes transtornos.

5 OBJETIVOS

5.1. OBJETIVOS GERAIS

Identificar, através de revisão sistemática, o efeito de perturbações da ritmicidade circadiana sobre a saúde humana e verificar o efeito da cronorruptura sobre a vulnerabilidade ao estresse em um modelo animal.

5.2. OBJETIVOS ESPECÍFICOS – ARTIGO 1

- Identificar as evidências atuais dos riscos à saúde e comportamentais associados ao jetlag social;
- Avaliar as evidências encontradas, através de revisão sistemática, quanto a:
    - Áreas de publicação e desenhos experimentais;
    - Heterogeneidade metodológica;
    - Risco de vieses;
    - Desfechos observados;

5.3. OBJETIVOS ESPECÍFICOS – ARTIGO 2

- Avaliar os efeitos de perturbação circadiana por encurtamento do ciclo claro-escuro (10h claro, 10h escuro) seguida de estresse crônico moderado em camundongos Balb/c nos seguintes parâmetros:
    - Ritmicidade circadiana dos ritmos de atividade-repouso e temperatura central;
    - Comportamento tipo-depressivo (teste de preferência por solução de sacarose);
    - Comportamento tipo-ansioso (teste de caixa claro-escuro)
    - Peso corporal;
    - Parâmetros metabólicos (glicose, triglicerídeos e colesterol total e HDL);

6 ARTIGO 1

SOCIAL JETLAG IN HEALTH AND BEHAVIORAL RESEARCH: A SYSTEMATIC REVIEW

(Artigo publicado na revista ChronoPhysiology and Therapy em 08/05/2017)

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ABSTRACT

Background: Even though light is considered the main cue that entrains inner biological rhythms according to circadian environmental rhythms, social organizations have the capacity to take the body "out of sync". An emergent field of research on the topic refers to what has been described as social jetlag, the biological misalignment that arises from alternated work and free days. However, to the present moment, there is still controversial evidence on the effects of such a phenomenon to human health. **Objective:** The aim of this study was to identify current peer-reviewed evidence of the health and behavioral risks associated with social jetlag. **Method:** We conducted a systematic review of the literature on PubMed, Scopus, Embase and LILACS electronic databases using the terms "social AND (jet lag OR jetlag)". The search was finalized on August 22, 2016, resulting in 26 research articles included in the review. **Results and discussion:** Our results point to a variety of health and behavioral outcomes that seem to be associated with the mismatch existent between work or study days and free days. They are epilepsy, minor psychiatric symptoms, aggression and conduct problems, mood disorders, cognitive impairment (eg, work and academic performance), substance use, cardiometabolic risk and adverse endocrine profiles. However, these results must be analyzed with caution because of the high methodological heterogeneity, the significant risk of bias of analyzed studies, as well as the low similarity among the populations described.

Keywords: chronobiology, biological rhythms, sleep, shift work.

Background

Living beings are regulated by oscillating biological systems that orchestrate physiologic and behavioral events, synchronizing them to external signals such as light–dark cycles and social routines.[1] Although several studies support that light is still the main zeitgeber (from German, zeit = time, geber = giver),[2] that is, the main environmental cue that entrains our bodily rhythms, a growing amount of evidence suggests that social cues might be of major importance influencing biological rhythms.[3,4]

Social routines are organized into conventional time periods (eg, business hours from 8 a.m. to 6 p.m. in most cities in Brazil). These routines are influenced by several factors, which include the demand for increased productivity and competitiveness, public security and other cultural aspects such as religion and ethnicity. Therefore, such routines are not in total synchrony with the endogenous circadian (from Latin, circa = about, diem = a day) rhythmicity of all individuals and, sometimes, not even with the natural day–night transitions.[5] Modern lifestyles changed the way we interact with our surroundings. The need to live in a society requires submission to these rules and structures, leading to obvious benefits from cooperation as a group, but providing situations that could represent a risk for some individuals who have to abandon photoentrainment (entrainment to the exogenous light–dark cycle) and follow routines in disagreement with their physiologic rhythmicity.[6]

Each person presents an individual phase of entrainment to the external days, referred to as chronotypes, which reflects on optimal daily times for different activities.[7] This derives from a combination of genetic factors, development and environment, which determine the timing of our endogenous clock.[8] Even though the majority of individuals are classified as intermediate chronotypes with no strong preferences, the distribution can vary from extreme early to extreme late chronotypes. The standard organization of our society favors those who tend to wake and sleep earlier (early types) and obliges those who tend to wake and sleep later (late types) to follow routines that differ from their internal time. In addition, the demand for productivity led to the emergence of practices such as shift work, which implies exposition to unnatural patterns of light–dark cycles. An increasing number of studies suggests that disruption of normal circadian rhythms can lead to a variety of health and behavioral outcomes, such as metabolic syndrome, obesity,[9,10] breast cancer[11] and mood disorders.[12] This relevant disturbance on circadian rhythms is also known as chronodisruption.[13] Several studies focus on sleep parameters as means to identify the mechanisms through which

circadian disruption occurs.[14,15] Despite the scientific development to the present moment, there is still no consensus on what it is that we should call "optimal sleep".[16,17] Most studies focus on sleep quality and daytime sleepiness, but these are not the only variables that are relevant to the study of animal's ideal sleep. In this scenario, one emergent field of research pertains to what has been called social jetlag (SJL). This phenomenon has been initially described as a biological misalignment that arises from alternated work and free days,[18] considering the discrepancies between the social and the biological clock that lead to sleep and wakefulness at inappropriate circadian times.[19] SJL has been studied in many health-related researches, measured by either subjective or objective assessments (eg, reported sleep times and actigraphy, respectively). The conventional formula to calculate SJL subtracts the Midpoint of Sleep on Work days from the Midpoint of Sleep on Free days (MSF).

A growing amount of evidence suggests that SJL is related to a series of conditions commonly associated with chronodisruption. However, the association of SJL to human health is still controversial, since no systematic review on the topic has been performed till now. Through a systematic review of the literature, we aimed to identify the current evidence of health and behavioral risks associated with SJL.

Method

Search method

We conducted a systematic review of the literature on PubMed, Scopus, Embase and Latin American and Caribbean Health Science Literature (LILACS) using the terms "social AND (jet lag OR jetlag)" on the default search fields of each electronic database. No advanced search tool was employed. We searched for peer-reviewed papers reporting empirical studies including data on health and behavioral outcomes associated with SJL in different populations. Given the heterogeneity of the body of literature on the topic, we included all studies with at least one health or behavioral outcome associated with SJL. However, studies that solely analyzed sleep parameters or circadian patterns, but did not assess health or behavioral outcomes were excluded from the review.

Due to the restricted number of empirical studies, we did not establish a minimum number of participants included in the studies. This way, we could avoid missing data from small sample studies. Looking for the highest methodological quality, we excluded non-peer-

reviewed references and unpublished data. We retained only observational studies with no language restriction. Review studies were excluded.

The search was finalized on August 22, 2016. Two authors (ACT and JCB) independently and blindly performed the search and screening of abstracts against the eligibility criteria. The other four authors were divided in pairs and they blindly read all full-length papers initially selected, applying a more rigorous eligibility. Any disagreement among authors was resolved by consensus. Figure 1 displays the systematic review process conducted according to Cochrane guidelines.[20]

Risk of bias assessment

In order to determine the level of evidence of included studies, six questions were proposed and blindly answered for each study by the same pairs of reviewers that read the full-length papers. Any disagreement among authors was resolved by consensus. Questions assessed the risk of selection bias (Q1 and Q2), detection bias (Q3), attrition bias (Q4) and reporting bias (Q5). An additional question (Q6) was included to assess biases not addressed in the previous questions. They are:

1. Q1: General characteristics of the individuals are assessed (eg, demographics, possible confounding factors)? If groups are compared, is there any significant difference among them?

2. Q2: Is there significant exclusion/noninclusion of individuals that could compromise results validity?

3. Q3: Are outcomes assessed ensuring internal validity of the study?

4. Q4: Are there significant withdrawals from the study?

5. Q5: Results reported are in agreement to the hypothesis?

6. Q6: Is there any additional risk of bias that could compromise study findings?

Three possible answers could be given to each question: “high risk of bias”, “low risk of bias” or “inability to determine due to lack of information”. The level of evidence for each study can be seen in Figure 2.

Results and discussion

In the past years, the term “social jetlag” has been increasingly used. Our initial search shows that, even though many articles were excluded from this review because they did not measure SJL, a significant part of studies mentioned it throughout the text.

Excluded studies and terminology

Several studies retrieved in our search correlate SJL with sleep disturbances. Although sleep disturbances are known to affect health and behavior,[6] we cannot presume a direct causal relationship between such outcomes and SJL, which appears mostly as a variable that characterizes the study samples. Thus, we chose to exclude studies that did not explicitly assess illness states or behavioral outcomes. Furthermore, it is very likely that sleep deficit correlates with SJL. However, they are not necessarily associated, as people can still have SJL even though they do not present sleep deficit during week/work days.

A reflection on terminology is inevitable. Although transmeridional travels also lead to misalignments between endogenous and social rhythms, the expression “jetlag” would not be the best to call such disruption provoked by social routines during work/study and free days, which may lead to some confusion or misunderstanding of the term SJL. It is important to emphasize that some studies might have used the same measure of difference between the midpoints of sleep on free and on work days, but did not use the term SJL, which was first described in 2006 by Wittmann et al,[18] to define it. Therefore, these studies could not be retrieved in our search. An effort should be made to standardize the nomenclature across all research fields in order to unify the literature regarding SJL. Furthermore, some studies did not meet our research criteria because they discussed SJL, but failed on reporting subjective or objective measurements of it. This reinforces the need for adequacy of what it is that we should call the biological misalignment that arises from alternated work and free days which reflects the discrepancies between the social and the biological clock. We believe that “social lag” would be more appropriate to describe this phenomenon, since the word “jet” relates to transmeridional travels. However, we suggest specialists of this area to form a joint commission to define the best nomenclature in order to guarantee the homogeneity of publications on the topic.

Summary of findings

Table 1 summarizes findings from the 26 studies included in the review.

Two cross-sectional studies evaluated obesity in large samples, measuring SJL with the Munich ChronoType Questionnaire (MCTQ), but reaching opposite conclusions. Roenneberg et al[21] found that SJL increased the chance of being one of the overweight participants, with a body mass index (BMI) above or equal to 25 kg/m^2. Conflictingly, Johnsen et al[22] found no association between SJL and obesity measures (BMI ≥25 kg/m^2, waist-to-hip ratio and abdominal obesity) when analysis was controlled for health, lifestyle and biological factors. This incongruence can be attributed to methodological differences, such as the use of self-reported data on height and weight in one study[21] versus assessment of BMI variables by staff members in another,[22] or to different sample characteristics, since Roenneberg et al[21] studied individuals primarily from central Europe with a broader age range, while Johnsen et al[22] investigated a population located at higher latitudes, above the Arctic Circle. Corroborating the hypothesis of the influence of latitude, Parsons et al[23] found an association between SJL and higher BMI, fat mass and probability of being obese (BMI ≥30 kg/m^2) in individuals from midlatitudes, while Polugrudov et al[24] found no association of SJL with BMI and waist circumference in a population living in the subarctic. Furthermore, none of these studies controlled the results for energy intake, energy expenditure or body fat. Thus, it is not possible to determine if SJL can alter BMI through an effect of increase in energy intake, or a decrease in energy expenditure, or even an impact on metabolism leading to increased fat storage. Hence, the link between SJL and obesity needs further research, but the evidence so far points to absence of such association among inhabitants of high latitudes.

Three cross-sectional studies investigated the association between SJL and metabolic risk in samples of similar age and latitude, also finding opposing results. Kantermann et al[25] found no association between SJL and metabolic risk parameters in male shift workers, which may be due to the small sample size. However, Parsons et al[23] found that individuals with higher SJL were more likely to meet the criteria for metabolic syndrome and Wong et al[26] found higher cardiometabolic risk markers associated with higher SJL in larger samples of both sexes. Regarding the risk of cardiovascular pathologies, a positive correlation between SJL and heart rate has been demonstrated.[27,28] Nonetheless, Kantermann et al[27] did not find associations with arterial stiffness in male shift workers, indicating that SJL is not associated with this measure of atherosclerotic risk or that the small number of participants was not enough to find statistically significant differences. On the other hand, Rutters et al[28] found higher cortisol levels and frequency of inactive periods linked to higher SJL and also reported no associations with BMI and waist measures.

Depression, one of the mood disorders most related to circadian and sleep disturbances, was evaluated in four selected studies. Levandovski et al[29] found an association between SJL and higher levels of depressive symptoms in a large rural sample aged from 18 to 65 years, while Polugrudov et al[24] found such an association in a population located in the subarctic with a mean age of 22 years. However, no association was found in young students aged between 12 and 21 years[12] or in undergraduate students of median age of 21 years.[30] Depressive symptoms in young individuals may be associated with factors other than circadian misalignment (eg, puberty, hormones, sexual or emotional abuse). The associations of SJL with such outcomes might appear at the beginning of the adult life, when chronotypes tend to be the latest and, consequently, SJL is at its highest.[31] This raises the question about how much time of exposure to SJL is enough to cause harm to an organism, suggesting a certain level of adaptation (like an allostatic load) that must be overcome before symptoms appear.

Findings from another study[32] suggest a stronger relationship between SJL and depressive mood in individuals living in highest latitudes where sunlight varies the most across seasons. This phenomenon of drastic changes in photoperiod (the amount of time of natural light within a day) is related to winter seasonal affective disorder, a common condition among inhabitants of high latitudes characterized by an experience of depressive symptoms, mainly during winter season.[33] Borisenkov et al[32] found higher indication of seasonal mood variation associated with SJL in young women of subarctic zones, suggesting that the stronger unconformity between chronotype and social obligations might be related to higher susceptibility to seasonal changes in length of sunlight availability.

Jankowski[34] demonstrated a positive association between SJL and tense arousal (nervous mood) in a cohort of undergraduate students, although the small sample size gives reasons to regard this findings with caution. Nonetheless, Schimitt et al[35] found no significant results assessing minor psychiatric symptoms in a sample of day-shift workers. Both Díaz-Morales[36] and Sheaves et al[30] conducted cross-sectional studies with students of midlatitudes and found no association between SJL and anxiety, assessed by different instruments in different age ranges. In addition, Polugrudov et al[24] found no associations of SJL with anxiety or anger in inhabitants of high latitudes, neither did Sheaves et al[30] with hallucinations, paranoia, mania and hypomania. Lin and Yi[37] demonstrated an association of SJL with poor academic performance in a cohort of junior-high school students. Yet, the assessment through open questions and inadequate calculation of SJL by subtracting weekday bedtime from

weekend bedtime make these results questionable. Kolomeichuk et al[38] reported that academic achievement of schoolchildren and college students from subarctic locations did not differ according to SJL. However, studies with midlatitude samples showed a link of SJL with lower cognitive performance and lower Grade Point Average in high school students[39] and poor grades during lecture term (with fixed timetables) in undergraduate students.[40] Haraszti et al[40] also found that this cohort of undergraduate students with higher SJL had better performance during final exams periods (with self-selected study times). This suggests that such intervals free of weekly fixed obligations provide suitable conditions for individuals that are normally out of synchrony because of regular social routines. Likewise, Yong et al[41] demonstrated that, in a sample of day and shift workers, work ability is decreased the most when SJL is associated with shorter sleep duration.

Other behavioral aspects were associated with SJL in some included studies. Tavernier et al[42] found no predictive effect of SJL on academic adjustment and substance use in a cohort of undergraduate students. On the other hand, substance use was a significant predictor of SJL in this study. Wittmann et al[18] also evaluated the use of stimulants and demonstrated a higher probability of smoking in individuals with higher SJL. Lin and Yi[37] demonstrated an association between SJL and defiant attitude, although, as mentioned above, this result must be considered with caution due to methodological inadequacies in SJL measurement. Likewise, Randler and Vollmer[43] found higher physical and verbal aggression in undergraduate students with higher SJL.

In view of the close relationship between SJL and the circadian biology, Polugrudov et al[24] assessed wrist temperature rhythm and Cortisol Awakening Response in order to evaluate circadian system state in a sample of individuals living in a subarctic location. Despite the small sample size, this study found higher wrist temperature at night, lower during the day, lower amplitude of wrist temperature rhythm and tendency for an increase in Cortisol Awakening Response in participants with SJL higher than or equal to 1 hour, indicating circadian system disturbances in this sample. Miller et al[44] investigated positive affect rhythm as a possible mediator in the relationship between evening chronotype and depression. However, this study found no association between such rhythm and SJL measured by actigraphy. This last study represents a good evidence due to the use of an alternative complementary method to assess SJL. Subjective data assessments estimate participants' general characteristics like elemental phenotypes. Actigraphy is a useful objective method to

be explored, but this technique usually assesses a limited period of time and does not represent a general pattern.

Finally, two studies evaluated distinct outcomes that could not be compared with other samples. Choi et al[45] studied epileptic and healthy participants and found lower SJL in focal epilepsy patients than healthy controls and general epilepsy patients. Silva et al[46] assessed the quality of diet in undergraduate students, resulting in a negative association of SJL with servings per day of beans. Yet, the use of self-reported measures for both outcomes and SJL in these studies represents a high risk of detection bias and raises doubt about the reliability of such evidences.

Publishing areas and research designs

Most articles addressing SJL were published in biological rhythms and sleep journals. Nevertheless, disturbances of internal rhythms are thought to be risk factors for several metabolic, endocrine, behavioral and psychiatric pathologies.[9–12] Thus, SJL and other forms of circadian disruption should gain more focus on journals of broader scope. The selective publication phenomenon clearly shows that discrepancies between endogenous circadian rhythms and social routines are still explored by a few research groups. SJL is a health issue that occurs on a large scale and deserves attention. For instance, the process of industrialization frequently neglects negative impacts on workers' health in order to improve profit. In addition, school schedules are dictated by parents' work schedules disregarding what would be more beneficial to students' health and academic performance. Considering the numerous epidemiological studies conducted by the scientific community, SJL is not a priority topic in general biomedical research. Thus, several health and behavioral risks associated with it might be neglected.

Another factor that must be taken into account is study design. About 80% of the articles analyzed are crosssectional studies. That is, measurements are performed in a single timepoint of participants' life. This observational design does not allow the establishment of causality between factors and outcomes assessed. In other words, it is not possible to determine if SJL is the cause of illness states, or if these health and behavioral variables lead to SJL. We can only assume that there is an association among some of them, but further longitudinal research is required to determine the direction of such association. Biological mechanisms involved in circadian system regulation are a growing area of study. Furthermore, most outcomes found in Table 1 are multifactorial. Therefore, establishing if

SJL acts as cause or consequence of ill health states is essential to acquire better knowledge on the pathophysiology of circadian disruption.

Methodological heterogeneity

A strong limitation that restricts the possibility of inferences from the present results is the high methodological heterogeneity. Studies methodologies mainly differ in four ways:

1. instruments used to calculate SJL, reflecting on the accuracy of information retrieved, ie, SJL measured objectively by actigraphy or self-reported by questionnaire data;

2. computation of SJL;

3. definition of what it is that should be considered SJL, ie, the total amount of mismatch time, or the establishment of cutoff values;

4. duration of the study and latitude of studies, comprising assessments in different photoperiods and seasons of the year.

Most studies used MCTQ or its validated adaptation for shift workers (MCTQ-shift), but some used open questions or adapted MCTQ questions. Although MCTQ psychometric properties have not yet been assessed,[47] studies have shown the reliability of its parameters in comparison to sleep logs,[48] actigraphy,[49] Morningness–Eveningness Questionnaire,[50,47] and Dim Light Melatonin Onset.[51] MCTQ considers the difference between bedtime and sleep onset time, and between rise time and wake up time. Furthermore, SJL is a construct derived from MCTQ-based sleep parameters. Therefore, the distinction of sleep and bed timing, which can be standardized by MCTQ, is crucial for a reliable measure. We believe that the studies that formulated their questions disregarding such distinction are at risk of reporting a biased SJL assessment.

One study[37] did not calculate SJL through the conventional formula, but by subtracting weekday bedtime from weekend bedtime. Someone who goes to bed at the same time on work and free days, but wakes up much later on free days has a strong misalignment between chronotype and social routine. This situation is contemplated by measuring differences in midpoints of sleep, but not by comparing bedtimes. Two other studies[36,39] also calculated midpoints of time in bed as a proxy for midpoint of sleep, and another[42] operationalized the midpoint of sleep as the halfway point between bedtime and wake time. Therefore, results on SJL from such studies should be regarded with caution. Additionally, some studies calculated

SJL using a correction of MSF for sleep debt.[22,38] This correction is typically used to estimate chronotype, but is not necessary (and not recommended) for measurement of SJL.

In addition, study results are based mostly on self-reported measures, such as questionnaires and sleep logs. People who volunteer to participate in researches regarding sleep might represent the population sample that is most concerned about the topic. Therefore, overestimation of sleep-related problems and wrong perceptions of sleep-awakening schedules can be important sources of bias. Furthermore, most people might also overestimate sleep latency, therefore interfering in selfreported sleep time. Only two studies used objective methods to evaluate SJL (eg, actigraphy). Although not as useful as questionnaires to assess general sleep features, actigraphy is a good alternative to objectively evaluate daily motor activity, light exposure and other parameters of importance to biological rhythms.[52,53]

Some studies attempt to establish a relevant and useful cutoff value, representing a certain number of hours (or minutes) of SJL that would have stronger association with adverse outcomes. For example, Rutters et al[28] showed that individuals suffering from an SJL ≥2 hours had worse endocrine and cardiovascular risk profiles, compared to those who had an SJL≤1 hour. Levandovski et al[29] also reported more depressive symptoms in participants with more than 2 hours of SJL in comparison to the rest of the population. Johnsen et al[22] excluded participants who presented an SJL ≤3 hours. Since there is still no consensus as to what would be the best cutoff value of SJL for health and behavioral research, future studies should analyze SJL both as a continuous and as a categorical variable.

Most cross-sectional studies had durations of more than 4 months, assessing SJL in different seasons of the year. Since the circadian system is strongly influenced by seasonality and photoperiod,[54] internal circadian rhythmicity and, consequently, SJL might change within a few months. This can be a confounding factor, increasing the risk of bias. In addition, Figure 3 shows the different settings of studies, highlighting the diversity of latitudes that might influence the study results because of photoperiod and seasonal variations. Notably, higher latitudes present more pronounced photoperiod changes throughout the year, when compared to equatorial and tropical zones.[32] It is important to either measure SJL controlling for season of assessment and latitude, or take these factors into consideration when performing statistical analysis and discussing results.

Accounts on the diversity of circadian biology

As chronotypes vary according to age,[31] it is very likely that outcomes associated with SJL also change depending on it. Therefore, studies investigating the same parameters in samples of different age ranges (eg, children and elderly) might reach different conclusions. For example, Levandovski et al[29] found an association between SJL and depression in an adult sample (from 18 to 65 years), while de Souza and Hidalgo[12] found no such association in young students (from 12 to 21 years). Even though several factors might play a role in this difference, SJL age dependence should always be considered.

Moreover, it is known that circadian typologies are different between men and women.[31] In addition, some work activities that are culturally attributed to women (eg, domestic services and babysitting) are not necessarily considered as formal labor activities. Such activities need to be taken into account for an accurate evaluation of SJL, as they are prevalent and tend to occur even on free days.

Since SJL is necessarily associated with contrasting social schedules within a week period, it is important to consider further characterization of sociodemographic data, including work status, education level, behaviors and traditions of samples. For example, some studies retrieved in our search distinguish rural from urban populations. These social organizations have marked differences in terms of social activity schedules. Rural populations have different lifestyles and circadian patterns that could influence measurements of SJL.[55,56] Most studies do not present a clear description of their samples in terms of research setting, cultural background and work situation. Part of these studies could have been conducted in rural populations and/or with unemployed individuals who had freedom to choose sleep timing. These characteristics can distort SJL analysis and its relation to health and behavior outcomes. Finally, any medical condition (including psychiatric affections) that could disturb sleep/wake schedules should be assessed as a confounding factor. It is likely that part of these studies was conducted in rural populations, therefore distorting SJL analysis and its relation to health and behavior outcomes.

Conclusion

Our results point to a variety of health and behavioral outcomes that seem to be associated with the mismatch existent between work or study days and free days. Our search highlights mainly neuropsychiatric disturbances. They are epilepsy, minor psychiatric symptoms, aggression and conduct problems, mood disorders, cognitive impairment (eg, work and

academic performance) and substance use. Additionally, SJL has been associated with increased cardiometabolic risk and adverse endocrine profiles in some studies.

However, there is still lack of consensus on the associations reported, methodological flaws and lack of high-level evidence. To the present moment, SJL was studied only in a restricted amount of samples. Current evidence on SJL regards to individuals thought to be at risk of circadian disruption because of weekly activity routines (eg, shift workers, undergraduate and college students). The incongruences in reported results might be due to the high heterogeneity of methodologies applied or to the low similarities among the populations studied (eg, young students from a rural setting versus shift workers from high-latitude locations). This highlights the need for methodological adequacy in order to determine 1) if differences observed are, in fact, reflecting singularities of samples, which would suggest the existence of factors modulating SJL differently in each of these populations or 2) if they are mere methodological or statistical artifacts. It is worth emphasizing that SJL measurement and calculation can be done in a simple, cheap and fast way. Given the growing concern about the effects of modern lifestyles on health, more resources should be destined to large, population-based longitudinal studies, preferably with instruments for objective measures of MSF. Future research should aim to prospectively identify health and behavioral outcomes associated with SJL and determine the time of exposure and the number of hours of SJL necessary to represent a risk.

Whereas humans are social beings and modern lifestyles are demanding gradually more adaptation of individuals to routines that do not respect chronotypes, social cues are increasingly gaining attention in chronobiology research. The effects of circadian disruption are new in the course of human evolution, but its possible hazards are of interest to several areas of medicine, such as psychiatry, endocrinology, immunology and oncology. Therefore, it should be included in future research agenda of different biomedical sectors.

Disclosure

The authors report no conflicts of interest in this work.

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FIGURES AND TABLES

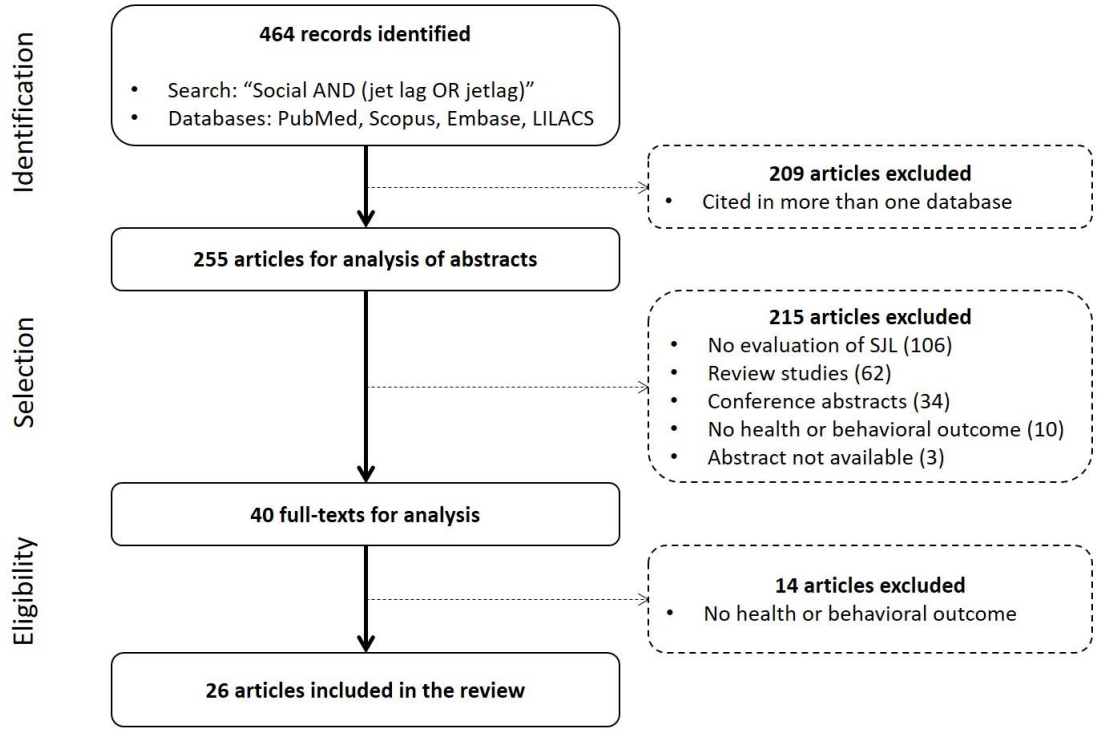
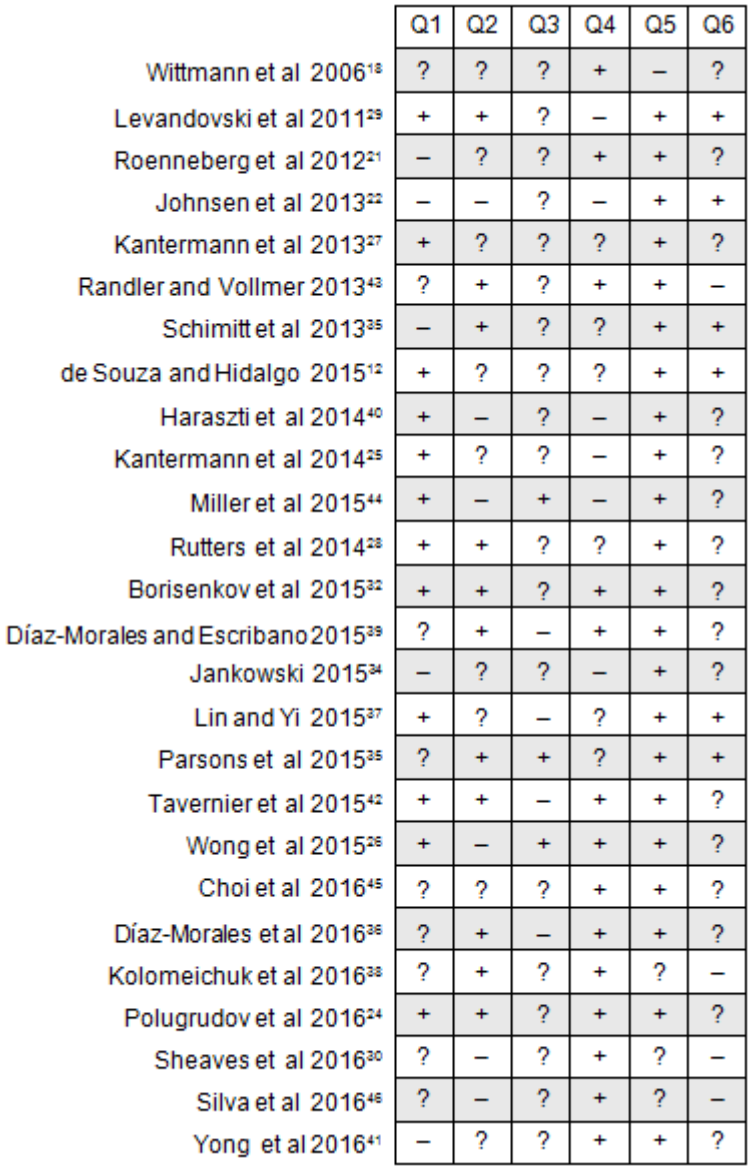


Figure 1 Systematic review process.

Abbreviations: SJL, social jetlag; LILACS, Latin American and Caribbean Health Science Literature.



	Q1	Q2	Q3	Q4	Q5	Q6
Wittmann et al 2006[18]	?	?	?	+	–	?
Levandovski et al 2011[29]	+	+	?	–	+	+
Roenneberg et al 2012[21]	–	?	?	+	+	?
Johnsen et al 2013[22]	–	–	?	–	+	+
Kantermann et al 2013[27]	+	?	?	?	+	?
Randler and Vollmer 2013[43]	?	+	?	+	+	–
Schimitt et al 2013[35]	–	+	?	?	+	+
de Souza and Hidalgo 2015[12]	+	?	?	?	+	+
Haraszti et al 2014[40]	+	–	?	–	+	?
Kantermann et al 2014[25]	+	?	?	–	+	?
Miller et al 2015[44]	+	–	+	–	+	?
Rutters et al 2014[28]	+	+	?	?	+	?
Borisenkov et al 2015[32]	+	+	?	+	+	?
Díaz-Morales and Escribano 2015[39]	?	+	–	+	+	?
Jankowski 2015[34]	–	?	?	–	+	?
Lin and Yi 2015[37]	+	?	–	?	+	+
Parsons et al 2015[35]	?	+	+	?	+	+
Tavernier et al 2015[42]	+	+	–	+	+	?
Wong et al 2015[26]	+	–	+	+	+	?
Choi et al 2016[45]	?	?	?	+	+	?
Díaz-Morales et al 2016[36]	?	+	–	+	+	?
Kolomeichuk et al 2016[38]	?	+	?	+	?	–
Polugrudov et al 2016[24]	+	+	?	+	+	?
Sheaves et al 2016[30]	?	–	?	+	?	–
Silva et al 2016[46]	?	–	?	+	?	–
Yong et al 2016[41]	–	?	?	+	+	?

Figure 2 Risk of bias assessment.

Notes: Questions 1 and 2 (Q1 and Q2) indicate selection bias, Q3 indicates detection bias, Q4 indicates attrition bias, Q5 indicates reporting bias, and Q6 indicates any additional bias. Crosses represent low risk of bias; dashes, high risk of bias; and interrogation marks, an inability to determine bias due to lack of information.

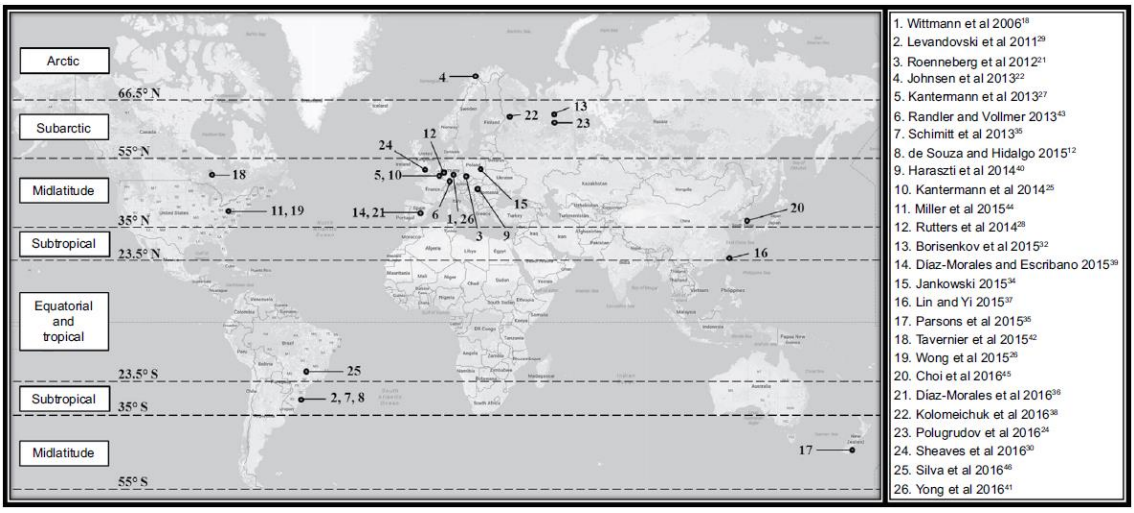


Figure 3 Distribution of studies across latitude zones.

Table 1 Summary of findings

Study	Population	Age range/mean age (SD)	Number of males/ females	Geographic location	Sample size	Design	Instrument used to define SJL
Wittmann et al 2006[18]	General population	14–94	190/303	Germany (midlatitude)	501	Cross-sectional	MCTQ
Levandovski et al 2011[29]	Rural population	44.1 (13.4)	1340/2711	Southern Brazil (subtropical)	4051	Cross-sectional	MCTQ
Roenneberg et al 2012[21]	General Internet-using public	16–65	NI	Primarily central Europe (midlatitude)	64,110	Cross-sectional	MCTQ
Johnsen et al 2013[22]	General population	30–65	3053/3356	Northern Norway (arctic)	6412	Cross-sectional	MCTQ
Kantermann et al 2013[27]	Shift workers of a steel factory	42 (7.6)	77/0	Belgium (midlatitude)	77	Cross-sectional	MCTQshift
Randler and Vollmer 2013[43]	Undergraduate students	23.8 (3.7)	320/112	Germany (midlatitude)	432	Cross-sectional	Open questions
Schimitt et al 2013[35]	Day shift workers	18–60	NI	Southern Brazil (subtropical)	143	Cross-sectional	MCTQ
de Souza and Hidalgo, 2015[12]	Adolescents	14.7 (1.86)	104/247	Southern Brazil (subtropical)	351	Cross-sectional	MCTQ
Haraszti et al 2014[40]	Undergraduate students	21.23 (3.12)	241/512	Hungary (midlatitude)	753	Cohort	MCTQ
Kantermann et al 2014[25]	Male shift workers	43.3 (6.8)	43/0	Belgium (midlatitude)	43	Cross-sectional	MCTQshift
Miller et al 2015[44]	General population	42.89 (7.33)	197/211	Northern USA (midlatitude)	408	Cross-sectional	Actigraphy
Rutters et al 2014[28]	University students and employees	Male: 28.6 (9.4) Female: 26.9 (8.5)	67/78	The Netherlands (midlatitude)	145	Cross-sectional	Adapted MCTQ
Borisenkov et al 2015[32]	High school students	14.8 (2.6)	1517/1918	Northern European Russia (subarctic and arctic)	3435	Cross-sectional	MCTQ
Díaz-Morales and Escribano 2015[39]	High school students	14.1 (1.48)	371/425	Spain (midlatitude)	796	Cross-sectional	School Sleep Habits Survey
Jankowski 2015[34]	Undergraduate students	22.2 (1.9)	16/101	Poland (midlatitude)	117	Cohort	Open questions
Lin and Yi 2015[37]	Junior-high school students	Wave 2: 14.3 (0.48) Wave 3: 15.3 (0.47) Wave 4: 16.3 (0.47)	1368/1315	Taiwan (subtropical)	2683	Cohort	Open questions
Parsons et al 2015[23]	General population	38	NI	New Zealand (midlatitude)	815	Cross-sectional	MCTQ
Tavernier et al 2015[42]	Undergraduate students	19.01 (0.9)	268/674	Southwestern Canada (midlatitude)	942	Cohort	Adapted MCTQ
Wong et al 2015[26]	General population	42.7 (7.4)	210/237	Northern USA (midlatitude)	447	Cross-sectional	Actigraphy
Choi et al 2016[45]	Epileptic and healthy control participants	Epilepsy: 33.0 (8.24) Control: 33.6 (8.80)	155/135	South Korea (midlatitude)	290	Cross-sectional	Sleep diary

Calculation of SJL	Calculation of midpoint of sleep	Outcomes related to SJL	Study results[a]
MSF–MSW	Midpoint between sleep onset and sleep end	Sleep quality, use of stimulants	Higher probability of smoking
MSF–MSW	Midpoint between sleep onset and sleep end	Depression	Higher BDIas (BDI corrected for age and sex) scores when SJL >2 hours independent of smoking status; age group 31–40 years with mild to severe BDI scores suffered from higher SJL
MSF–MSW	Midpoint between sleep onset and sleep end	Obesity	Higher probability of being overweight (BMI ≥25) and weight increase in the overweight group
MSFsc–MSW	Midpoint between sleep onset and sleep end	Obesity	No association between SJL, BMI ≥25 kg/m^2, waist-to-hip ratio and abdominal obesity when controlled for health, lifestyle and biological factors
MSF–MSW	Midpoint between sleep onset and sleep end	Atherosclerotic risk	Positive correlation with heart rate, but no association with arterial stiffness measured by pulse wave velocity
MSF–MSW	Midpoint between sleep onset and sleep end	Aggression	Higher physical and verbal aggression scores measured by Buss–Perry Aggression Questionnaire
MSF–MSW	Midpoint between sleep onset and sleep end	Social rhythm and minor psychiatric symptoms	Inverse correlation with Index of Regularity of activities in bivariate, but not in multivariate analysis; no associations with SRQ-20
MSF–MSW	Midpoint between sleep onset and sleep end	Depression	No association with depressive symptoms assessed by BDI scores
MSF–MSW	Midpoint between sleep onset and sleep end	Academic performance	Poor academic grades during lecture term and better performance during exams period (no weekly fixed obligations)
MSF–MSW	Midpoint between sleep onset and sleep end	Metabolic risk	No associations with blood glucose, insulin, apolipoprotein-A and -B, HDL and LDL, total cholesterol, triglycerides, minimally oxidized LDL, C-reactive protein, IL-8 and 25-OH D
MSF–MSW	Midpoint between sleep onset and sleep end	Mediation in the relationship between positive affect rhythms and chronotype	No significance found in correlations of acrophase and amplitude of PANAS with CSM
MSF–MSW	Midpoint between sleep onset and sleep end	Adverse endocrine and cardiovascular risk profile	Subjects with ≥2 hours SJL had higher 5 hour cortisol levels, were more often physically inactive and had higher resting heart rate, compared with those who had ≤1 hour SJL; no differences in BMI, waist circumference and blood pressure
MSF–MSW	Midpoint between sleep onset and sleep end	Winter seasonal affective disorder	Higher winter scores of SPAQ in women
MSF–MSW	Midpoint between bedtime and rising time	Cognitive performance and academic achievement	Lower general cognitive ability; lower scores on Thurstone's primary mental abilities (except for verbal fluency); lower GPA. Greater effects on women
MSF–MSW	Midpoint between sleep onset and sleep end	Psychological well-being	Positive association with nervous mood, assessed by TA subscale of UMACL
Weekend bedtime–weekday bedtime	SJL calculation did not use midpoint of sleep	Conduct problems	Poor academic performance and defiant attitude assessed by open questions
MSF–MSW	Midpoint between sleep onset and sleep end	Obesity and metabolic disorder	Higher BMI and fat mass; higher probability of being obese and meet the criteria for metabolic syndrome
MSF–MSW	Midpoint between bedtime and wake time	Academic adjustment and substance use	No predictive effect on substance use or academic adjustment assessed by a subscale of the SACQ; greater substance use was a significant predictor of SJL
MSF–MSW	Midpoint between sleep onset and sleep end	Cardiometabolic risk	Higher triglycerides, fasting insulin, insulin resistance estimated by the HOMA-IR, waist circumference and BMI; lower HDL
MSF–MSW	Midpoint between sleep onset and sleep end	Prevalence in epileptic compared to healthy control subjects and among epilepsy subtypes	Larger SJL in healthy controls and in general epilepsy compared to focal epilepsy

(*Continued*)

Table 1 (*Continued*)

Study	Population	Age range/mean age (SD)	Number of males/ females	Geographic location	Sample size	Design	Instrument used to define SJL
Díaz-Morales et al 2016[24]	High school students	13.95 (1.69)	690/716	Spain (midlatitude)	1406	Cross-sectional	Open questions
Kolomeichuk et al 2016[28]	Schoolchildren and college students	10–18	808/858	Northern European Russia (subarctic)	1666	Cross-sectional	MCTQ
Polugrudov et al 2016[54]	General population	22 (2)	27/35	Northern Asian Russia (subarctic)	62	Cross-sectional	MCTQ
Sheaves et al 2016[20]	Undergraduate students	21 (20–23)[c]	612/780	Southern UK (midlatitude)	1403	Cross-sectional	MCTQ
Silva et al 2016[44]	Undergraduate students	21.6 (3.9)	92/112	Central Brazil (tropical)	204	Cross-sectional	Open questions
Yong et al 2016[41]	Day and shift workers	42 (10)	1831/643	Germany (midlatitude)	2474	Cross-sectional	Modified version of MCTQ

Notes: [a]Only outcomes related to SJL are described. [b]In the case of shift workers, midsleep on day shifts, night shifts and free days were considered. [c]Median age (IQR).
Abbreviations: 25-OH D, 25-hydroxyvitamin D; BDI, Beck Depression Inventory; BMI, body mass index; CAR, cortisol awakening response; CSM, composite scale of morningness; DASS-21, Depression, Anxiety and Stress Scales; FFQ, Food Frequency Questionnaire; GPA, grade point average; HDL, high-density lipoprotein; HOMA-IR, homeostatic model assessment of insulin resistance; IL-8, interleukin-8; IQR, interquartile range; LDL, low-density lipoprotein; MCTQ, Munich ChronoType Questionnaire; MSF, midpoint of sleep on free days; MSFsc, midpoint of sleep on free days corrected for sleep debt; MSW, midpoint of sleep on work days; NI, not informed; PANAS, positive affect negative affect schedule form; SACQ, Student Adaptation to College Questionnaire; SD, standard deviation; SJL, social jetlag; SPAQ, Seasonal Pattern Assessment Questionnaire; SPEQ, Specific Psychotic Experiences Questionnaire; SRQ-20, Self-Report Questionnaire-20; STAI, state–trait anxiety inventory; TA, tense arousal; UMACL, University of Wales Institute of Science and Technology mood adjective checklist; WAI, work ability index.

Calculation of SJL	Calculation of midpoint of sleep	Outcomes related to SJL	Study results[a]
MSF–MSW	Midpoint between bedtime and rising time	Anxiety	No correlation found with the STAI
MSFsc–MSW	Midpoint between sleep onset and sleep end	Academic achievement	No influence in academic achievement (assessed by an open question) in a linear regression model together with average sleep duration, chronotype, age and sex
MSF–MSW	Midpoint between sleep onset and sleep end	Circadian system state, depression, anxiety, anger, obesity and cognitive function	Higher incidence of depression measured by BDI, higher wrist temperature at night and lower during the day, lower wrist temperature rhythm amplitude and a tendency for increase in CAR; no association with BMI, waist circumference, Spielberger Anxiety Inventory and Clinical Anger Scale
MSF–MSW	Midpoint between sleep onset and sleep end	Hallucinations, paranoia, anxiety, depression, mania and hypomania	No associations found for the following outcomes: hallucinations or paranoia assessed by subscales from the SPEQ; depression or anxiety, assessed by two subscales from the DASS-21; mania or hypomania assessed by the Mood Disorder Questionnaire
MSF–MSW	Midpoint between sleep onset and sleep end	Inadequate diet	Negative association with servings per day of beans assessed by the FFQ
MSF–MSW[b]	Midpoint between sleep onset and sleep end	Work ability	Poor WAI when SJL was associated with short sleep duration

7 ARTIGO 2

Is chronodisruption a vulnerability factor to stress?

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Abstract

Since circadian system modulates stress responses, rhythmicity can be a determinant of resilience/vulnerability to the development of psychiatric disorders. The aim of this study was to evaluate if the combination of shortened light-dark (LD) cycles and chronic mild stress (CMS) results in rhythm, behavioral and metabolic changes in Balb/c mice. In one experiment, mice were submitted to 3 weeks of CMS for rest-activity and temperature rhythms and body weight measurements. In experiment 2, sucrose preference test (SP), black and white test (BW) and dosage of metabolic parameters (serum glucose, triglycerides, total and HDL cholesterol) were performed after 2 weeks of CMS. The CMS protocol was applied alone (NL-S) or in combination to shortened LD cycles (L-S), and compared to mice submitted to shortened light-dark cycles alone (L-NS) or none (NL-NS) interventions. The relative amplitude of body temperature was increased after CMS by the combination of stress and shortened LD cycles, while body weight gain was only reduced by stress combined with shortened LD cycles. Increases in temperature amplitude were clearly associated with decreased body weight. No significant changes were seen in behavior. Our findings suggest that circadian disruption might increase the vulnerability to stress. This is of relevance considering the potential increased risk for developing psychiatric and/or metabolic disorders in individuals at circadian strain. The lack of effects in behavioral measures may be associated with methodological issues. Identifying suitable research designs to investigate our hypothesis that circadian disturbances may increase vulnerability to stress-induced depressive-like and anxiety-like behavior are warranted.

Keywords: chronic mild stress; sucrose preference; light-dark; rest-activity; temperature; allostasis.

1. Introduction

Circadian systems regulate physiological rhythms by synchronizing them to environmental signals, a process known as entrainment [1]. Since light is the main *zeitgeber* (environmental cue of passage of time), changes in light regimens can in turn lead to disruptions in the nexus of internal and external times [2–4]. It has been previously reported that mice under shortened light-dark (LD) cycles (10 h light, 10 h dark) present anxiety-like behavior and changes in the morphology of medial prefrontal neurons [5,6]. 10:10h LD cycles also resulted in increased body weight gain and increased levels of insulin and leptin in mice [6], suggesting that circadian disruption may lead to a deregulation of energy homeostasis mechanisms. Genetic disruption of rhythmicity by knocking-down clock genes in the suprachiasmatic nucleus (SCN) leads to increased body weight gain, immobility in the tail suspension test, behavioral despair and anxiety-like behavior [7], corroborating that circadian disruption might be an important contributing factor to the etiology of psychiatric and metabolic disorders.

Chronic stress and/or maladaptive responses to stressors are thought to prompt affective disorders, especially major depression. Studies using experimental models of chronic mild stress (CMS) [8,9] and early life stress [10] showed association between stress and depression-like behavior, changes in corticosterone levels and gene expression in the nucleus accumbens of mice. Even though stress is a well-established model to disturb homeostasis, some individuals are more resilient than others, but the factors that account for individual differences in stress vulnerability are only starting to be scrutinized [11,12]. The hypothesis that circadian modulatory systems may be a contributing factor to resilience/vulnerability to stress arises from the close relationship and interplay between the circadian and stress systems [13]. Given that most modulators of allostasis are rhythmic, circadian disruption could lead to impaired response to stress [14,15]. Accordingly, CMS protocols reduce the amplitude of clock genes rhythms in the SCN, as well as the rest-activity and body temperature rhythms amplitude in mice [8], though only susceptible animals display circadian alterations after a social defeat protocol [16] .

Although there is evidence that LD cycle manipulation and chronic mild stress can lead to depressive-like behavior in rodents [17,18] and increased cardiometabolic risk in experimental subjects and patients [19–21], to the best of our knowledge there are not studies investigating the combination of LD cycle changes and CMS. We reported that Balb/c mice, a

strain particularly sensitive to stress, are also more susceptible to shortened LD cycles than C57BL/6N or CF1 mice [22]. The aim of this study was to evaluate if the combination of shortened LD cycles and CMS results in rhythm, behavioral and metabolic changes in Balb/c mice. We hypothesized that exposure to shortened LD cycles increases vulnerability to CMS. The end point observations included circadian rhythmicity (rest-activity and body temperature rhythms), body weight gain, sucrose preference (as a measure of depressive-like behavior), black and white test (as a measure of anxiety-like behavior) and metabolic parameters (glucose, triglycerides, total and HDL cholesterol).

2. Materials and Methods

2.1. Mice

Male Balb/c mice aged 38 to 58 days (n = 56) were acquired from Universidade Federal de Pelotas. Mice were housed in the animal facility of Hospital de Clínicas de Porto Alegre (Unidade de Experimentação Animal, UEA - HCPA), under standard conditions (12:12h LD cycles) and in groups of 3 - 5 animals for 16 days before the beginning of the experiments. Animals were then isolated in transparent acrylic home-cages (Panlab Harvard Apparatus; 25 x 15 x 25cm) lined with zeolites, since the equipment for activity detection does not allow the use of wood shavings. Food and water were provided *ad libitum*. Animals remained in a photoperiod station with four separate independent chambers containing digital electronic timers (model TT34, COEL), which allow different photoperiod programming under the same controlled temperature (22 ± 2 °C), humidity and noise exposure. A total of 3 animals died due to surgical complications and undetermined causes. All procedures were carried out according to institutional policies on animal use in research. This study was approved by the Ethics Committee of the institution (#12-0313 GPPG/HCPA).

2.2. Study Design

Experimental design can be seen at Figure 1. Randomization aimed that the composition of all 4 experimental groups included at least one subject from each initial litters (3-5 mice), and none of the groups had more than 2 subjects from the same litter. Animals were randomized in such manner into 4 experimental groups:

- NL-NS (No Light, No Stress): no light manipulation (12:12h LD cycles) and no stress (CMS) protocol;

- L-NS (Light, No Stress): kept for 4 cycles under 10:10h LD cycles, but not submitted to the stress protocol;
- NL-S (No Light, Stress): no light manipulation, but subjected to the CMS protocol;
- L-S (Light, Stress): kept for 4 cycles under 10:10h LD cycles and submitted to the CMS protocol.

After the standard period of adaptation to the facilities (16 days), animals were isolated and remained undisturbed until LD cycles were advanced as follows: groups NL-NS and NL-S were advanced in 1 hour; L-NS and L-S were advanced in 9 hours, so that LD timing was the same for all groups after the 10:10h LD cycles. Animals underwent surgery for intraperitoneal (i.p.) implantation of temperature sensors (iButtons) 6-8 days after the LD cycles adjustment. Experiments begun after a week for recovery from this surgery.

Experiment 1: Effects of shortened LD cycles and CMS on circadian rhythms and body weight

Rest-activity and core body temperature rhythms were recorded for 45 days (n = 4 - 5). L-NS and L-S groups were exposed to 4 shortened LD cycles (10:10 h) starting on day 11. From day 13- 34, NL-S and L-S groups were submitted to 3 weeks of CMS. Rhythms recording proceeded from day 37-45, and animals were euthanized on day 45.

Starting on day 6, animals were weekly weighted for 6 weeks at key experimental times: before any intervention (week 1, day 6), before the CMS protocol (week 2, day 13), after the first and second weeks of CMS (week 3/day 20 and week 4/day 27, respectively), after the CMS (week 5, day 34) and after all interventions (week 6, day 41).

Experiment 2: Effects of shortened LD cycles and CMS on depressive-like and anxiety-like behavior

Mice were treated exactly as in Experiment 1. Sucrose Preference (SP) test was performed (day 10). After 10 undisturbed days L-NS and L-S groups were exposed to 4 shortened LD cycles (10:10 h). From day 13-27 NL-S and L-S groups were submitted to 2 weeks of CMS, so that behavior would be assessed at the time point where differences in weight were more pronounced as noted in Experiment 1. On day 28, the SP test was performed to assess depressive-like behavior, and on day 29, the Black and White (BW) test

was performed to assess anxiety-like behavior. On the following day, animals were fasted for 4h and blood samples were collected at euthanasia. Animals were weighted throughout the 4 weeks of experiment at the same time points as Experiment 1: week 1 at day 6; week 2 at day 13; week 3 at day 20; and week 4 at day 27.

2.3. Rhythm Characterization

2.3.1. Rest-activity: The activity rhythm of each animal was monitored daily every 10 minutes by actigraphy (ADNplin-©Antoni Díez Noguera, Barcelona, CT, Spain). The actimeter emits two infrared beams that cross the animal's home-cage longitudinally and transversely and are detected by receivers on the opposite face of the cage. One locomotion unit is recorded when a beam is interrupted by the interposition of the animal. The data can be accessed with the DAS192USB software from a computer to where data is continually sent from each cage. Only Experiment 1 activity recordings from days 1 to 9 and 37 to 45 were analyzed since CMS procedures (days 13-34) interfered with the activity data acquisition.

2.3.2. Core body temperature: The temperature rhythm of each animal was measured by internal temperature sensors (Thermochron iButtons, Dallas, TX, USA) implanted into the peritoneal cavity. The surgery consisted in an incision of approximately 2 cm under isoflurane anesthesia (5% induction and maintenance of 2-3%); 4.0 Vycril thread was used for internal suture and 5.0 Nylon for external suture. Animals were kept at warmed incubators and under observation until full recovery and were returned to their home-cages. Analgesia was obtained with Tramadol (ip, 10mg/kg) every 12 hours on the day of surgery and on the two following days. Body temperature was recorded every 45 minutes. The iButtons were recovered after euthanasia and data were extracted through specific equipment and software (1-Wire® devices, Dallas, TX, USA). Only Experiment 1 temperature recordings from days 1 to 9, 13 to 34 and 37 to 45 were analyzed.

2.4. Light and Stress Interventions

2.4.1. Shortened Light-Dark Cycle

The LD cycle shortening protocol was adapted from Karatsoreos et al. [6], as described by Pilz et al. [5]. NL-NS and NL-S groups remained under 12:12h LD cycles throughout the experiment. From day 11 to day 13, L-NS and L-S groups were submitted to 4 cycles of 10:10h LD, after which they returned to 12:12h LD cycles for the rest of the experiment.

2.4.2. Chronic Mild Stress

The CMS protocol was adapted from Haridas et al. [9]. Animals were subjected daily to three different randomized stressors, in three periods of the subject' light phase, indicated in zeitgeber times (ZT). ZT 0 was considered the moment the lights were turned on and ZT 12 was the moment they were turned off. The three daily stressors were administered at the first half of light phase (ZT 3.5 to ZT 5.5), the second half of light phase (ZT 7.5 to ZT 9.5) and overnight (ZT 11.5 to ZT 2.5 of the following day).

Stressors (and its order) are described at Table 1. Briefly: Cold, in which mice were placed on icepacks bedding; Space Reduction, by placing a partition which limited the cage space to 50%; Cage Tilt, in 45°; Wet Bedding, by adding 100 ml of water to the zeolites; No Bedding, by placing the animal in a zeolites-free home-cage; Rat Bedding, using wood shavings previously used in rats home-cages; and Restraint Stress, performed with a perforated tube of 10 cm height and 3 cm diameter ending in a cone of 2 cm height.

2.5 Behavioral Tests

2.5.1 Sucrose Preference (SP) was performed according to Haridas et al. [9]. A decreased sucrose preference is considered a correlate of depressive-like behavior in rodents. Briefly, animals were habituated to the test equipment by having their usual drinking bottles replaced by two test bottles filled with tap water during the 24 hours preceding the test. A bottle containing 2% sucrose solution (Labsynth, Diadema, SP, Brazil) and another containing tap water were placed in the cage for 6 hours during the dark phase (ZT 15 to ZT 21). After the initial 3 hours, the position of the bottles was switched to avoid position specific bias [9]. Intake of sucrose and water were assessed by weighting the bottles before and after the 6 hours. Sucrose ingestion was calculated according to the following formula:

$$Sucrose\ Ingestion\ (\%) = \frac{sucrose\ intake\ (g)}{sucrose\ intake\ (g) + \ water\ intake\ (g)} \times 100$$

Sucrose ingestion variation was calculated by subtracting the sucrose ingestion at day 10 from the sucrose ingestion at day 28.

2.5.2 Black and White Test: BW was performed according to Costa-Campos et al [23]. Animals were habituated to the dimly lighted experimental room in their home-cages for 1 hour before the test (performed between ZT 17 and ZT 19). The black and white apparatus

consists of a wooden rectangular box (46×27×30 cm), divided into two compartments: one small (18×27 cm) and one large (27×27 cm), with an opening (7.5×7.5 cm) in the center of the partition at floor level. The small compartment is painted black and devoid of light, whereas the large compartment is white and illuminated with a 60-W cold light source during the experiment. Mice were individually placed in the center of the white compartment, facing away from the opening, and the test was video recorded for 5 minutes. The apparatus was cleaned with alcohol immediately after each test. Two investigators blinded to the experimental groups analyzed the videos with the Behavioral Observation Research Interactive Software (BORIS) [24]. Latency of the first crossing to the black compartment, time spent in the white zone and numbers of crossings between the white and black compartments were noted.

2.6 Body Weight Measurement

Animals were weekly weighted for 6 weeks in Experiment 1 and for 4 weeks in Experiment 2. Two animals of Experiment 1 died before all weight measures could be performed and were removed from the analysis. Body weight change (weight - initial weight) was calculated at each week. From day 13, after the shortening of LD cycles, ZT 0 occurred at the same time for all animals and weighting was performed at the light phase. However, the time when lights were turned on were different between groups until day 11, resulting in weighting during the light phase for NL-NS and NL-S groups and during the dark phase for L-NS and L-S groups.

2.7 Metabolic Parameters

Subjects from Experiment 2 were fasted for 4 hours (ZT 20 to ZT 0) on day 29 (after the BW test) and euthanized at the beginning of light phase on day 30. Euthanasia by decapitation was performed without anesthesia to avoid interference with blood analysis [25]. The order of euthanasia was randomized between groups to avoid time of blood collection bias and all animals were euthanized from ZT 0 to ZT 2 of day 30. Trunk blood was immediately collected in 0.8 mL sterile tubes with clot activator (MiniCollect®, Greiner Bio-One) and centrifuged at 3670×g for 10 min after approximately 20 min of clotting at room temperature. Serum was transferred to sterile cups and biochemical analyses of glucose, triglycerides, total cholesterol and HDL cholesterol were performed by the Department of Clinical Pathology of Hospital de Clínicas de Porto Alegre (Serviço de Patologia Clínica, SPC – HCPA) using the Cobas® 8000 c702 module chemistry analyzer (Roche Diagnostics International LTD.,

Rotkrewz, Switzerland). Serum glucose was measured using the enzymatic-ultraviolet test with hexokinase; triglycerides and total cholesterol were determined through colorimetric enzymatic methods; and HDL cholesterol was assessed by a homogeneous colorimetric enzymatic assay. All results are expressed as mg/dL of serum. One sample was lost in processing.

2.8 Data analysis

2.8.1 Serial analysis (circadian rhythms)

Rest-activity and temperature rhythms were evaluated using the integrated Chronobiology software El Temps (©Antoni Díez-Noguera, Barcelona, CT, Spain). Double-plotted actograms were generated for rest-activity and body temperature data. The actograms for each experimental group were derived from calculating the mean value for each time point. Waveform analysis was performed to compute the acrophase (estimated by fitting the data to a sinusoidal function and calculating the time when this function reaches its maximum value), the relative amplitude (the difference between the values of the least active period and the values of the most active period), the Intradaily Variability (IV, which quantifies the rhythm fragmentation) and the Interdaily Stability (IS, which estimates the synchronization to the 24 h LD cycle). Serial analyses were used to calculate the sum of activity and the mean of temperature in light and dark phases and in the total period of each day, which were then used to calculate the mean activity sum and the mean temperature of each desired period.

2.8.2 Statistics

Data from Experiment 1 was analyzed using non-parametric tests due to the reduced sample size (n = 4-5). For Experiment 2 (n = 8-9), tests were chosen according to results of Shapiro-Wilk's test of normality. One-way ANOVA followed by Tukey post-hoc test was performed to analyze sucrose ingestion pre and post-intervention, sucrose ingestion variation, serum glucose and serum triglycerides. Paired-samples t-test was performed to compare sucrose ingestion pre-interventions with ingestion post-interventions for each group. Kruskal-Wallis followed by Dunn's post-hoc and Bonferroni correction for multiple comparisons was used to compare serum total cholesterol, HDL cholesterol and rhythms parameters (relative amplitude, IV, IS, sum of activity and mean temperature). We chose not to perform correction for multiple comparisons in rhythms parameters analyses due to the small sample size for these variables (n= 4-5). Spearman's correlation was used to check for correlations between

body weight and relative amplitude of temperature rhythm. Generalized Estimating Equations (GEE) with linear distribution were used to evaluate the effects group and time and their interaction on body weight and body weight change; Bonferroni was used for pairwise comparisons. Levene's test was used to assess the variances on sucrose ingestion variation. Statistical analyses were performed using SPSS 23.0 software (SPSS Inc, Chicago, IL, USA), with statistical significance set at $p \leq 0.05$. Graphs were generated using Graph Pad Prism 6, with data presented as: mean ± standard deviation (SD) for variables with Gaussian distribution; median ± interquartile range (IQR) for non-parametric variables; and estimated marginal means ± standard error (SE) calculated for the GEE model of the interaction between group and time on body weight and on body weight change.

3 Results

3.5 Rest-activity rhythm

Rest-activity recordings for the 45 days of Experiment 1 are shown in double-plotted actograms for each experimental group in Figure 2. Due to actimeter malfunction, activity data for one animal could not be used. Data from the CMS period (days 13 to 34) demonstrates the interference of stressors on rest-activity recordings of NL-S and L-S groups.

At the period from day 1-9 of Experiment 1, rest-activity IV, total sum of activity and sum of activity at dark phase were similar between groups (data not shown). However, all other rest-activity parameters analyzed differed between groups: 1) rest-activity acrophase (H_3=12.176, $p<0.01$), which occurred later for L-NS than for NL-NS group ($p<0.01$) and NL-S group ($p<0.05$) and also occurred later for L-S than for NL-NS group ($p<0.05$) and NL-S group ($p<0.05$); 2) rest-activity IS (H_3=9.361, $p<0.05$), which was higher in L-NS group than in NL-NS group ($p<0.01$) and NL-S group ($p<0.05$); 3) sum of activity at the light phase (H_3=8.537, $p<0.05$), which was lower in L-NS than in NL-S group ($p<0.05$) and was also lower in L-S group than in both NL-NS ($p<0.05$) and NL-S groups ($p<0.05$); and 4) rest-activity amplitude (H_3=12.461, $p<0.01$), which was higher in L-NS group than in both NL-NS ($p<0.005$) and NL-S groups ($p<0.01$) and also higher in L-S than in NL-NS group ($p<0.05$) (Figure 3a).

Rest-activity rhythm parameters (amplitude, acrophase, IV, IS, total sum of activity and sum of activity at light phase and at dark phase) could not be assessed from day 13-34 and did not differ between groups on the period from days 37-45 (data not shown).

3.6 Core body temperature rhythm

Core body temperature recordings for the 45 days of Experiment 1 are shown in double-plotted actograms for each experimental group in Figure 2. Due to iButton malfunction, temperature data of one animal could not be extracted.

At the first 9 days of Experiment 1, total mean temperature and mean temperature at light and at dark phases were similar in all groups (data not shown). Nonetheless, the following parameters were different: 1) temperature acrophase (H_3=13.596, $p<0.005$), which occurred later in L-S group than in both NL-NS ($p<0.005$) and NL-S groups ($p<0.05$) and also occurred later in L-NS than in NL-NS group ($p<0.01$); 2) temperature IV (H_3=12.429, $p<0.01$), which was lower in L-S group than in NL-NS ($p<0.01$) and NL-S groups ($p<0.05$) and was also lower in L-NS group than in both NL-NS ($p<0.01$) and NL-S groups ($p<0.05$); 3) temperature IS (H_3=12.096, $p<0.01$), which was higher in L-S group than in NL-NS ($p<0.05$) and NL-S groups ($p<0.05$) and was also higher in L-NS group than in both NL-NS ($p<0.01$) and NL-S groups ($p<0.05$); and 4) temperature amplitude (H_3=10.088, $p<0.05$), which was higher in L-S group than in both NL-NS ($p<0.05$) and NL-S groups ($p<0.01$) (Figure 3b).

Concerning the period from day 13 to day 34, temperature IV, total mean temperature and mean temperature at light and at dark phases did not differ between groups (data not shown). Temperature IS was different between groups (H_3=11.469, $p<0.01$), being lower in L-NS group than in both stressed groups, NL-S ($p<0.005$) and L-S ($p<0.01$). Temperature acrophase differed among groups (H_3=12.176, $p<0.01$), occurring earlier for L-S group compared to both non-stressed groups, NL-NS ($p<0.01$) and L-NS ($p<0.005$) and also earlier for NL-S group compared to L-NS group ($p<0.05$). Lastly, temperature amplitude also differed between groups (H_3=12.523, $p<0.01$) and was significantly lower in L-NS group than in both stressed groups, NL-S ($p<0.005$) and L-S ($p<0.005$) (Figure 3b).

In the last 9 days of Experiment 1 (days 37-45), most temperature rhythm parameters (acrophase, IV, IS, total mean temperature, mean temperature in light phase and in dark phase) were similar between groups (data not shown). However, temperature amplitude was different between groups (H_3=8.014, $p<0.05$), being higher in L-S group than both NL-NS ($p<0.05$) and L-NS groups ($p<0.05$) (Figure 3b).

3.7 Body weight

Body weight measures from Experiment 1 and 2 are depicted in Figure 4 (4a and Figure 4b, respectively). Concerning Experiment 1, the GEE analysis showed a significant effect of group (Wald X^2=9.405, df=3, p<0.05), time (Wald X^2=462.465, df=5, p<0.001) and interaction of these factors (Wald X^2=398493.52, df=14, p<0.001). Pairwise comparisons of the interaction groups*time (Figure 4a) revealed that L-S group had lower body weight than both non-stressed groups (NL-NS and L-NS) throughout the three weeks of CMS (week 3: Bonferroni: L-S vs. NL-NS, p<0.05; L-S vs. L-NS, p<0.01; week 4: Bonferroni: L-S vs. NL-NS, p<0.05; L-S vs. L-NS, p<0.01; week 5: Bonferroni: L-S vs. NL-NS, p<0.005; L-S vs. L-NS, p<0.05).

Regarding Experiment 2, GEE analysis did not show effects of group (Wald X^2=3.901, df=3, p>0.05), but significant effect of time (Wald X^2=67.763, df=3, p<0.001) and the interaction of group and time (Wald X^2=108.075, df=9, p<0.001). However the post-hoc is unable to determine differences among groups due to lack of statistical power.

Body weight change analyses showed similar results to the ones observed in body weight and these results are depicted in Figures 4c and 4d (Experiment 1 and Experiment 2, respectively) to facilitate visualization.

3.8 Correlation between temperature rhythm and body weight

Correlations between temperature relative amplitude and weekly body weight can be seen at Figure 5. There was a significant negative correlation between temperature amplitude at days 13 to 34 and body weight assessed at week 5 (Spearman's r = -0.584, p<0.05), suggesting the effects of CMS on temperature rhythm. No significant correlation was found at pre (days 1-9 and weight at week 1 [Spearman's r = 0.13, p>0.05]) or post (days 37-45 and weight at week 6 [Spearman's r = -0.239, p>0.05]) stress periods.

3.9 Sucrose ingestion

Sucrose ingestion at day 10 was similar to sucrose ingestion at day 28 for all groups (data not shown). Sucrose ingestion was similar among groups before and after interventions (data not shown); groups were also similar in regard to sucrose ingestion variation ($F_{3,31}$=0.306, p>0.05) (Figure 6a). ANOVA showed that sucrose ingestion variation was not different among groups ($F_{3,31}$=4.997, p>0.05) and pairwise Levene's tests showed that L-NS

group displayed higher variance as compared to NL-NS ($F_{1,16}$=12.1, p<0.005) and NL-S ($F_{1,15}$=6.272, p<0.05) groups.

3.10 Black and white

Although all animals of groups NL-S and L-S moved between the compartments of the BW box during the test, several animals from the NL-NS group and most animals from L-NS group never left the compartment where they were initially located (Figure 6b). This fact skewed the results (with obvious longer time the white compartment and fewer crossings) preventing a sensible analysis.

3.11 Metabolic parameters

No significant differences were found between groups regarding fasting serum glucose ($F_{3,30}$=2.426, p>0.05), triglycerides ($F_{3,30}$=0.721, p>0.05) and total cholesterol (H_3=6.179, p>0.05); only HDL cholesterol levels were statistically different (H_3=11.159, p<0.05), being higher in NL-S than in L-NS group (p<0.05).

4 Discussion

The relevance of discerning if rhythm disruption can increase vulnerability to stress is unequivocal given that the majority of human populations are now frequently exposed to artificial irregular LD cycles and stress. We used an animal model that combined established circadian-disruption (shortened LD cycles) [5,6] and chronic mild stress [9] protocols. The study shows that: (1) stress increased temperature amplitude, an effect lasting longer than the stress period per se only when CMS was combined with shortened LD; (2) the combination of shortened LD cycles and stress, but none of these alone, caused a decrease in body weight gain; (3) increases in temperature amplitude were clearly associated with decreased weight gain; (4) shortened LD cycles significantly increased variability in the sucrose preference test.

The study shows that the combination of altered light-dark cycles and stress had a higher impact on temperature rhythms than either shortened LD cycles or stress alone. During the CMS temperature amplitude was higher in the L-S group than in the group with shortened LD cycles alone; after the CMS, only the group exposed to both shortened LD cycles and CMS displayed higher temperature amplitude in comparison to non-stressed groups. Suggestive of a meaningful relationship between the clock control of temperature rhythms and weight gain, it is reported that obese women display a flattened 24 h rhythm of wrist temperature in

comparison to normal-weight women [26]. Supporting the existence of such relationship and its interaction with stress, our data show an inverse correlation between temperature amplitude and body weight during the CMS period, but not before or after it. A limitation of this rationale is the small sample size of this study, which prevented correction for multiple comparisons. Nevertheless, since statistical significant differences were seen despite the small samples the findings still seem germane. No differences in rest-activity rhythms were observed among experimental groups after the stress period in experiment one; rest-activity rhythms could not be assessed during the CMS since most stressors directly interfere on actigraphy recordings. The groups that were later exposed to 10:10h LD cycles were different from the others groups even before being submitted to the shortening of LD cycles and CMS. A possible explanation is the adjustment to the phase advanced 9h before the experiment, or that environment disturbances (e.g., animal facility maintenance) were less disturbing to those in 10:10h LD cycles.

A significant finding of this study is the interaction of circadian disturbance and stress on weight gain. Animals exposed to shortened LD cycles were the most affected by the CMS protocol, with lower body weight gain during the three weeks CMS in comparison to non-stressed or just shortened LD cycle groups. Stress or the administration of the stress hormone glucocorticoids (GCs) induce weight loss or reduced weight gain [8,18,27,28]. On the contrary, when sustained for longer periods (over 6 weeks), circadian disruption induce body weight gain [6,7]. The desynchronization protocol used in this study (shortened LD for 3-4 cycles) induced alterations in the body weight regulation mechanisms in a way that aggravated the CMS-induced decrease in weight gain. These findings were replicated in experiment 1 and 2, supporting a meaningful impact of circadian system perturbations on stress-induced changes in weight gain. Stress-induced hyperactivity (hence increased energy expenditure) during the CMS could be a possible explanation for the reduction in body weight gain observed in the stressed groups [29]. The literature points also to a negative effect of stress on food consumption and weight gain in rodents in the absence of access to high calorie food [30,31]. Though there were no biologically relevant differences among groups in the metabolic parameters analyzed, the research design used in this study does not illuminate if the change in weight gain is the result of metabolic changes or food ingestion.

CMS is an animal model extensively used in the study of depression as well as the effects of antidepressants [32]. Nevertheless, the method is difficult to establish and often fail to replicate even in the same research group [33]. The lack of a standard CMS protocol adds

difficulties to compare results and to replicate findings [34]. The impact of CMS on sucrose preference seems to be especially inconsistent, with some animals displaying decreased while others showing increased preference [32]. A potential explanation to the opposite outcomes often observed in CMS is the resilience/ vulnerability theories. In fact, it has been shown that only susceptible animals display reduced sucrose preference after social defeat stress [16] and CMS [33]. We did not observe changes in sucrose ingestion among groups, though shortened LD cycles clearly increased variability in this behavior. The lack of CMS effect on sucrose ingestion may result from an insufficient CMS duration, stressors not sufficiently deleterious, a sample mainly comprised of resilient mice, difficulties with measuring sucrose ingestion, or a combination of these factors. Due to the lack of a clear effect of CMS (NL-S), our hypothesis that shortened LD cycles would increase vulnerability to CMS cannot at this point be accepted or rejected. The meaning of the finding that shortened LD cycles increase sucrose ingestion variability remains to be explored.

Results from the BW, aimed to assess anxiety-like behavior, were skewed by the high number of animals in non-stressed groups (NL-NS and L-NS) that did not leave the white compartment during the test. It has been shown that Balb/c mice naïve to the BW test display freezing behavior upon being placed in the white compartment and take longer than 5 min (the time limit of our BW protocol) to overcome the freezing and begin exploring the apparatus [35]. Because the white compartment has a lid that is closed during the test session, at this point we can only hypothesize that the high number of non-stressed animals that remained in the white compartment were in a freezing state. Mice from the NL-S and L-S groups had no trouble in moving between compartments. The CMS protocol may have induced a change in behavior in these animals, leading to a shift in coping strategies from passive to active, resulting in hyperactivity [36].

In testing if chronodisruption can be a vulnerability factor to stress, we show that body temperature amplitudes were only increased after CMS when combined with shortened LD cycles and, likewise, that body weight was only reduced by stress if preceded by shortened LD cycles. There was a clear association between increase in temperature amplitude and reduced body weight. These findings suggest that altered circadian cycles, for instance by the use of artificial lighting, might increase vulnerability to stress. The finding is of relevance considering the potentially increased risk for the development of psychiatric and/or metabolic disorders in individuals at circadian strain. Identifying suitable research designs to further

investigate our hypothesis that chronodisruption may increase vulnerability to stress-induced depressive-like and anxiety-like behaviors are warranted.

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FIGURES AND TABLES

Table 1. Stressors applied every week of chronic mild stress.

	1st half (ZT 3.5 - 5.5)	**2nd half** (ZT 7.5 - 9.5)	**Overnight** (ZT 11.5 - 2.5)
Day 1	Cold	Cage-Switch	Space Reduction
Day 2	Cage Tilt	Wet Bedding	No Bedding
Day 3	Space Reduction	Restraint Stress	Rat Bedding
Day 4	No Bedding	Space Reduction	Cage Tilt
Day 5	Cage-Switch	Cold	Space Reduction
Day 6	Wet Bedding	Cage Tilt	No Bedding
Day 7	Restraint Stress	No Bedding	Rat Bedding

ZT: zeitgeber time. ZT 0: lights on.

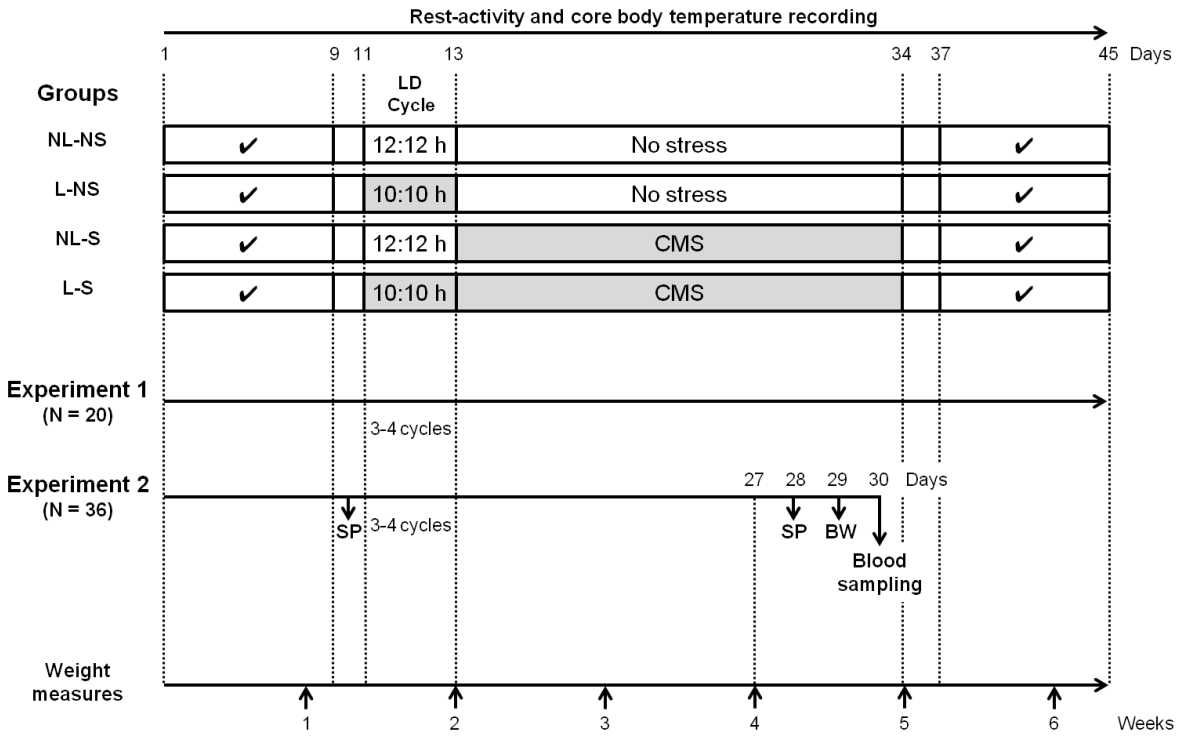

Figure 1. Experimental design. NL-NS: No Light, No Stress; L-NS: Light, No Stress; NL-S: No Light, Stress; L-S: Light, Stress; LD: Light-Dark; CMS: Chronic Mild Stress; SP: Sucrose Preference Test; BW: Black and White Test.

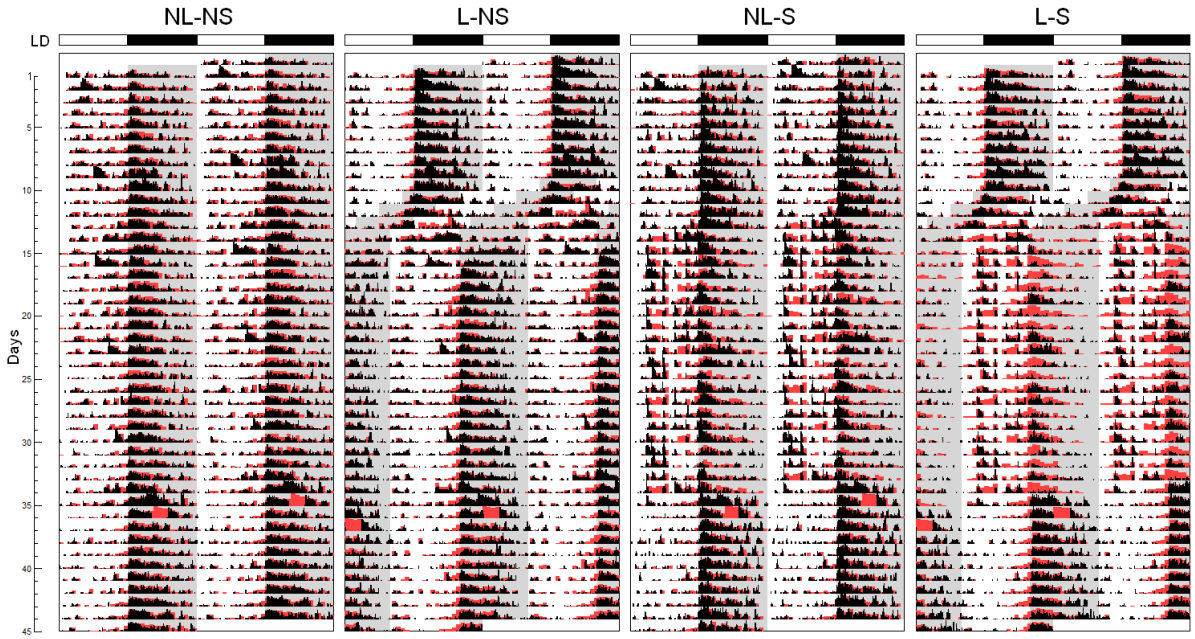

Figure 2: Effect of shortened LD cycles and/or stress in rest-activity and body temperature rhythms. Double-plotted actograms derived from experimental groups, depicting daily variance of rest-activity (black) and core body temperature (red) rhythms throughout the experiment. Lights-off periods are shown in gray. NL-NS: No Light, No Stress; L-NS: Light, No Stress; NL-S: No Light, Stress; L-S: Light, Stress; LD: Light-Dark. n = 4-5.

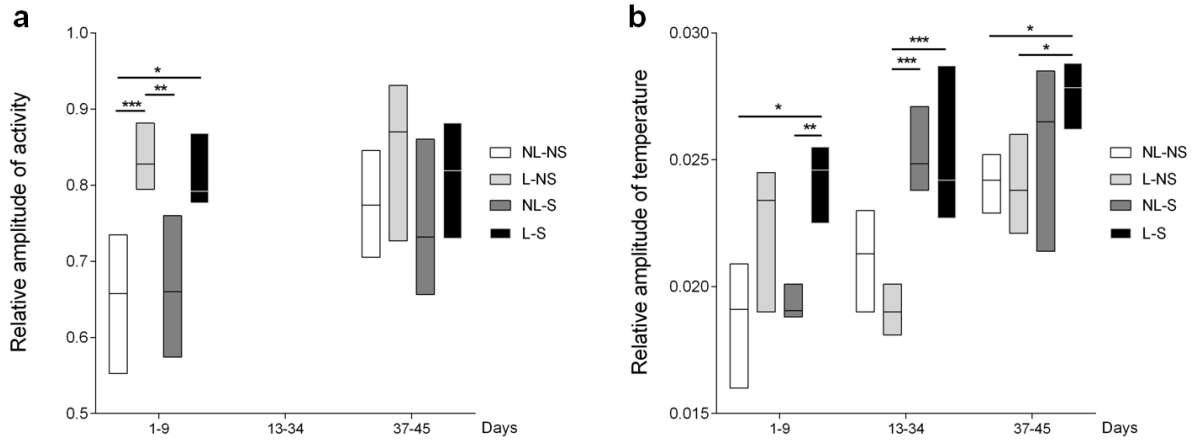

Figure 3: Rest-activity and body temperature amplitude. Relative amplitude of rest-activity (a) and of body temperature (b) rhythms for each group from days 1 to 9 (before interventions), 13 to 34 (when stressed groups underwent CMS) and 37 to 45 (after interventions). NL-NS: No Light, No Stress; L-NS: Light, No Stress; NL-S: No Light, Stress; L-S: Light, Stress; CMS: Chronic Mild Stress. n = 4-5. Data expressed as median ± minimum and maximum values. *p<0.05, **p<0.01, ***p<0.005, Kruskal-Wallis/Dunn.

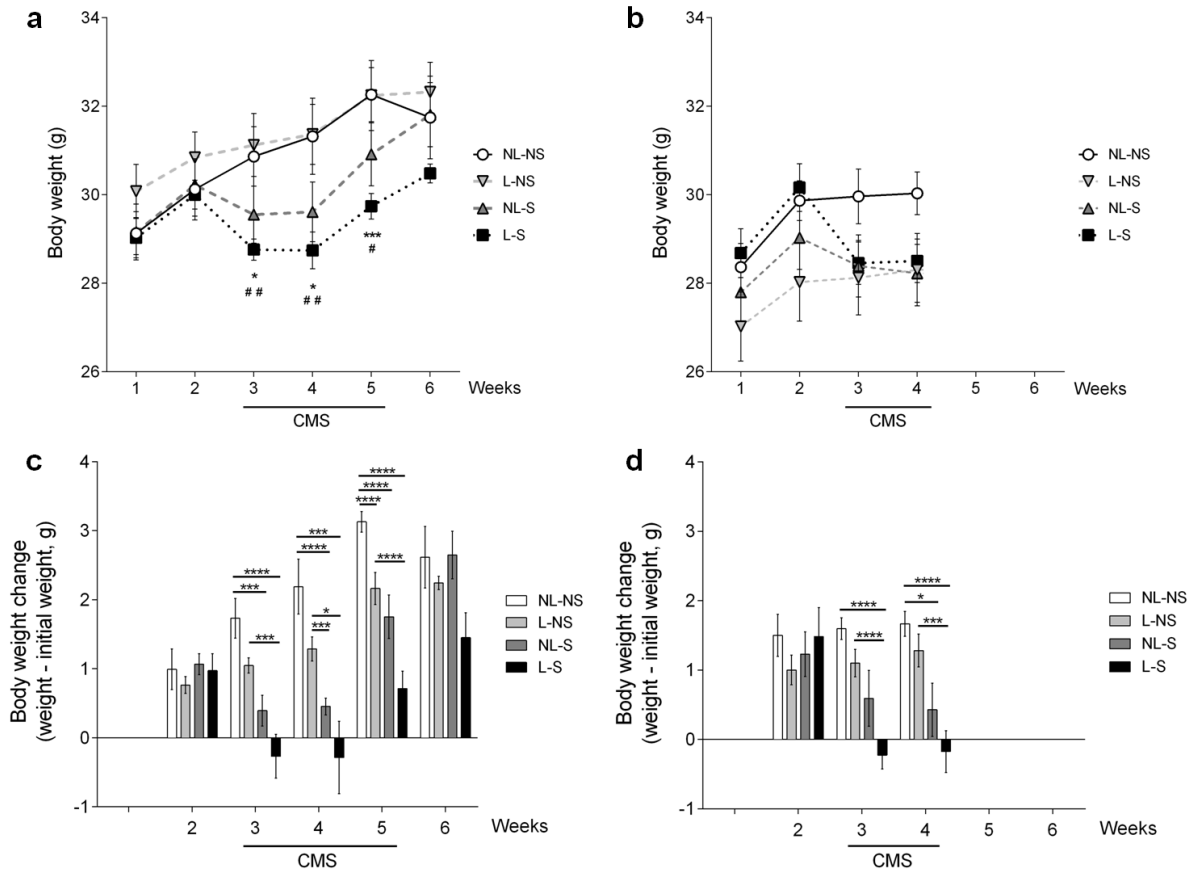

Figure 4: Body weight. Body weight and body weight change for Experiment 1 (a, c) and Experiment 2 (b,d). NL-NS: No Light, No Stress; L-NS: Light, No Stress; NL-S: No Light, Stress; L-S: Light, Stress; CMS: Chronic Mild Stress. Experiment 1: n = 4-5. Experiment 2: n = 9. Data expressed as estimated marginal mean ± standard error calculated for the GEE model of interaction group*time. *Body weight:* *p<0.05, L-S compared to NL-NS; ***p<0.005, L-S compared to NL-NS; $^{\#}$p<0.05, L-S compared to L-NS; $^{\#\#}$p<0.01, L-S compared to L-NS, GEE/Bonferroni. *Body weight change:* *p<0.05, ***p<0.005, ****p<0.001, GEE/Bonferroni.

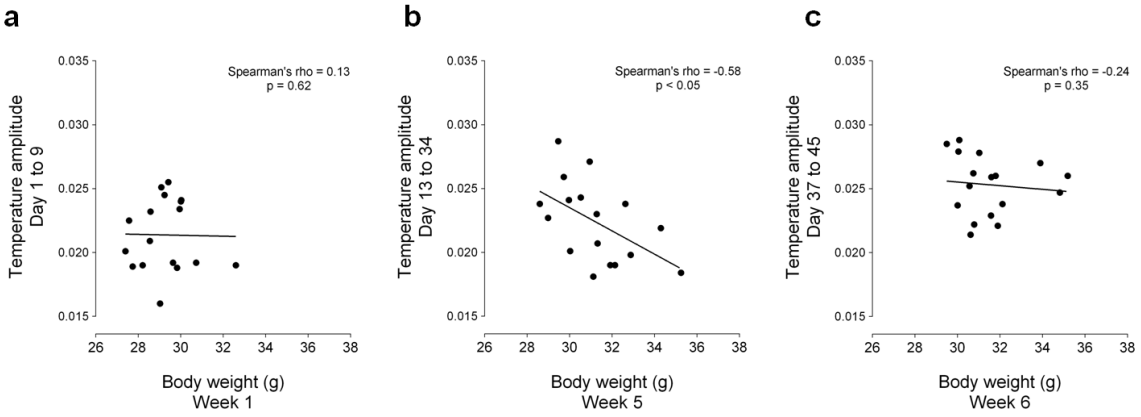

Figure 5: Correlation between temperature amplitude and body weight. Correlations between (a) temperature amplitude from the beginning of the experiment (days 1 to 9) and body weight assessed at week 1, (b) temperature amplitude from the period when stressed groups underwent CMS (days 13 to 34) and body weight measured at week 5 and (c) temperature amplitude at the end of the experiment (days 37 to 45) and body weight assessed at week 6. NL-NS: No Light, No Stress; L-NS: Light, No Stress; NL-S: No Light, Stress; L-S: Light, Stress; CMS: Chronic Mild Stress. N = 17. Spearman's correlation.

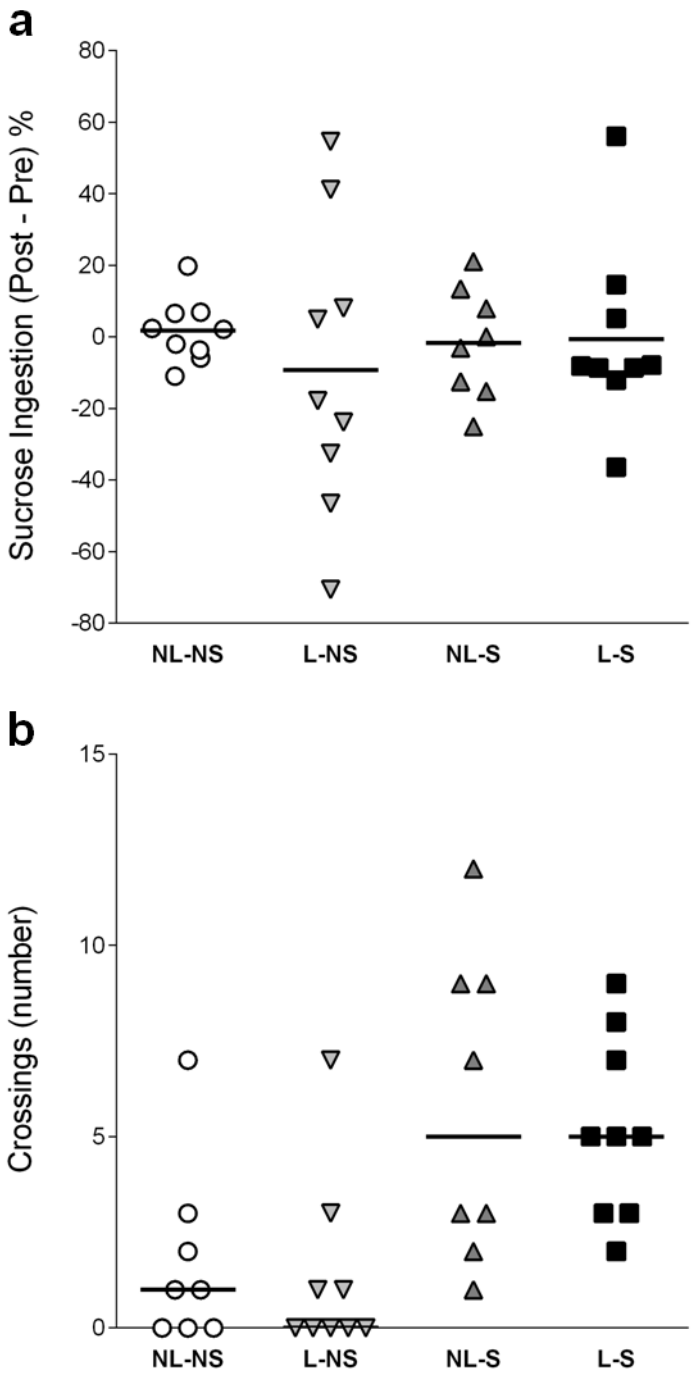

Figure 6: Depression- and anxiety-like behavior. (a) Sucrose ingestion in the sucrose preference test. NL-NS: No Light, No Stress; L-NS: Light, No Stress; NL-S: No Light, Stress; L-S: Light, Stress. Data expressed as mean and individual data points for each group. n = 8-9. Variance was higher in L-NS group than in NL-NS ($p<0.005$) and NL-S groups ($p<0.05$), Levene's test. (b) Number of crossings between compartments in the black and white test. Data expressed as median and individual data points for each group. n = 8-9.

8 DISCUSSÃO

O ritmo da vida na sociedade contemporânea tem sido ditado principalmente pelo mercado de trabalho que, diante dos grandes avanços tecnológicos das últimas décadas, exige uma produtividade igualmente avançada dos trabalhadores, sejam eles chefes ou funcionários. Em função desta demanda, a organização social também tem se adaptado de modo a estabelecer um ambiente que permita a otimização da mão de obra e a maior produtividade a todo momento, influenciando profundamente a relação do ser humano com o relógio, ditador do tempo social. Os horários de início das aulas nas escolas, por exemplo, costumam ser muito mais cedo do que os horários de despertar naturais das crianças e adolescentes que nelas estudam (54). Essa interrupção do sono dos estudantes, porém, é necessária para manter a estrutura da sociedade, uma vez que os horários das escolas são resultado da necessidade dos pais de deixarem seus filhos sob os cuidados dos professores para poderem ir cumprir sua rotina de trabalho. Além disso, buscamos otimizar a produção criando jornadas de trabalho que se sobrepõem de modo a manter a população trabalhando 24 horas por dia e 7 dias por semana. Para isso, indivíduos se submetem a trabalhos noturnos, trocando o dia pela noite e, muitas vezes, sacrificando seu bem estar e sua saúde para executar funções essenciais ao funcionamento da sociedade em que hoje vivemos (49).

Essa nova relação do homem com o tempo tem nos distanciado cada vez mais das condições naturais em que nossos antepassados viveram e para as quais nosso organismo se adaptou ao longo de milhares de anos de evolução. Antigamente, nossos horários de dormir e descansar e de acordar e produzir eram regulados pelo nascer e pelo pôr do sol, uma vez que nosso trabalho era principalmente voltado à subsistência e a escuridão à noite representava um grande perigo e vulnerabilidade a predadores. Hoje em dia, porém, permanecemos em ambientes fechados e iluminados durante o dia e durante a noite com o mesmo tipo de iluminação artificial e nos tornamos algo como “mestres do tempo”, podendo decidir a duração de nossos dias e noites (48). Porém, o fator que de fato decide a duração de nossos dias e noites é a estrutura social atual que, frequentemente, não respeita as necessidades individuais e fisiológicas de cada um. Essa disparidade que surge entre o ritmo do ambiente e o ritmo biológico intrínseco do organismo – e que pode influenciar negativamente a saúde – é conhecida como cronorruptura, ocorrendo em diferentes intensidades dependendo da rotina de cada um, mas sendo um fenômeno sem distinção de idade ou classe social (53).

Um dos exemplos de cronorruptura mais difundido atualmente é o jetlag social, que representa a diferença oriunda das obrigações sociais entre os horários de dormir e acordar nos dias livres e nos dias de trabalho (56). Neste estudo, buscando melhor compreender as consequências da cronorruptura na saúde humana, executamos uma revisão sistemática das evidências de associação entre jetlag social e desfechos de saúde e comportamento. Verificamos que estudantes e trabalhadores de turno são populações mais suscetíveis ao jetlag social, mas que este fenômeno é um problema observado na população em geral. Apesar de haver heterogeneidade quanto à metodologia utilizada para aferir o jetlag social e o tipo de população estudada, as evidências apontam para a existência de associação entre o jetlag social e obesidade (apenas em habitantes de menor latitude), síndrome metabólica, sintomas depressivos em adultos, depressão sazonal e pior desempenho acadêmico e capacidade de trabalho. A partir destes resultados, podemos nos questionar o quanto a maneira como organizamos nosso tempo e nossas atividades pode estar prejudicando o desempenho de estudantes e trabalhadores e até mesmo contribuindo para os grandes índices atuais de obesidade, depressão e tentativas de suicídio.

Contudo, a cronorruptura não é o único fenômeno com potencial prejuízo à saúde ao qual nos expomos diariamente na sociedade contemporânea. Simultaneamente, o estresse psicológico causado pela competitividade e pela cobrança, tanto no ambiente de trabalho/escolar quanto em família ou em sociedade, é intenso e constante em todos os níveis socioeconômicos (76,77). Também há violência crescente nos centros urbanos, decorrente da grande desigualdade social e de políticas ineficientes de manutenção da segurança, promovendo uma sensação de insegurança e medo na população (78). Essa condição de estresse que vivenciamos atualmente representa um desafio diário ao nosso organismo, que geralmente é capaz de se adaptar, retornar ao equilíbrio e permanecer saudável (85). Porém, indivíduos sob rotinas inadequadas às suas necessidades fisiológicas já apresentam uma carga alostática devido às alterações nas funções do organismo em decorrência da cronorruptura. Devido à relação próxima existente entre o sistema de regulação dos ritmos biológicos e o sistema de resposta ao estresse, organismos expostos à cronorruptura poderiam, frente a um evento estressante agudo ou a condições de estresse crônico, atingir um estado de sobrecarga alostática e potencialmente desenvolver patologias, podendo ser o mecanismo por trás da grande prevalência de depressão e alterações metabólicas (10).

Apesar de esta hipótese já ter sido discutida na literatura, modelos animais até então foram desenvolvidos de modo a investigar apenas os efeitos individuais da cronorruptura ou do

estresse sobre os processos de saúde-doença. Neste estudo, buscamos desenvolver um modelo animal capaz de melhor mimetizar as condições vividas pelos seres humanos contemporâneos, expostos a ambos os fatores cronorruptura e estresse. Confirmando nossa hipótese, verificamos que apenas os animais expostos à combinação dos fatores apresentaram elevação na amplitude do ritmo de temperatura corporal e redução no ganho de peso e que estes desfechos estavam associados. Este resultado reforça a noção sobre a conexão entre os ritmos biológicos e o metabolismo e indica que o mecanismo pelo qual a cronorruptura está associada à obesidade e à síndrome metabólica poderia ser o desenvolvimento de vulnerabilidade aos efeitos do estresse. A hipótese de que este mecanismo de vulnerabilidade ao estresse em decorrência da cronorruptura poderia ser responsável pelo desenvolvimento de alterações de humor e de comportamento, como depressão e ansiedade, ainda deverá ser adequadamente testada.

9 CONCLUSÕES E CONSIDERAÇÕES FINAIS

Destacamos os seguintes achados dos estudos realizados para esta dissertação:

- Os desfechos de saúde e comportamento associados ao jetlag social são diversos (epilepsia, sintomas psiquiátricos menores, agressão e problemas de conduta, transtornos de humor, prejuízo cognitivo, uso de substâncias, risco cardiometabólico e perfil endócrino adverso), mas a grande variabilidade de metodologias e populações e o risco de viés dos estudos analisados tornam necessário cautela na análise crítica das evidências;

- A exposição à perturbação da ritmicidade circadiana (10h claro, 10h escuro) seguida de estresse crônico moderado resultou em aumento na amplitude do ritmo de temperatura, mantido mesmo após o término do protocolo de estresse, e redução do peso corporal, havendo uma clara associação entre estes desfechos.

Os achados do estudo experimental apresentam um potencial translacional, uma vez que:

- As evidências relacionadas à alteração no peso corporal sugerem haver um efeito de vulnerabilidade aos efeitos do estresse no metabolismo em organismos submetidos à cronorruptura;

- Parâmetros como a amplitude do ritmo de temperatura podem ser importantes na investigação dos mecanismos relacionados às alterações de peso em resposta ao estresse e à cronorruptura;

- Deve-se considerar um replanejamento das estruturas sociais atuais, de modo a reduzir a exposição dos indivíduos a condições de cronorruptura e potencialmente contribuir para a redução dos índices de alterações metabólicas;

Portanto, as nossas perspectivas são:

- Desenvolver um desenho experimental que permita investigar adequadamente o efeito da cronorruptura na vulnerabilidade aos efeitos do estresse sobre comportamentos tipo-depressivo e tipo-ansioso;
- Investigar o mecanismo envolvido na associação entre o aumento da amplitude do rimo de temperatura e a redução do peso nos animais expostos à cronorruptura e ao estresse;
- Desenvolver um modelo experimental que simule a condição de desalinhamento circadiano imposta pelo jetlag social, de modo a verificar a associação com os desfechos de saúde e comportamento verificados na revisão sistemática.

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ANEXO A – Carta de aprovação do projeto pela CEUA/HCPA referente ao Artigo 2

HOSPITAL DE CLÍNICAS PORTO ALEGRE RS

UFRGS UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

GRUPO DE PESQUISA E PÓS GRADUAÇÃO
COMISSÃO DE ÉTICA NO USO DE ANIMAIS

Certificamos que o projeto abaixo, que envolve a produção, manutenção ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto humanos), para fins de pesquisa científica, encontra-se de acordo com os preceitos da Lei nº 11.794, de 8 de outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA), e foi aprovada pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA) e pelas áreas de apoio indicadas pelo pesquisador.

Projeto: 120313 **Data de Aprovação do Projeto:** 12/06/2017

Título: ESTUDO DOS EFEITOS DO ESTRESSE NOS RITMOS CIRCADIANOS E DO ENVOLVIMENTO DESTES EM ESTADOS DEPRESSIVOS **Data de Término:** 31/12/2018

Pesquisador Responsável: MARIA PAZ LOAYZA HIDALGO

Equipe de pesquisa:
CAROLINE LUÍSA QUILES JULIANA CASTILHOS BEAUVALET LUÍSA KLAUS PILZ MELISSA ALVES BRAGA DE OLIVEIRA

Submissão	Documento	Espécie/Linhagem	Sexo/Idade	Qtd.	Data Reunião	Situação
12/12/2012	APROVAÇÃO	CAMUNDONGO - BALB/c	M/60dias	60	08/01/2013	APROVADO
24/09/2014	EMENDA		/	0	14/10/2014	APROVADO
03/12/2014	EMENDA		/	0	09/12/2014	APROVADO
21/06/2017	EMENDA	CAMUNDONGO - BALB/c	M/60dias	58	13/06/2017	APROVADO
15/12/2017	EMENDA		/	0	09/01/2018	APROVADO

Total de Animais: 118

Coordenador
Comissão de Ética no Uso de Animais

- Os membros da CEUA/HCPA não participaram do processo de avaliação onde constam como pesquisadores.
- Toda e qualquer alteração do Projeto deverá ser comunicada à CEUA/HCPA.
- O pesquisador deverá apresentar relatórios semestrais de acompanhamento e relatório final ao CEUA/HCPA.

ANEXO B – Comprovante de recebimento do Artigo 2 pela revista Behavioural Brain Research

Gmail

Juliana Beauvalet <juju.beauvalet@gmail.com>

Track your co-authored submission to Behavioural Brain Research

Behavioural Brain Research <EviseSupport@elsevier.com> 26 de fevereiro de 2018 12:32
Responder a: EviseSupport@elsevier.com
Para: juju.beauvalet@gmail.com

Dear Ms Beauvalet,

Submission no: BBR_2018_260
Submission title: Is chronodisruption a vulnerability factor to stress?
Corresponding author: Dr M. Paz Hidalgo
Listed co-author(s): Dr Elaine Elisabetsky, Ms Juliana Beauvalet, Ms Luísa Pilz

Dr Hidalgo has submitted a manuscript to Behavioural Brain Research and listed you as a co-author. This email is to let you know we will be in contact with updates at each decision stage of the submission process.

The link below takes you to a webpage where you can sign in to our submission system using your existing Elsevier profile credentials or register to create a new profile. You will then have the opportunity to tailor these updates and view reviewer and editor comments once they become available.

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Behavioural Brain Research

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ANEXO C – Abstracts em anais de eventos, apresentações e premiações

Pôster: Beauvalet, J. C.; Pilz, L. K. ; Elisabetsky, E. ; Hidalgo, M. P. L. Estudo do efeito do estresse e do fotoperíodo sobre ritmos de temperatura central e de atividade e repouso. 35ª Semana Científica do Hospital de Clínicas de Porto Alegre, Porto Alegre, RS, 2015.

Apresentação oral: Beauvalet, J. C.; Pilz, L. K. ; Elisabetsky, E. ; Hidalgo, M. P. L. Estudo do efeito do estresse e do fotoperíodo sobre ritmos de temperatura central e de atividade e repouso. XXVII Salão de Iniciação Científica da UFRGS, Porto Alegre, RS, 2015.

Premiação: Destaque na sessão Psiquiatra II do XXVII Salão de Iniciação Científica da UFRGS, 2015.

Apresentação oral: Beauvalet, J. C.; Pilz, L. K. ; Elisabetsky, E. ; Hidalgo, M. P. L. Shortened light-dark cycles enhance vulnerability to chronic mild stress. Congresso da Associação Brasileira do Sono, São Paulo, SP, 2016.

Pôster: Santos, D. P. S.; Beauvalet, J. C.; Pilz, L. K. ; Elisabetsky, E. ; Hidalgo, M. P. L. Estudo do efeito do estresse e do fotoperíodo sobre ritmos de temperatura central e de atividade e repouso. 36ª Semana Científica do Hospital de Clínicas de Porto Alegre, Porto Alegre, RS, 2016.

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