UNIVERSIDADE FEDERAL DO PARANÁ

BRUNO JACSON MARTYNHAK

ESTUDO DA RELAÇÃO ENTRE RITIMICIDADE CIRCADIANA E COMPORTAMENTO TIPO-DEPRESSIVO

CURITIBA 2015

BRUNO JACSON MARTYNHAK

ESTUDO DA RELAÇÃO ENTRE RITIMICIDADE CIRCADIANA E COMPORTAMENTO TIPO-DEPRESSIVO

Tese apresesentada como requisito parcial para a obtenção do grau de doutor em farmacologia, Curso de Pós-Graduação em Farmacologia, Setor de Ciências Biológicas, Universidade Federal do Paraná.

Orientador: Roberto Andreatini

CURITIBA 2015

ATA DO JULGAMENTO DA 32ª DEFESA DE TESE DE DOUTORADO

Ao décimo segundo dia do mês de dezembro do ano de dois mil e quatorze, às nove horas, no Auditório do Departamento de Farmacologia, Anexo I, do Setor de Ciências Biológicas da Universidade Federal do Paraná, reuniu-se a Comissão Examinadora da Tese de Doutorado do Programa de Pós-Graduação em Farmacologia, de autoria do pós-graduando BRUNO JACSON MARTINHAK, intitulada "ESTUDO DA RELAÇÃO ENTRE RITIMICIDADE CIRCADIANA E COMPORTAMENTO TIPO-DEPRESSIVO.", sob orientação do Prof. Dr. Roberto Andreatini e banca composta por: Prof. Dr. Roberto Andreatini (Presidente – Farmacologia – UFPR), Prof.ª Dr.ª Janaína Menezes Zanoveli (Farmacologia – UFPR), Prof. Dr. Marcelo de Meira Santos Lima (Fisiologia – UFPR), Prof.ª Dr.ª Sâmia Regiane Lourenço Joca (Física e Química – USP) e Prof. Dr. Reinaldo Naoto Takahashi (Farmacologia – UFSC). A Banca Examinadora iniciou os trabalhos e o candidato teve quarenta e cinco minutos para expor oralmente seu trabalho, sendo em seguida arguido durante quinze minutos por cada um dos membros da Banca, e tendo trinta minutos para responder a cada uma das arguições. No final a Comissão Examinadora emitiu o seguinte parecer: Aprovado. Para a publicação o trabalho deverá sofrer as modificações sugeridas que serão conferidas por seu orientador. Nada mais havendo a tratar, o Presidente deu por encerrada a sessão, da qual foi lavrada a presente ata, que será assinada pelo Presidente e pelos demais membros da Comissão Examinadora, em Curitiba, 12 de dezembro de 2014.

Prof. Dr. Roberto Andreatini (Presidente – Farmacologia – UFPR)

Prof.ª Dr.ª Janaína Menezes Zanoveli (Farmacologia – UFPR)

Prof. Dr. Marcelo de Meira Santos Lima (Fisiologia – UFPR)

Prof.ª Dr.ª Sâmia Regiane Lourenço Joca (Física e Química – USP)

Prof. Dr. Reinaldo Naoto Takahashi (Farmacologia – UFSC)

Ministério da Educação
UNIVERSIDADE FEDERAL DO PARANÁ
Setor de Ciências Biológicas
Programa de Pós-Graduação em Farmacologia

TERMO DE APROVAÇÃO DA VERSÃO FINAL DE TESE

Declaro que aprovo a versão final da tese de doutorado do aluno Bruno Jacson Martynhak intitulada *"Estudo da relação entre ritimicidade circadiana e comportamento tipo-depressivo"*.

Curitiba, 21 de janeiro de 2015

Roberto Andreatini
Orientador
Departamento de Farmacologia
Setor de Ciências Biológicas
Universidade Federal do Paraná
randreatini@ufpr.br
41-3361 1538

AGRADECIMENTOS

Agradeço a todos os envolvidos, direta ou indiretamente, na realização deste trabalho e às bolsas fornecidas pela CAPES e Ciência sem Fronteiras.

APRESENTAÇÃO

Esta tese apresenta os resultados sob a forma de trabalhos publicados, submetidos e a serem submetidos. Após a Introdução e Objetivos Gerais, os itens Material e Métodos, Resultados, Discussão e Referências encontram-se em cada um dos artigos e representam a íntegra deste trabalho. Em sequência, são realizadas as Considerações Finais, seguidas de todas as referências utilizadas.

SUMÁRIO

RESUMO

O presente estudo buscou estudar a relação entre ritimicidade circadiana e comportamento tipo-depressivo em roedores. Animais expostos ao claro constante (LL) expressam o período circadiano endógeno da atividade locomotora com uma longa duração ou então deixam de apresentar ritimicidade. Neste estudo, estratégias que visam prevenir esta perda da ritimicidade também preveniram o comportamento-tipo depressivo. A exposição neonatal de ratos ao LL previne a perda da ritimicidade quando os mesmos são re-expostos ao LL durante a vida adulta. O mesmo ocorre quando há co-habitação entre um rato previamente exposto ao LL e outro que foi mantido em ciclo claro/escuro regular (LD). Observamos que a co-habitação também previne o comportamento tipo-depressivo quando os animais são expostos ao LL durante a vida adulta, desde que um dos ratos do par tenha sido previamente exposto ao LL neonatal. Além da luz, a disponibilidade de alimento é um dos mais importantes sincronizadores do sistema de temporização circadiana. Também observamos em ratos que a restrição alimentar (6 h de disponibilidade) previne o comportamento tipo-depressivo induzido pelo LL. Adicionalmente, quando o alimento é fornecido apenas durante a fase clara, mas não quando é fornecido apenas durante a fase escura, também promove comportamento tipo-anedônico. Em camundongos, a alimentação durante apenas a fase clara também promoveu comportamento tipo-depressivo, que não foi revertido por tratamento crônico com imipramina, agomelatina ou melatonina, mas sim por uma única administração de quetamina, uma droga com ação antidepressiva eficaz em pacientes refratários ao tratamento. A luz pode afetar o comportamento não apenas através da ritimicidade circadiana, mas também através de efeitos diretos. Os camundongos deficientes para o gene *Per3* (*Per3*$^{-/-}$) apresentam menor sensibilidade aos efeitos diretos da luz, especialmente quanto ao mascaramento, definido como inibição da atividade motora induzida pela luz. Quando expostos cronicamente à luz de baixa intensidade à noite, os camundongos *Per3*$^{-/-}$ se mostraram mais sensíveis aos efeitos tipo-depressivos em relação aos camundongos selvagem. Possivelmente este efeito ocorre devido ao menor comportamento de escape à luz (sono, fechar os olhos ou se enterrar no cepilho), fazendo com que os camundongos *Per3*$^{-/-}$ sejam mais expostos à luz, sofrendo mais seus efeitos diretos. Os efeitos da luz foram associados ao aumento da corticosterona plasmática, aumento da expressão hipocampal de *Bdnf* em ambos os genótipos e aumento da expressão hipocampal de *Tnf-α* apenas nos camundongos *Per3*$^{-/-}$, indicando que além da maior exposição à luz, fatores relacionados à resposta inflamatória e à sensibilidade à corticosterona também podem estar envolvidos com a maior sensibilidade ao efeito da luz de baixa intensidade à noite sobre o comportamento tipo-depressivo. Em conclusão, os dados apresentados sugerem que a manutenção da ritimicidade circadiana, bem como redução da poluição visual noturna podem ser fatores protetores para o desenvolvimento da depressão.

ABSTRACT

The present study sought to evaluate the relation between circadian rhythms and depressive-like behaviour in rodents. Animals exposed to constant light (LL) express either lengthened intrinsic circadian period of the locomotor activity or show arrythmicity. In this study, strategies aiming to prevent this loss of rhythmicity also prevented depressive-like behaviour. Neonatal exposure to LL prevents the arrythmicity when the animals are re-exposed to LL during adulthood and the same occurs with co-habitation between a rat previously exposed to LL with a rat kept under regular light/dark cycle (LD). We observed that co-habitation also prevents LL-induced depressive-like behaviour, as long as at least one rat of the pair had been previously exposed to LL during lactation. Besides light, food availability is one of the most important synchronizers of the circadian system. We also observed in rats that food restriction (6 h of food availability) prevents LL-induced depressive-like behaviour. Additionally, when food is provided only during the light phase, but not when it is provided only in the dark phase, also promotes anhedonic-like behaviour. In mice, food restriction also induced depressive-like behaviour, which was not rescued by chronic imipramine, agomelatine or melatonin treatment, but it was improved by a single administration of ketamine, a drug with antidepressant effect in refractory patients. Not only light affects behaviour through the circadian system, but also through a direct pathway. Mice deficient for the *Per3* gene (*Per3*$^{-/-}$) show reduced sensibility to the direct effects of light, particularly the masking effect, defined as light-induced inhibition of locomotor activity. *Per3*$^{-/-}$ were more sensitive to the depressive-like effect of being chronically exposed to dim light at light (5 lux) than the wild-type mice, possibly due to reduced escape behaviour, such as sleeping, closing the eyelids or burying themselves in the bedding, so that they were more exposed to light, suffering more its direct effects. The dim light at night effects were associated with increased plasmatic corticosterone and increased hippocampal *Bdnf* expression in both genotypes and increased hippocampal *Tnf-α* expression in *Per3*$^{-/-}$ mice only, indicating that factors related to inflammatory process and increased sensitivity to corticosterone might also be involved with the increased response to dim light at night in promoting depressive-like behaviour. In conclusion, the presented data suggest that maintenance of the circadian rhythms as well as reduction of light pollution can be protector factors for the development of depression.

INTRODUÇÃO

Transtornos de humor

Humor pode ser definido como uma emoção sustentada que influencia o comportamento de uma pessoa e a sua percepção do mundo, enquanto que a expressão do humor é denominada afeto (Feinstein *et al*, 1988)

Portanto, os transtornos de humor (ou transtornos afetivos) são transtornos psiquiátricos que envolvem alterações do humor, conforme o relato do paciente. O humor reduzido é a característica principal da depressão. Já o humor elevado é característica da mania. A separação em polos de humor levou aos termos depressão unipolar e depressão bipolar. A depressão unipolar é caracterizada pelo presença apenas do polo do humor deprimido, sem que ocorram episódios de mania. Além disso, existem evidências para casos de mania unipolar, sem a ocorrência de episódios depressivos (Yazici, 2014). De acordo com o DSM-5 (*Diagnostic and Statistical Manual of Mental Disorders - 5th Ed.*), a presença de cinco sintomas caracteriza a depressão maior (ver abaixo), enquanto que a presença de 2-4 sintomas caracteriza a distimia. Dentro do espectro de transtornos bipolar do humor, existem pelo menos outras três importantes classificações. Pacientes com transtorno bipolar tipo I apresentam episódios de mania completos, enquanto que pacientes tipo II apresentam episódios de mania menos intensos (hipomania). Quando o episódio de mania ocorre quatro ou mais vezes ao ano, o paciente é chamado de ciclador rápido. Sintomas depressivos e maníacos podem ocorrer dentro do mesmo quadro, sendo chamados de episódios mistos. Finalmente, o transtorno da ciclotimia é caracterizado por episódios de distimia intercalados por episódios de hipomania.

Importante ressaltar que nem todas as variações de humor são patológicas e que, assim como a distribuição de outras variáveis biológicas, como a altura, o humor também é sujeito a variações individuais dentro do que é considerado normal. Estas variações podem ser denominadas temperamento, e fazem parte da personalidade. Entretanto, alguns traços de temperamento estão associados com maior risco para desenvolvimento de

transtornos psiquiátricos. Por exemplo, o traço de esquiva a danos, caracterizado principalmente por pessimismo, é considerado fator de risco para transtornos do humor e de ansiedade (Mochcovitch *et al*, 2012).

Dados epidemiológicos da população europeia mostram alta prevalência em 12 meses para transtornos de ansiedade (14%) e transtornos de humor (7,8%) (Wittchen *et al*, 2011). A prevalência em 12 meses representa o percentual da população que dentro de um período de 12 meses é diagnosticada com o transtorno. Apenas a depressão unipolar apresenta prevalência de 6,8%, sendo aparentemente de considerável maior prevalência que os transtornos de humor do espectro bipolar. Entretanto, até o momento em que pacientes com o transtorno bipolar venham a apresentar o primeiro episódio de mania, estes são classificados dentro da depressão unipolar. Dados similares são encontrados para a população brasileira. Em uma revisão sistemática, a prevalência em 12-meses para depressão maior foi estimada em 8%, sendo maior em mulheres (11%) em comparação com homens (4%) (Silva *et al*, 2014). Além disso, a prevalência ao longo da vida foi estimada em 17% (Silva *et al*, 2014).

Segundo o DSM-5, para o diagnóstico da depressão maior, são necessários cinco dos sintomas listados abaixo, por ao menos duas semanas, sendo que é obrigatório que um deles seja humor deprimido ou anedonia:

1. Humor deprimido
2. Anedonia (redução na capacidade de sentir prazer). Perda de interesse em atividades
3. Perda ou ganho de peso. Diminuição ou redução do apetite
4. Insônia ou hipersonia
5. Retardo ou agitação psicomotora
6. Fadiga ou perda de energia
7. Sentimento de inutilidade ou sentimento de culpa
8. Redução na capacidade de se concentrar
9. Pensamentos recorrentes de morte ou ideação suicida

Como pode ser observado, diferentes combinações de cinco ou mais sintomas podem ser formadas desta lista com nove itens. Além disso, alguns

sintomas podem tomar direções opostas, como retardo ou agitação psicomotora. Desta forma, mesmo utilizando um diagnóstico padronizado, existe uma grande variabilidade psicopatológica em pacientes diagnosticados com depressão maior. O diagnóstico padronizado é uma ferramenta importante para nomenclatura e estudos clínicos. Entretanto, por exemplo, cinco sintomas é um número arbitrário, assim como o período de suas semanas de sintomatologia. Desta forma, a cada edição, o DSM é sujeito a críticas de especialistas (Nemeroff *et al*, 2013). Outros sintomas também são bastante relacionados com depressão, porém não se enquadram na lista dos nove itens, como por exemplo, o sentimento de desesperança (Maiden, 1987).

A lista de sintomas pode ser deceptiva quanto o impacto do episódio depressivo. Os pacientes em quadro depressivo descrevem os sintomas com uma qualidade distinta em relação a emoções normais de tristeza e luto (Ramos-Brieva *et al*, 1987). Frequentemente, os sintomas emocionais negativos são descritos em termos de dor agonizante. Em quadros graves, atividades do cotidiano, como se levantar da cama poder passar a ser excruciantes (Cabello *et al*, 2012). Assim, a perda de motivação ou interesse para atividades é ainda agravada pela dificuldade em se engajar em atividades devido à fraqueza ou perda de energia. Cerca de 2/3 dos pacientes apresentam ideação suicida e 10-15% cometem suicídio.

Além da morte por suicídio, ocorre maior morte por problemas cardiovasculares em pacientes deprimidos em comparação com a população sem transtornos de humor. Adicionalmente, ocorre a perda de anos de vida saudáveis devido à depressão. Para levar em conta tanto a morte prematura quanto a invalidez, foi criado um índice denominado anos de vida perdido ajustado pela invalidez (DALY, *disability adjusted life years lost*). Um DALY representa um ano de vida saudável perdido por morte prematura ou por se encontrar em estado de invalidez. O último relatório da Organização Mundial da Saúde (GBD, 2012) aponta a depressão unipolar como a maior causa de anos perdidos por invalidez, à frente de dor no pescoço e coluna lombar, representando 10,3% das causas de invalidez (Ferrari *et al*, 2013). O mesmo relatório aponta o transtorno bipolar ocupando o 17º lugar, atrás de esquizofrenia, ansiedade e uso de etanol.

A depressão maior não apresenta uma causa única bem definida. Assim como outros transtornos, tanto fatores genéticos e ambientais podem contribuir para o desenvolvimento do episódio. Apesar do fator genético ter participação no quadro depressivo, incluindo hereditariedade entre 30-40% (Sullivan *et al*, 2000), estudos de associação entre polimorfismos genéticos e depressão em pacientes falham frente a tentativas subsequentes de replicação (Bosker *et al*, 2011). Dentre os fatores não-genéticos envolvidos com o desenvolvimento do quadro depressivo, os eventos da vida, particularmente o estresse, se destacam. Entretanto, não são todos os indivíduos submetidos à situações/períodos de vida altamente estressantes que vem a desenvolver depressão. Estas pessoas são denominadas resilientes. A resiliência vem sendo bastante estudada em modelos animais de depressão, com forte associação à epigenética e à experiência durante a fase neonatal (Russo *et al*, 2012). Além disso, há pacientes com diagnóstico de depressão com ausência de um fator estressante bem delimitado (van Praag, 2004). Neste caso, diversos fatores externos, bem como genéticos podem interagir para o início e manutenção do quadro.

Tratamento e neurobiologia

O tratamento farmacológico para depressão usualmente envolve o aumento da transmissão monoaminérgica no sistema nervoso central (SNC). Desde a descoberta que os fármacos que aumentam a transmissão monoaminérgica melhoram o humor, ocorreu um grande investimento para a síntese de novas drogas com mecanismo similar. O inibidor da monoamino oxidase (IMAO) iproniazida (Zeller and Barsky, 1952) se mostrou eficaz em melhorar o humor dos pacientes, apesar de estar sendo testada para o tratamento da tuberculose (Selikoff and Robitzek, 1952). A imipramina também foi descoberta por acaso em testes devido à possível propriedade antipsicótica por apresentar estrutura similar à clorpromazina. Entretanto, observou-se que a imipramina, apesar de leve efeito sedativo, não apresentava ação antipsicótica. Porém, a imipramina melhorou o humor de pacientes deprimidos (Kuhn, 1958).

A imipramina, cujo mecanismo de ação é inibir a recaptação de serotonina e/ou noradrenalina na fenda sináptica, apresenta uma ampla gama de reações adversas, tais como constipação, sedação, ganho de peso e boca seca. Estas reações são em parte atribuídas à atuação não específica em outros sistemas de neurotransmissão, como antagonista dos receptores histaminérgicos, α-1 adrenérgicos e muscarínicos, além de bloquearem canais de sódio. Este último efeito pode levar à depressão cardíaca, apresentando grave risco em pacientes com insuficiência cardíaca (Glassman *et al*, 1993).

A partir do mecanismo de ação da imipramina e outros antidepressivos como a amitriptilina e clomipramina, novos antidepressivos mais seletivos para os transportadores de monoaminas foram desenvolvidos. Os inibidores seletivos da recaptação da serotonina (ISRS) incluem a fluoxetina, o citalopram, a paroxetina e a sertralina, enquanto que a reboxetina inibe seletivamente a recaptação de noradrenalina. A venlafaxina é um fármaco que inibe tanto a recaptação de serotonina como de noradrenalina. Além destes fármacos, também existem os chamados antidepressivos atípicos, os quais incluem a mirtazapina (antagonista α-2) e a trazodona (antagonista 5-HT_2).

O mecanismo de ação dos antidepressivos clássicos (i.e., aumento da neurotransmissão monoaminérgica) levou à hipótese monoaminérgica da depressão. De acordo com a versão original desta hipótese, a diminuição da sinalização monoaminérgica no sistema nervoso central levaria ao quadro depressivo. Além disso, a reserpina, um antigo antihipertensivo que depleta as reservas de monoaminas, produz sintomas depressivos em alguns pacientes. No entanto, embora a depleção experimental de monoaminas possa piorar o humor de pacientes deprimidos não medicados, o mesmo não ocorre com pacientes saudáveis, demonstrando que a simples redução de monoaminas não é o suficiente para induzir um quadro depressivo (Ruhe *et al*, 2007). Além disso, apesar do mecanismo de ação dos inibidores da recaptação de monoaminas, a tianeptina apresenta ação antidepressiva, ainda que seu efeito seja diminuir a neurotrasmissão monoaminérgica (Lucki and O'Leary, 2004), embora o efeito antidepressivo da tianeptina também possa ser atribuído à modulação da transmissão glumatérgica (McEwen *et al*, 2010)

Antidepressivos podem produzir aumentos imediatos na neurotransmissão monoaminérgica, entretanto o efeito na melhora do humor

requer semanas de tratamento (Berton and Nestler, 2006). O tratamento crônico com inibidores da recaptação de monoaminas dessensibiliza receptores 5-HT_{1A} pré-sinápticos, sendo esta uma resposta implicada no efeito antidepressivo (Kreiss and Lucki, 1995). Além disso, o aumento sustentado de monoaminas nas fendas sinápticas produzido pelos antidepressivos leva secundariamente a processos de neuroplasticidade que podem ser responsáveis pelo efeito antidepressivo (Krishnan and Nestler, 2008; Pittenger and Duman, 2008).

Antidepressivos administrados cronicamente em roedores alteram a expressão do fator de transcrição CREB (proteína que se liga à região CRE do DNA, ou elemento de resposta ao AMPc). A expressão de CREB está diferentemente afetada em modelos animais de depressão de acordo com a região cerebral. Por exemplo, a expressão de CREB no hipocampo encontra-se reduzida, sendo restaurada após tratamento com antidepressivo (Carlezon *et al*, 2005; Nestler and Carlezon, 2006). Por outro lado, expressão de CREB é aumentada no núcleo accumbens em modelos animais após administração crônica de drogas de abuso e de estresse crônico através da derrota social (Pittenger *et al*, 2008).

Durante situações de estresse ocorre ativação do eixo hipotálamo-hipófise-adrenal (HPA) e consequente aumento dos níveis de cortisol. Visto que eventos estressantes são fatores relacionados com o quadro depressivo, associa-se o aumento da liberação de glicocorticóides como um dos fatores desencadeantes da depressão. O tratamento agudo com dexametasona, agonista de receptores glicocorticóides, induz quadro de anedonia em roedores, verificada através da diminuição da preferência por solução de sacarose em relação à água, sendo revertido pelo tratamento repetido com paroxetina (Casarotto and Andreatini, 2007). Dentre os modelos de depressão mais estudados relacionados ao estresse, encontram-se o estresse crônico brando e imprevisível (Katz *et al*, 1981; Willner *et al*, 1987), o desamparo aprendido (Seligman and Beagley, 1975) e a derrota social (Kudryavtseva *et al*, 1991). Outras formas de estresse incluem o isolamento prolongado (Wallace *et al*, 2009) e alterações do ciclo claro/escuro (Martynhak *et al*, 2011).

O estresse crônico brando e imprevisível é considerado um modelo animal de alta validade de face e constructo (Willner *et al*, 1987).

Primeiramente, o comportamento tipo-depressivo é causado por fator causal também associado com a depressão em humanos, o estresse. Adicionalmente, como mencionado acima, o estresse crônico induz ou predispõe a alterações que são vistas como comorbidades em pacientes depressivos. Além disso, alterações neurobiológicas observadas em pacientes depressivos também são observadas em animais após o estresse crônico, sendo estas implicadas com o desenvolvimento do quadro. Por exemplo, a expressão do fator neurotrófico derivado do encéfalo (BDNF, *brain-derived neurotrophic fator*) é reduzida tanto no hipocampo de roedores (Song *et al*, 2006) como no soro de pacientes deprimidos (Shimizu *et al*, 2003). Assim como para a validade preditiva do modelo em relação ao comportamento anedônico, o tratamento crônico com antidepressivos também restaura as concentrações hipocampais de BDNF em roedores (Song *et al*, 2006). Em pacientes depressivos, a concentração plasmática de BDNF se mostrou maior em pacientes tratados com antidepressivos em comparação com pacientes não tratados (Shimizu *et al*, 2003), embasando a hipótese neurotrófica da depressão. O estresse crônico, além de promover alterações na expressão de BDNF também reduz a fosforilação de seu receptor (pTrkb), se tratando de uma medida interessante, pois avalia a funcionalidade da sinalização via BDNF (Kubera *et al*, 2011).

Entretanto, apesar da compreensão de alguns mecanismos associados à ação dos antidepressivos e da introdução de novos medicamentos, importantes limitações permanecem na clínica: o retardo na ação antidepressiva (2 a 4 semanas), baixa eficácia do tratamento e alta prevalência de recorrência (Zarate *et al*, 2013). Dentre os novos fármacos, a quetamina se destaca. A quetamina é utilizada como anestésico dissociativo, atuando como antagonista dos receptores glutamatérgicos NMDA. A administração intravenosa de quetamina promove ação antidepressiva algumas horas após a administração, sendo efetiva em pacientes refratários ao tratamento com inibidores da receptação de monoaminas (Zarate *et al*, 2006).

Em 2009, a ANVISA aprovou o uso do antidepressivo agomelatina no Brasil. A agomelatina é um agonista dos receptores melatonérgicos MT1 e MT2, além de ser antagonista do receptor serotonérgico 5-HT_{2C} (Loo *et al*, 2002). Tendo em vista que novas drogas com promissor efeito tipo-antidepressivo na pesquisa pré-clínica falham em demonstrar eficácia em

pacientes (Belzung, 2014), o desenvolvimento da agomelatina se baseou em uma estratégia baseada na junção de um mecanismo antidepressivo conhecido por ser eficaz (serotonérgico) com um novo potencial mecanismo (melatonérgico) na mesma molécula (Millan, 2006).

Visto que a melatonina é uma das eferências do sistema de controle da ritimicidade biológica, a investigação da relação entre ritimicidade circadiana e depressão pode vir a revelar novos alvos terapêuticos, ou estratégias não-farmacológicas, tanto para tratamento como para prevenção da depressão.

Introdução aos ritmos biológicos

Os ritmos circadianos, ritmos fisiológicos com duração aproximada de 24h, são encontrados em praticamente todos os seres vivos, incluindo bactérias, plantas e animais. A principal característica dos ritmos circadianos é o seu caráter endógeno. Os ritmos circadianos são gerados endogenamente e sincronizados às 24h do dia da Terra principalmente pelo ciclo claro/escuro. Mesmo em condições ambientais constantes, como o escuro constante para roedores noturnos e o claro constante para humanos, os ritmos ainda são expressos com um período próximo do ciclo da Terra, o que evidencia seu caráter endógeno. Os organismos que expressam a ritmicidade nestas condições constantes são ditos em livre-curso, referenciando-se à ausência de pistas temporais do ambiente (Golombek and Rosenstein, 2010).

Visto que existe a geração de um ritmo endógeno e que este é sincronizado pelo ciclo claro/escuro, pode-se supor que exista uma estrutura que integre estas informações para então ajustar os ritmos de todo o organismo. Nos mamíferos, esta estrutura se localiza nos núcleos supraquiasmáticos (NSQs) do hipotálamo. Os NSQs são compostos por cerca de 10.000 neurônios cada, esquerdo e direito, e recebem fibras glutamatérgicas da retina através do trato retinohipotalâmico. A informação luminosa que se dirige ao hipotálamo é recebida pelo pigmento melanopsina localizado nas células ganglionares da retina. Entretanto, recentemente foi observado que os bastonetes também podem fornecer esta informação (Altimus *et al*, 2010; Li *et al*, 2010). Além de fibras aferentes da retina, os NSQs

também recebem aferências serotonérgicas dos núcleos da rafe, expressando receptores 5-HT_{1A} e 5-HT_7 (Pickard and Rea, 1997).

Os NSQs constituem o oscilador central do sistema de temporização. Assim como em todas as células nucleadas, os NSQs expressam conjunto de genes que se autorregulam através de alças de *feedback* positivo e negativo (Shearman *et al*, 2000b). Estes genes são denominados genes-relógio. Os genes *CLOCK* e *BMAL1* codificam proteínas que se unem formando um heterodímero. Este promove, então, a transcrição dos genes *PER1*, *PER2*, *PER3*, *TIM*, *CRY1* e *CRY2*. Os dímeros PER-TIM, PER-PER, TIM-CRY e PER-CRY unem-se à Caseína Kinase ε ou δ, são fosforilados e, após penetrarem no núcleo, bloqueiam o heterodímero CLOCK-BMAL1, formando assim, uma alça de retroalimentação negativa (Fig. 1). Outra alça regulatória consiste na ativação dos receptores órfãos nucleares relacionados ao ácido retinóico, *REV-ERBα* e *RORα*, pelo heterodímero CLOCK-BMAL1. Estes receptores competem pela ligação no promotor do gene *BMAL1*, sendo que REV-ERBα atua inibindo a transcrição do gene e RORα ativando (Ko and Takahashi, 2006).

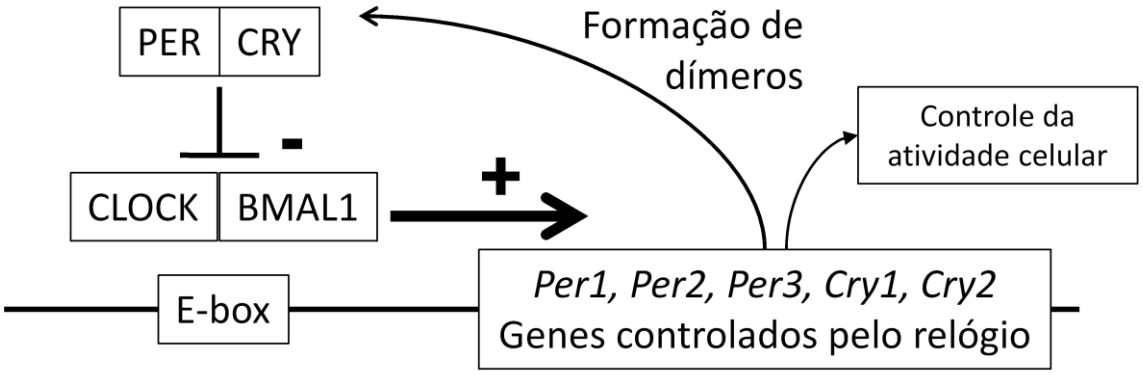

Figura 1 – Alça de retroalimentação negativa que constitui a base para o relógio molecular. O heterodímero CLOCK-BMAL1 promove a transcrição dos genes Per e Cry, que por sua vez, após transcritos e traduzidos, inibem a ação de CLOCK-BMAL1.

A expressão dos genes relógio, que por sua vez controlam a expressão dos genes controlados pelo relógio, gera um padrão circadiano nas eferências dos NSQs. As eferências dos NSQs são diversas, como, por exemplo, para os núcleos paraventricular e arqueado, participando então da modulação da hipófise (Panda and Hogenesch, 2004). Uma das projeções do núcleo

paraventricular é o gânglio simpático da raiz cervical da medula, que por sua vez, envia fibras para a pineal, controlando a secreção de melatonina, cuja secreção é inibida pela luz (Wurtman and Ozaki, 1978).

É possível fazer uma distinção anatômica dos NSQs e de acordo com os neurotransmissores expressos (Antle and Silver, 2005). A porção dorsomedial é pouco inervada pela retina, sendo então o componente essencial para a geração do ritmo endógeno. A porção ventrolateral recebe fibras da retina através do trato retino-hipotalâmico, levando ao sistema de temporização as informações sobre o ambiente. O desacoplamento entre as porções dorsomedial e ventrolateral pode ser induzido por meio do protocolo de dessincronização forçada. Em ratos, um ciclo claro-escuro de 22 h é imposto ao animal, de forma que ele passe a exibir dois ritmos locomotores, um sincronizado ao ciclo claro-escuro imposto e outro obedecendo ao período circadiano endógeno (de la Iglesia *et al*, 2004).

Esta dissociação entre as porções dorsomedial e ventrolateral dos NSQs também pode ser observada em roedores quando o horário de apagar ou acender das luzes do biotério é adiantado em três horas (Nagano *et al*, 2003). Portanto, é provável que mudanças bruscas de horários possam levar a esta dessincronização em humanos também, mais notavelmente em trabalhores em turno e pessoas que atravessam fusos horários em voos, sofrendo o chamado jet lag.

Tanto o jet lag quanto o trabalho em turno podem trazer problemas para a saúde. Por exemplo, o trabalho em turno é associado a diversas patologias, como aumento da incidência de câncer, dispepsias gástricas, hipertensão e infarto do miocárdio (Foster and Wulff, 2005). Dentre elas ainda se enquadram questões de saúde mental, envolvendo depressão e ansiedade, tópico discutido a seguir.

Associação entre alterações da ritimicidade circadiana e transtornos de humor

Episódios depressivos são associados a alterações do ritmo circadiano. Variações no humor ao longo do dia são comuns em pacientes deprimidos (Gordijn *et al*, 1994). Alterações nos ritmos circadianos, incluindo o ciclo

vigília/sono, são heterogêneas na depressão maior, como evidenciado pelo próprio critério diagnóstico sugerido pelo DSM-5, em que pode haver tanto insônia ou hipersonia. Outras alterações incluem alta temperatura corporal durante a noite, secreção de cortisol com avanço de fase, além de redução no sono de ondas lentas, adiantamento de fase do sono REM, bem como o aumento da duração total do sono REM (McClung, 2013). A temperatura corporal pode até mesmo deixar de apresentar a ritmicidade de 24h em alguns pacientes deprimidos (van Londen *et al*, 2001). De forma geral, pode-se dizer que os pacientes apresentam uma dessincronização interna e/ou encontram-se em livre-curso (Ehlers *et al*, 1988; Li *et al*, 2013; Stetler *et al*, 2004). Uma das formas de se conceber esta dessincronização interna é a relação de fase entre o ciclo vigília/sono com a secreção de melatonina. Muitos pacientes deprimidos apresentam a secreção de melatonina muito adiantada em relação ao horário de dormir, sendo inclusive importante para as abordagens terapêuticas envolvendo luz intensa (Wehr *et al*, 1979). Além disso, ratos submetidos ao CMS apresentam uma alteração dos ritmos circadianos, como, por exemplo, diminuição da amplitude do ritmo da atividade motora (Gorka *et al*, 1996).

Distúrbios nas propriedades de sincronização do sistema de temporização circadiano também são observados em mutações de genes relógio. Além de seu papel potencial na preferência por horários de sono e na síndrome da fase atrasada de sono (Archer *et al*, 2003; Pereira *et al*, 2005), o gene relógio *PER3* também foi associado a características dos transtornos de humor, como a responsividade ao tratamento com antidepressivo e a idade do aparecimento do transtorno (Artioli *et al*, 2007).

Diferenças individuais nas preferências por horários de sono também são relacionadas com depressão. Existe uma associação entre a vespertinidade, preferência por horários de dormir e acordar mais tardios, com a depressão maior (Merikanto *et al*, 2013). Indivíduos vespertinos estão frequentemente privados de sono (Korczak *et al*, 2008) e também apresentam maior prevalência para outros transtornos, como esquizofrenia, depressão bipolar (Wood *et al*, 2009) e déficit de atenção (Caci *et al*, 2009). Entretanto, apesar da privação crônica de sono, insônia e vespertinidade aparentemente são fatores independentes de risco para depressão (Chan *et al*, 2014). A associação entre vespertinidade e depressão é curiosa devido à indivíduos

deprimidos frequentemente apresentarem a ritmicidade mais adiantada na secreção de cortisol, e não mais atrasada. Por outro lado, estes dados reforçam a hipótese da dessincronização temporal interna, visto que os horários de sono estão com menor acoplamento com o ritmo da temperatura, melatonina e cortisol. Além disso, a exposição à luz intensa pela manhã, conhecida por adiantar os ritmos, também apresenta efeito antidepressivo (Golden *et al*, 2005).

A privação de uma noite de sono, assim como a privação da última metade da noite de sono, também promove efeito antidepressivo em pacientes deprimidos. Assim como pacientes bipolares diagnosticados com depressão maior que iniciam o tratamento com antidepressivos podem apresentar um episódio de mania, pacientes bipolares também podem entrar em quadro de mania quando são privados de sono (Riemann *et al*, 2002). A privação de uma noite de sono em roedores, assim como o tratamento com antidepressivos, aumenta a neurogênese no hipocampo (Grassi Zucconi *et al*, 2006). Entretanto, a privação de 72h de sono promove o efeito oposto: ocorre diminuição da neurogênese (Mirescu *et al*, 2006). Caso seja imposta uma restrição crônica de sono, roedores passam a apresentar comportamento depressivo (Novati *et al*, 2008).

Tanto manipulações dos ritmos biológicos podem levar a efeitos antidepressivos, como o tratamento com fármacos antidepressivos pode alterar os ritmos circadianos. Um dos efeitos do tratamento com inibidores da recaptação de serotonina é o adiantamento dos ritmos (Cuesta et al., 2008). Os neurônios dos NSQs apresentam receptores para a serotonina, podendo então ser diretamente sensíveis ao aumento de serotonina na fenda sináptica. Além disso, a agomelatina atua diretamento na regulação da ritimicidade circadiana, ao ativar receptores melatonérgicos.

Em humanos, a depressão também é associada com a redução do fotoperíodo que ocorre durante o inverno. A depressão sazonal ocorre mais frequentemente em países localizados em altas latitudes, ou seja, em áreas do mundo onde há pouca luz solar por extensos períodos de tempo (Booker *et al*, 1991; Mersch *et al*, 1999). A maioria dos pacientes apresenta atraso nos seus ritmos. Entretanto, um pequeno subgrupo apresenta avanços de fase durante a depressão de inverno (Lewy *et al*, 2006). Em concordância, ratos diurnos

mantidos em fotoperíodos curtos ou que receberam administrações de melatonina durante a fase clara do ciclo apresentaram comportamento depressivo nos testes de preferência por sacarose e natação forçada (Ashkenazy *et al*, 2009b). Além disso, exposição por uma hora de luz intensa foi suficiente para reverter o comportamento depressivo no teste da natação forçada (Ashkenazy *et al*, 2009a). O uso da luz intensa pela manhã vem sendo utilizada tanto para depressão sazonal como para pacientes com depressão maior (Oldham and Ciraulo, 2014).

A completa privação de luz, ou seja, o escuro constante, após seis semanas também promoveu a indução de comportamento depressivo em ratos, além de acarretar danos em neurônios monoaminérgicos, principalmente noradrenérgicos, sendo ambos os efeitos revertidos por tratamento com desipramina, um antidepressivo noradrenérgico (Gonzalez and Aston-Jones, 2008). Os autores observaram aumento no tempo de imobilidade e diminuição no comportamento de escalada no teste da natação forçada. A diminuição do comportamento de escalada e o maior dano dos neurônios noradrenérgicos reforçam a distinção do comportamento de escalada e natação como relacionados predominantemente com um componente noradrenérgico e serotonotérgico, respectivamente (Detke *et al*, 1995).

No outro extremo, camundongos ou ratos expostos à luz constante apresentaram comportamento tipo-depressivo (Fonken *et al*, 2009; Martynhak *et al*, 2011; Tapia-Osorio *et al*, 2013). Exposição prolongada à iluminação constante também promove arritmicidade na atividade locomotora (Eastman and Rechtschaffen, 1983; Honma and Hiroshige, 1978). Porém, a luz também pode exercer efeitos diretamente sobre o humor independentemente da ritimicidade circadiana. A luz é recebida tanto por cones e bastonetes, como também por células ganglionares fotossenstivas contendo o fotopigmento melanopsina (Altimus *et al*, 2010; Berson *et al*, 2002). A luz pode exercer efeitos no humor de forma indireta, como através da regulação da ritimicidade circadiana via conexão com os NSQs, assim como também de forma direta, através da ativação de áreas como a amígdala e habênula (LeGates *et al*, 2014). A disponibilidade de um tubo opaco foi capaz de prevenir parcialmente o comportamento tipo-depressivo induzido pela luz constante (Fonken *et al*, 2009), argumentando para o papel direto da luz sobre o humor. Entretanto,

exposição neonatal ao claro constante previne tanto a perda da ritimicidade (Cambras *et al*, 1998), como o comportamento tipo-depressivo induzidos pela luz constante (Martynhak *et al*, 2011), argumentando em favor do efeito indireto da luz sobre a regulação do afeto. Camundongos expostos a um ciclo de 7 h (3,5:3,5 h, claro:escuro) não são capazes de se sincronizarem ao ciclo claro/escuro, entrando em livre-curso, ou seja, expressando o período circadiano endógeno, próximo de 24 h (LeGates *et al*, 2012). Apesar de manterem a ritimicidade, a exposição prolongada a este ciclo aberrante leva a comportamento tipo-depressivo, que é prevenido em animais que não expressam células ganglionares fotorreceptoras. Desta forma, pode-se concluir para este modelo a luz exerce seus efeitos sobre o humor de forma direta, sem participação da ritimicidade circadiana (LeGates *et al*, 2012).

Visto a relação entre ritimicidade circadiana e depressão, além do uso terapêutico da agomelatina e da luz intensa, este trabalho visa aprofundar os estudos acerda da relação entre alterações na exposição ao ciclo claro/escuro e o comportamento tipo-depressivo em modelos animais.

OBJETIVOS ESPECÍFICOS

- Avaliar o efeito da co-habitação entre um animal que expressa ritimicidade circadiana em claro constante e outro que não a expressa em relação ao comportamento tipo-depressivo
- Avaliar o efeito da restauração da ritimicidade circadiana sobre o comportamento tipo-depressivo em animais expostos ao claro constante através da restrição alimentar
- Avaliar o efeito da dessincronização entre o ciclo claro/escuro e a disponibilidade de alimento sobre o comportamento tipo-depressivo
- Avaliar a resposta sobre o comportamento tipo-depressivo em alterações do ciclo/claro escuro em camundongos menos sensíveis aos efeitos diretos da luz (deficientes para o gene relógio *Per3*).

CAPÍTULO 1

Social interaction with rat exposed to constant light during lactation prevents depressive-like behavior induced by constant light in adulthood

Bruno Jacson Martynhak[1]*, Luiz Kae Sales Kanazawa[1], Guilherme Messias do Nascimento[1], Roberto Andreatini[1]

Departamento de Farmacologia, Universidade Federal do Paraná Cel. Francisco H. dos Santos, Centro Politécnico, 81530-900 – Curitiba, Paraná, PR,Brazil

* Corresponding author.
Contact: brunojm@ymail.com,
Tel.: +55 41 3361 1693.

Departamento de Farmacologia, Universidade Federal do Paraná Cel. Francisco H. dos Santos, Centro Politécnico, 81530-900 – Curitiba, Paraná, PR,Brazil

Nota: Manuscrito aceito para publicação no periódico Neuroscience Letters.

ABSTRACT

Circadian rhythm disruptions are often observed in depressed patients, and changes in the light/dark cycle promote depressive-like behavior in animal models. Prolonged exposure to constant light (LL) is known to lead to arrhythmicity of circadian locomotor activity and depressive-like behavior in rats. Interestingly, neonatal exposure to LL prevents both arrhythmicity and depressive behavior in adulthood. Arrhythmic rats under LL conditions that cohabitate with a rhythmic rat exhibit improvement in circadian rhythms. Therefore, we tested whether such cohabitation also protects against LL-induced depressive-like behavior. Wistar rats were assigned to conditions of either neonatal constant light (neonatal-LL) on postnatal days 10-22 or a regular light/dark cycle (neonatal-LD). On day 45, the animals were assigned to three possible pair combinations. After the baseline sucrose preference test, half of the pairs were placed under LL conditions. Weekly sucrose preference tests were used to evaluate depressive-like behavior. The animals were isolated by an aluminum wall on the test day. At week 2 of LL, sucrose preference was reduced in neonatal-LD/neonatal-LD pairs of animals. At week 5, neonatal-LD/neonatal-LD pairs exhibited anhedonic-like behavior, but the pairs with at least one neonatal-LL rat did not. The LL cycle was changed back to an LD cycle, and 2 weeks later the neonatal-LD/neonatal-LD pairs exhibited a restoration of sucrose preference. We conclude that social interaction can prevent depressive-like behavior induced by circadian rhythm disruption as long as one of the animals is more prone to present a strong rhythm.

Keywords: depression, depressive-like, anhedonia, circadian rhythms, constant light, neonatal

1. Introduction

The disruption of circadian rhythms has long been known to be related to mood disorders. Although the causality of this relationship is not well established, the manipulation of circadian rhythms via light schedules, meals, or social interaction might provide a valuable tool for improving treatments for mood disorders or even preventing their onset.

Constant light (i.e., conditions of a 12 h/12 h light/light [LL] cycle, in contrast to the usual light/dark [LD] cycle) has been shown to induce depressive-like behavior (Fonken *et al*, 2009; Martynhak *et al*, 2011) and reduce hippocampal neurogenesis in animal models (Fujioka *et al*, 2011). Prolonged exposure to LL also induces arrhythmicity in circadian locomotor activity (Eastman *et al*, 1983). Neonatal exposure to LL prevents this arrhythmicity in adulthood (Cambras *et al*, 1998). This protocol has been shown to prevent LL-induced depressive-like behavior (Martynhak *et al*, 2011). Therefore, arrhythmicity appears to be essential for the effects of LL.

Rats under LL conditions that cohabitate with a rhythmic rat (i.e., a rat that was exposed to LL during weaning) exhibit improvements in circadian patterns of motor activity (Cambras *et al*, 2012). Therefore, we sought to determine whether this cohabitation can also protect against depressive-like behavior induced by LL. We hypothesized that pairs of rats that consist of at least one rat that was exposed to neonatal LL would be protected from LL-induced depressive-like behavior. We also hypothesized that pairs that consist of both animals that were not previously exposed to LL would be more resilient to the effects of LL than single-housed animals, given that social isolation has already been used as a model of depression (Wallace *et al*, 2009).

2. Materials and Methods

Adult male and female Wistar rats were obtained from the Federal University of Paraná and maintained under a controlled temperature (22 ± 3°C) and 12 h/12 h light/dark (LD) cycle (lights on 7:00 AM-7:00 PM). Food and water were available *ad libitum*. All of the animal procedures were approved by the Ethical Committee of Animal Experimentation of the Federal University of Paraná (protocol no. 600) and were in accordance with the Guide for the Care and Use of Laboratory Animals adopted by the Department of Pharmacology,

Federal University of Paraná. The mating procedure involved placing each male in a cage with three female rats for 1 week as described previously (Martynhak *et al*, 2011). For this experiment, 18 females were used and 13 of them had litters.

2.1. Experimental design

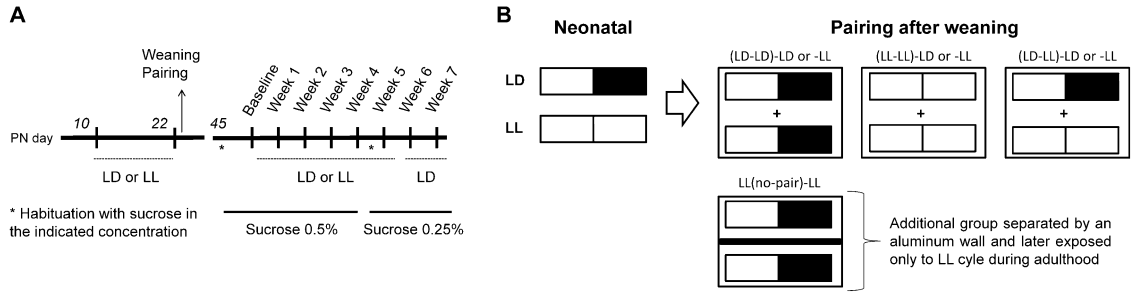

Fig. 1. (A) Experimental timeline and (B) group distribution. All pair combinations were set according to the neonatal light schedule and later divided into LD and LL groups in adulthood. *One additional group consisted of pairs of two rats that were housed in the same cage separated by an aluminum wall. These animals were all exposed to LL during adulthood, as in a previous study (Martynhak *et al*, 2011).

On postnatal day 10-22, a total of 13 litters were assigned to two groups: neonatal-LD (control) group and neonatal-LL group (~200 lux) (Martynhak *et al*, 2011). This period has been reported to be the most sensitive time period for preventing arrhythmicity in adulthood (Canal-Corretger *et al*, 2001). The litters were randomized into neonatal-LD and neonatal-LL groups according to the total number of pups within each litter to minimize possible litter size effects. Seven litters were exposed to the regular LD cycle (36 males), and six litters (30 males) were exposed to LL. The behavior and weight of the dams were not evaluated during or after the LL exposure. The LL-exposed rats were maintained in a separate room that was used specifically for this experiment. The neonatal-LD group was maintained in a common room. After weaning, we randomly distributed the male rats into pairs. To evenly distribute the influence of eventual fighting across groups, we avoided placing siblings together. During distribution of the pairs at weaning, the rats were weighted and no differences were found comparing LD and LL animals. The animals were not further weighted throughout the experiment. As depicted in Fig. 1, the final experimental pairings were the following: neonatal-LD housed with neonatal-LD

(LD-LD), neonatal-LD housed with neonatal-LL (LD-LL), and neonatal-LL housed with neonatal LL (LL-LL). We then randomly distributed the cages into the LD room ([LD-LD-LD], [LD-LL]-LD and [LL-LL]-LD) and a room that would later be placed under LL conditions ([LD-LD]-LL, [LD-LL]-LL and [LL-LL]-LL). Finally, for rats in adulthood, we had five cages for each pair combination. Moreover, six neonatal-LD rats were assigned to an additional group. These rats were housed two per cage but separated by an aluminum wall throughout the entire experiment. These no-pair animals were later placed under LL conditions (LD[no-pair]-LL). This group was included as an internal control, given that it was similar to our previous experiment with LL (Martynhak *et al*, 2011).

In a previous experiment, imipramine treatment was able to rescue LL-induced anhedonic-like while the rats were still kept in LL (Martynhak *et al*, 2011). For this experiment, after detection a reduction in sucrose preference in week 5, the rats that were under the LL cycle were transferred back to a regular LD cycle to evaluate possible spontaneous improvements in depressive-like behavior (Fig. 1).

2.2. Sucrose preference test

To evaluate anhedonic-like behavior, sucrose preference tests were conducted (Willner *et al*, 1987). The tests consisted of a modified two-bottle choice procedure. When the rats were 45 days old, they were habituated to the sucrose solution (0.5%, w/v) for 2 days, during which they were allowed to choose freely between the sucrose solution and water. After 2 days, a baseline test was performed. To measure individual intake for each animal of the pair, the rats were separated by an aluminum wall for the duration of the test. The bottles were weighed before they were offered to the animals and weighed again 24 h later. Sucrose preference was calculated as the percentage of the volume of sucrose intake over the total volume of fluid intake. Subsequently, sucrose preference tests were performed weekly in the same manner.

In a previous experiment, the no-pair group exhibited a reduction of sucrose preference at week 3 (Martynhak *et al*, 2011). Given that no reduction was observed in the current experiment at week 4, we sought to increase the sensitivity of the test by devaluating the reward. At week 5 of exposure to LL,

we reduced the sucrose concentration by half (0.25%). The animals were first habituated to this concentration to minimize possible negative contrast effects (Justel *et al*, 2012). For the habituation, animals were offered two bottles, one with water and other with the new sucrose centration for 24 h. The test was then performed 1 day after the end of habituation. The tests at weeks 6 and 7 were also performed using this concentration.

2.3. Statistical analysis

The data are expressed as mean ± SEM and were analyzed using two-factor (group × week) repeated-measures analysis of variance (ANOVA) followed by Duncan's *post hoc* test. Differences were considered statistically significant at $p < 0.05$.

3. Results

The ANOVA revealed significant main effects of group ($F_{8,57} = 5.80$, $p < 0.001$) and week ($F_{7,399} = 15.79$, $p < 0.001$) and a significant group × week interaction ($F_{56,399} = 3.73$, $p < 0.001$). In week 2, neonatal-LD animals that were housed in pairs with one another (other neonatal-LD rat) and exposed to LL in adulthood (LD[LD-LD]-LL) exhibited a reduction of sucrose preference compared with baseline ($p < 0.05$) and the equivalent LD group (LD[LD-LD]-LD; $p < 0.05$), but this difference did not persist in weeks 3 or 4, during which preference returned to control levels (Fig. 2B).

After reward devaluation (weeks 5-7), neonatal-LD animals that were exposed to LL in adulthood, both animals that were housed in pairs with similar animals (i.e., LD[LD-LD]-LL; $p < 0.001$) and animals that were separated by the aluminum wall (i.e., LD[no-pair]-LL; $p < 0.01$), exhibited a reduction of sucrose preference in week 5 compared with baseline (Fig. 2B). In addition, LD(LD-LD)-LL and LD(no-pair)-LL rats also had reduced sucrose preference compared with the control group, LD(LD-LD)-LD and to the other groups exposed to LL, LD(LD-LL)-LL and LL(LL-LL)-LL ($p < 0.05$ for all comparisons).

One week after changing the light schedule from LL back to LD in week 6, all of the previous groups that were protected from LL-induced anhedonia (i.e., all of the groups with at least one neonatal-LL rat in the cage) exhibited a reduction of sucrose preference ($p < 0.01$) (Fig. 2B,C). In week 6, LD(LD-LL)-LL

and LL(LD-LL)-LL groups showed reduced sucrose preference compared with LD-(LD-LD)-LD ($p < 0.001$), but the LL(LL-LL)-LL did not. Additionally, the LD(LD-LD)-LL group exhibited an increase in sucrose preference compared with the previous week ($p < 0.005$), although sucrose preference in this group was significantly lower compared with baseline ($p < 0.05$). In the second week under LD conditions (week 7), sucrose preference was at baseline levels in all of the groups that were previously exposed to the LL cycle and there were no differences compared with LD(LD-LD)-LD group.

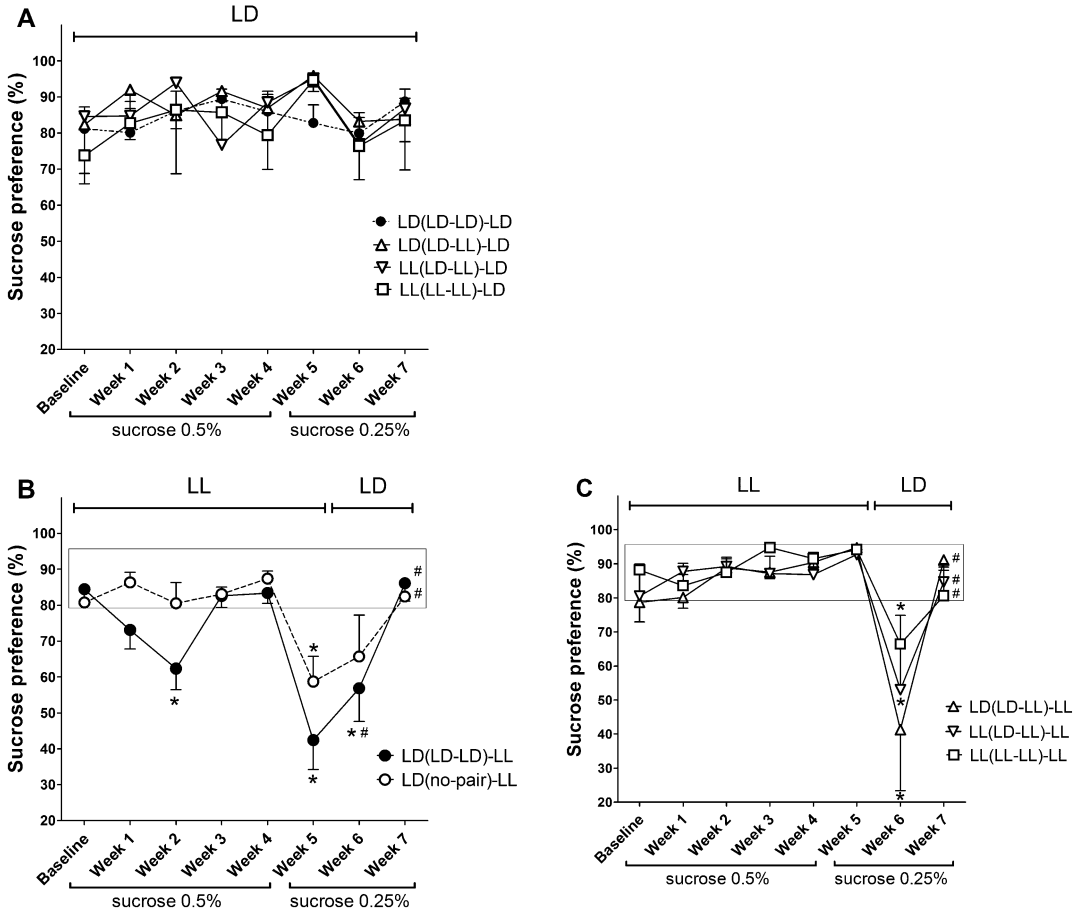

Fig. 2. Sucrose preference in rats subjected to constant light (LL) during the neonatal period and adulthood. (A) Control groups exposed to LD during all of adulthood. (B,C) Groups exposed to LL during weeks 1-5 and LD during weeks 6 and 7. The data are expressed as mean ± SEM (n = 5-10 rats/group). The boxes in B and C represent the maximum and minimum 95% confidence intervals (calculated from each week) for the control group (i.e., LD[LD-LD]-LD). *$p < 0.05$, compared with baseline in the same group; $^{\#}p < 0.05$, compared with previous week in the same group. LD, standard light/dark cycle; LL, light/light cycle (lights on for entire 24 h). $^{\dagger}p < 0.05$ compared with LD(LD-LD)-LD in the same week. The group abbreviations are the following: LD cycle during the neonatal phase (the pair in the cage during adulthood refers to the neonatal cycle)-LD cycle in adulthood. For example, LD(LD-LL)-LL indicates an animal that was under LD conditions during the neonatal phase and shared a cage with an animal that was under LL conditions during the neonatal phase, and both animals were exposed to LL during adulthood.

4. Discussion

The main finding of the present study was that anhedonia-like behavior induced by LL could be prevented by social interaction with an animal that was presumed to be rhythmic. These results reinforce the influence of circadian rhythms on mood and extend our previous data that showed that LL induces anhedonia, which can be reversed by imipramine treatment (Martynhak *et al*, 2011). In this study, not only the rat with stronger rhythm under LL is protected from depressive-like behavior, as previously found (Martynhak *et al*, 2011) but its presence also protects the other rat in the cage. Considering that the LL was switched to LD immediately after detection of anhedonic-like behavior, we cannot affirm that the social interaction rendered the animals fully protected from depressive-like behavior induced by LL. However, a late onset for the reduction of the sucrose preference can be considered a form of resilience.

However, LL with standard constant light (200 lux) is not the only manipulation that promotes depressive-like behavior. Another type of LL that employs a dim light (~5 lux) rather than a normal light at night also decreases sucrose preference and increases the latency to float in the Nile grass rat, which is a diurnal rodent (Fonken *et al*, 2012). Moreover, citalopram is able to reverse depressive-like behavior in the forced swim test in ovariectomized female hamsters that are exposed to dim light at night (Bedrosian *et al*, 2012).

Cohabitation with a rhythmic rat has been shown to improve locomotor activity and temperature rhythms in a previous study (Cambras *et al*, 2012) and prevent anhedonia in arrhythmic rats in the present study. In the previous study, social interaction commenced after long-term exposure to LL, and the animals that were born under the LD condition exhibited gradual increases in the stability of their temperature rhythms. In the present study, the animals were already in the same cage at the time LL was imposed to prevent the weakening of circadian rhythm stability rather than rescue such stability later. Another difference between these studies was that we examined all possible combinations of neonatal-LL and neonatal-LD pairings, whereas the previous study (Cambras *et al*, 2012) examined only one group, which involved social interactions between animals with strong and weak rhythms under LL conditions. Notably, interactions with neonatal-LD rats increased rhythm stability

in neonatal-LL rats during cohabitation (Cambras *et al*, 2012). However, as addressed in another study, social interaction itself does not delay the time at which the animals become arrhythmic under LL conditions (Cambras *et al*, 2011). Accordingly, we observed that social interactions between two animals that were expected to show weak or absent rhythms were insufficient to prevent LL-induced anhedonia. Therefore, to prevent or rescue the depressive phenotype, social interaction must strengthen circadian rhythmicity. In order to further support this conclusion, after long exposure to LL, the pairs of protected animals could be switched (i.e., replacing a neonatal-LL rat for a neonatal-LD). Thus, accordingly to our hypothesis and observed results, we could expect that the new pair would become more susceptible to depressive-like behavior. Instead, we opted to test whether the anhedonic-like behavior induced by LL would be restored by switching the light/dark cycle back to LD.

In fact, the groups under LL with anhedonic-like behavior had their sucrose preference restored to baseline levels after two weeks under regular LD. Discontinuation of stress in the unpredictable chronic mild stress has also been reported to spontaneously recover the sucrose consumption in rats with ‘optimistic’ trait, but not in ‘pessimist’ rats (Rygula *et al*, 2013). Considering the high rate of recurrence in remitted patients and that not only stress, but also other factors are implicated in the maintenance and relapse of depressive episodes, a non-spontaneous recovery would be a unique feature in an animal model of depression. However, here we showed that the depressive-like behavior is reversible when the normal light/dark cycle is restored. Unexpectedly, sucrose preference in anhedonia-protected animals was reduced after their light/dark cycles were changed from LL to the regular LD schedule. Because this reduction occurred in LL animals that were supposed to be rhythmic and exhibited high sucrose preference, this shift may have been too abrupt and caused temporary mood changes. For example, after a delay of 10 h in the light/dark schedule, male Wistar rats exhibited desynchronization of the dorsomedial and ventrolateral portions of the suprachiasmatic nucleus that lasted for approximately 6 days (Nagano *et al*, 2003). Additionally, chronic light/dark shifts have been shown to be stressful and reduce immune responses in rats (Kort and Weijma, 1982).

The reduction of the sucrose concentration effectively increased the sensitivity of the sucrose preference test. Importantly, all of the control groups (always under the LD cycle during adulthood) were unaffected by this reduction (Fig. 1A). In our previous study (Martynhak *et al*, 2011), we observed reductions of sucrose preference in the third week of LL while sucrose concentrations remained constant. Therefore, the difference between the present and previous results may be attributable to a borderline effect of LL exposure (i.e., when a 0.5% sucrose solution is used, the results are more variable). Another possibility is that the high hedonic value of the sucrose solution could have masked differences between groups. Depressive-like effects of LL after 4 and 8 weeks have been reported using a 5% sucrose concentration (Tapia-Osorio *et al*, 2013). However, this previous study used a different procedure for the sucrose preference test that included food and water deprivation, which we chose to avoid. Moreover, other unknown factors might have influenced the sensitivity to LL and responses to the sucrose concentration. Interestingly and consistent with our hypothesis, a recent study confirmed that these animals were arrhythmic under LL conditions (Tapia-Osorio *et al*, 2013).

The present results may be related to the loss of circadian rhythms or changes in these rhythms. Other light/dark cycle manipulations, such as shortening the photoperiods for both diurnal and nocturnal animals (Einat *et al*, 2006; Prendergast and Nelson, 2005), exposure to constant darkness (Gonzalez *et al*, 2008), and changing the 24 h period to a 7 h period with 3.5 h of light and 3.5 h of dark (LeGates *et al*, 2012), have also been shown to cause depressive-like behaviors. A common feature of these studies is that these manipulations do not lead to the loss of circadian locomotor activity rhythms. Thus, although the loss of circadian rhythms can induce depressive-like behaviors, such loss is not necessary to induce depression. Moreover, the mechanism of the induction of depressive-like behaviors does not appear to involve reductions of neurogenesis. One study did not detect a reduction of neurogenesis after arrhythmicity induced by prolonged LL exposure (Mueller *et al*, 2011).

Not only changes in the light/dark cycle can influence mood by modulation of the circadian rhythms, but light can also influence the affect through a direct pathway (LeGates *et al*, 2014). For example light has acute

arousal effect in humans. Interestingly, this effect is also observed in a small portion of blind patients, suggesting a role for the melanopsin-expressing retinal ganglion-cells (Vandewalle *et al*, 2013). In addition, mice that did not express photosensitive retinal ganglion cells also did not show depressive-like behavior or memory impairments after exposure to a 7 h light/dark period (LeGates *et al*, 2012). Interestingly, the depressive-like behavior induced by constant light in mice is partially reversed by providing an opaque tube for escape of the light (Fonken *et al*, 2009). Therefore, constant light might also lead to anhedonia through the direct effect of light. Although the animals exposed to LL during lactation have normal synchronization to the light/dark cycle (Cambras *et al*, 1998), one cannot rule out reduced sensitivity to light as an explanation to the protection of LL-induced anhedonia. In fact, response to light pulse-induced phase shift in animals under constant darkness was reported to be reduced depending on the amount of days exposed to LL during lactation, although this effect was only observed in a specific phase of the subjective day on which the light pulse was administered (Canal-Corretger *et al*, 2000). In this current study, not only rats exposed to LL during lactation, but also the cage conspecifics exposed to LD during lactation were protected from the reduction of sucrose preference under LL in adulthood, reinforcing the putative role of improvement in circadian rhythmicity as the protective factor from anhedonia.

Unexpectedly, pairs of rats under a regular LD cycle during lactation that were exposed to a LL cycle in adulthood exhibited a reduction of sucrose preference in the second week of LL (i.e., before reward devaluation), whereas the single-housed animals did not. Perhaps social interactions between two arrhythmic animals have more deleterious effects compared with rats that are arrhythmic and single-housed. However, the reduction of sucrose preference was transient. The preference level was restored to normal levels by the third week, and both groups exhibited reduced sucrose preference after devaluation of the sucrose solution. Additionally, the aluminum wall did not provide complete separation, so that the animals might still have influenced each other through movement and noise.

The sucrose preference test protocol used in this experiment spanned over 24 hours for behavioral evaluation during the entire circadian period of the animals. Animals exposed to LL during lactation are expected to have

lengthened circadian period under LL in adulthood (Cambras *et al*, 1998). On the other hand, control animals in LL initially show lengthened period and gradually become arrythmic. Moreover, not necessarily all animals exposed to LL become arrhythmic, as they might be free-running with lengthened circadian period as well. Therefore, the sucrose preference test did not span over the whole cycle for all animals in this experiment, which might have influenced the sucrose preference test. However, the groups that were more prone to show the lengthened circadian period in LL were the pairs that contained at least one rat exposed to LL during lactation, the same pairs that were protected from the depressive-like behavior. Rats not only ingest more liquid during the activity phase, but were also shown to have increased sucrose preference in the dark phase, although this effect faded after repeated testing (Tonissaar *et al*, 2006). Thus, not covering part of inactive phase would not impact significantly the result of the sucrose preference test, whereas not covering part of the active phase could result in a lower sucrose preference. However, the groups of animals which were expected to have lengthened circadian period were also the groups that did not reduce the sucrose preference.

In conclusion, the present results are consistent with previous studies that showed that disrupting circadian rhythms can trigger depressive-like behavior (Tapia-Osorio *et al*, 2013). Considering the effects of constant light and cohabitation (Cambras *et al*, 2012), our results also suggest that the maintenance of circadian rhythms caused by social interaction can restore depressive-like behavior. This result should be relevant for non-pharmacological treatments that seek to recover circadian rhythms for mood disorders, such as Interpersonal Social Rhythm Therapy (Frank, 2007) and bright light therapy (Golden *et al*, 2005).

CAPÍTULO 2

Modulation of antidepressant and pro-depressive-like effect of food restriction according to the light/dark cycle schedule

Bruno Jacson Martynhak[1], Diego Correia[2], Cristina A. Jark Stern[3], Thiago Rodrigues da Silva[1], Roberto Andreatini[1]

[1]. Pharmacology Department, Federal University of Paraná, Cel. Francisco H. dos Santos, Centro Politécnico, 81530-900 – Curitiba, Paraná, PR, Brazil.

[2]. Genetics Department, Federal University of Minas Gerais – Belo Horizonte, Minas Gerais, MG, Brazil.

[3]. Pharmacology Department, Federal University of Santa Catarina – Florianópolis, Santa Catarina, Brazil.

Nota: Manuscrito a ser submetido

ABSTRACT

Constant light (LL) exposure in rodents is known to either lengthen the circadian period or to promote arrythmicity of the circadian pattern of the locomotor activity. Prolonged exposure to LL also induces depressive-like behaviour in rodents. The depressive-like behaviour might be prevented either by strategies that prevent arrythmicity or by providing an opaque tube for escaping the light. Besides the light/dark cycle (LD), food availability is the stronger synchronizer of the circadian rhythms. Food availability might take priority over the LD cycle when given at the inactive phase of activity. In this study, Wistar rats submitted to LL showed reduced sucrose preference, an effect that was prevented by food restriction, in which animals received food dayly for a 6 h interval. Interestingly, rats under the regular light/dark (LD) cycle submitted to food restriction also showed anhedonic-like behavior when food was available in only the light phase, but not when it was available only in the dark phase. The same effect of food restriction was observed in mice. Although neither melatonin, imipramine or agomelatine treatment in mice were able to prevent the reduction in sucrose preference due to food restriction, a single administration of ketamine had antidepressant-like effects. Thus, food restriction is an effective way to synchronize the circadian rhythms in constant light conditions. However, food restriction might also promote desynchrony between feeding schedule and the light/dark cycle. Altogether, we showed that both arrythmicity and desynchronization of the circadian rhythms might lead to depressive-like behaviour. As the depressive-like behaviour induced by food restriction was not responsive to antidepressant treatment, except ketamine, this protocol might comprise a treatment-resistant preclinical model of depression.

Key words: depressive-like behaviour, food restriction, constant light, circadian rhythms, anhedonia

Introduction

The etiology of depression is multifactorial, including genetic mechanisms, life events and even the presence of other disorders. In pre-clinical models, stress is a key component for inducing depressive-like behaviour in rodents (Willner *et al*, 1987). Among several stressors, changes in the light/dark cycle constitute a promising approach to study one of the possible causal factors for depression. Changes in sleep architecture and in circadian rhythms are often observed in depressed patients. Core body temperature rhythm might have flattened amplitude or no detectable circadian rhythmicity (van Londen *et al*, 2001). The latency for REM sleep is reduced, occurring earlier in the night. Conversely, the slow wave sleep, that is usually more concentrated in the first half of the night, is also reduced. Moreover, the phase angle between different rhythms might also be altered, which means that not only phase delays or advances might be present in depressed patients, but also shortening or lengthening the time interval between two peaks of different rhythms (Lewy *et al*, 2006).

Prolonged exposure to constant light (LL) has been shown to induce depressive-like behaviour in mice and rats (Fonken *et al*, 2009; Martynhak *et al*, 2011; Tapia-Osorio *et al*, 2013). The availability of an opaque tube that provides an escape from the light prevents the effect of LL in the forced swimming test (Fonken *et al*, 2009). Exposure to LL leads to a lengthening of the endogenous circadian period and a prolonged exposure to LL leads to loss of circadian rhythmicity. It has been shown that rat pups exposed to LL during lactation are more resilient to the effect of LL when adults (Cambras *et al*, 1998). Accordingly, these animals are also resilient to the effects of LL in reducing the sucrose preference (Martynhak *et al*, 2011). Moreover, co-habitation with a rhythmic rat under LL can improve the rhythmicity of a rat that has not been exposed to LL during lactation (Cambras *et al*, 2012). In line, we recently showed that the same co-housing protects the rat exposed to normal light/dark cycle (LD) during lactation from the LL-induced anhedonia-like behaviour (Martynhak et al., submitted).

Apart from light, one of the strongest synchronizers of the circadian timing system is food availability. When food is available during a restricted and predictable time of the day, animals exhibit an increase in locomotor activity

preceding the presentation of food. Importantly, if food is presented during the light phase, the animals shift their activity phase to the time of food availability, despite the light/dark cycle (Mistlberger, 2009).

Considering the synchronizing effects of food restriction, we hypothesized that depressive-like behaviour induced by LL could be prevented by food-restricting the animals by rescuing the arrythmicity/abnormal long circadian period. In addition, we hypothesized that food restriction, when food is available during the light phase in animal under regular LD, would also lead to depressive-like behaviour due to the desynchronization between locomotor activity and metabolism in relation to the light/dark cycle. One of the signals of the dark phase is the hormone melatonin, whose secretion is inhibited by light. Thus, we also tested whether exogenous melatonin during the light phase would have antidepressant effect due to the synchronization between plasma melatonin and food availability. Additionally, food-restricted animals were also treated with the antidepressant agomelatine, which is an antagonist of the serotonergic 5-HT_{2C} receptors and agonist of the melatonergic MT1 and MT2 receptors (Millan *et al*, 2003).

Materials and Methods

Animals

Male Wistar rats (60 days) and Swiss mice (45 days) were obtained from the Federal University of Paraná and kept under controlled temperature (22±3 ◦C) and 12 h/12 h light/dark (LD) cycle (lights on 0700–1900 h), unless specified. ZT0 and ZT12 are defined as the lights on and off times, respectively. Food and water were available *ad libitum*, unless specified. All animal procedures were approved by the Ethical Committee of Animal Experiment (protocol no. 818) and in accordance with the Guide for the Care and Use of Laboratory Animals adopted by the Department of Pharmacology, Federal University of Paraná.

Rats and mice were singly-housed in all experiments, except for the acute test for antidepressant action in the forced swimming test, in which mice were house in cages with 7-12 animals.

Locomotor monitoring

Rats had their locomotor activity continuously recorded through a passive infrared detector connected to computer software named SAP, kindly provided and developed by Marconi Camara (UFRN). Rats were randomized into four groups. Half of the rats were kept in LD and the other half in LL. After six weeks, half of the rats from each group was food restricted (food availability from ZT2-08 for the LD group) for another four weeks (n=5 per group).

Sucrose preference test

Sucrose preference test was performed similarly in rats and mice, except that the sucrose concentration for rats was 0.25% (w/v) and 1% (w/v) for mice. Animals were habituated with a sucrose solution bottle as only drinking option for one day. After one day interval with a water bottle, animals had the choice between water and sucrose solution bottles for two days. After another day of interval with only water bottle, animals were tested for the baseline sucrose preference.

The sucrose preference consisted in offering both bottles of water and sucrose solution for 24h. The bottles were weighted before and after placing. The sucrose preference is expressed as a percentage of the total liquid ingestion. The test was performed weekly throughout the experiments.

Drugs

Melatonin (acute and chronic treatment) and imipramine (acute treatment) were dissolved in ethanol PA and then diluted in saline (0.9%) to 1% ethanol (v/v). The used vehicle was also saline solution in 1% ethanol. Agomelatine (acute and chronic treatment) and imipramine (chronic treatment) were dissolved in saline (0.9%) and the vehicle was also saline. All drugs were administered intraperitoneally at a volume of 10 ml/kg.

Experimental design

1. Constant light and food restriction in rats

According to the baseline sucrose preference, a group of 40 rats was randomized into 4 groups, with either LD or LL light schedule and with *ad libitum* food or food restriction (with ZT2-8 of food availability, two hours after lights on).

In a second experiment, a group of 20 rats was randomized according to the baseline sucrose preference into 2 groups, *ad libitum* food and food restriction (with ZT12-18 of food availability, at lights off).

In a third experiment, a group of 30 rats was randomized into 10 rats with *ad libitum* food and 20 animals with food restriction (with ZT2-8 of food availability). After detection of reduction in the sucrose preference, the 20 food restricted rats were further randomized according to the sucrose preference of the last test into be kept at the same light/dark cycle or to phase advance 10 hours, so that food would be given at the same external clock. Therefore, the phase advanced group had food availability at the same external time, but matching with the onset of the dark phase for the rats (ZT12-18).

2. Food restriction and pharmacological treatment in mice

In order to verify whether mice were also susceptible to the depressive-like effects of food restriction, a group of 26 mice were randomized according to the sucrose preference into food-restricted and ad libitum food for five weeks.

In a second experiment, a group of 60 mice was divided into 6 groups, with either food ad libitum or food restriction (with ZT2-8 of food availability, two hours after lights on). In addition, at ZT2, animals received vehicle or melatonin (1 µg/kg and 10 mg/kg) intraperitoneally.

The lowest dose of melatonin was reported to be sufficient to synchronize the circadian locomotor activity in pinealectomized rats under constant darkness (Warren *et al*, 1993). In rats, this dose achieves plasma levels around 10 times higher than physiological levels (Cassone *et al*, 1986). Importantly, a dose 100 times higher did not show antidepressant action, whereas 10 mg/kg had antidepressant effect in the forced swim test (Raghavendra *et al*, 2000). Immediately after the removal of the bottles of the week 4 test, the vehicle-treated animals were administered ketamine (10 mg /kg i.p), whereas the other groups were kept being treated with imipramine or

agomelatine. A new sucrose preference test was performed 24 h after ketamine treatment.

A third experiment was performed similarly to the previous one, were all 30 mice were food restricted. Animals received daily either vehicle, imipramine (10 mg/kg) or agomelatine (10 mg/kg) intraperitoneally.

3. Acute antidepressant effect of melatonin, imipramine and agomelatine

In order to test the acute antidepressant-like effect of the adopted doses of melatonin and agomelatine, mice (n=9-10) were treated with vehicle, melatonin (1 µg/kg and 10 mg/kg) or imipramine (30 mg/kg) 30 minutes prior to be submitted to open field and forced swimming test. Imipramine was used as a positive control. . In a second experiment, animals received vehicle or agomelatine (10 and 20 mg/kg) and were tested in the open field and forced swimming test after 30 min.

Statistical analysis

Sucrose preference tests were analysed by repeated measures two-way ANOVA. For the experiment in mice with agomelatine treatment, in which all animals were food-restricted, repeated measures ANOVA was performed. Open field and forced swimming test were analysed by one-way ANOVA. Body weight was analysed by one-way ANOVA or by independent t-test accordingly. When pertinent, tests were followed by Newman-Keuls post-hoc analysis. Significance levels were set at $p<0.05$. Data are represented as mean ± standard error of mean (SEM).

Results

LL-induced arrythmicity of the circadian locomotor activity is rescued by food restriction

It was observed that LL lengthens the circadian period, leading to arrythmicity after a few weeks (Fig. 1). Food restriction restores the rhythmicity in LL exposed animals. Rats under LD and food restriction prioritize the food interval over the dark phase the most active phase in the circadian cycle.

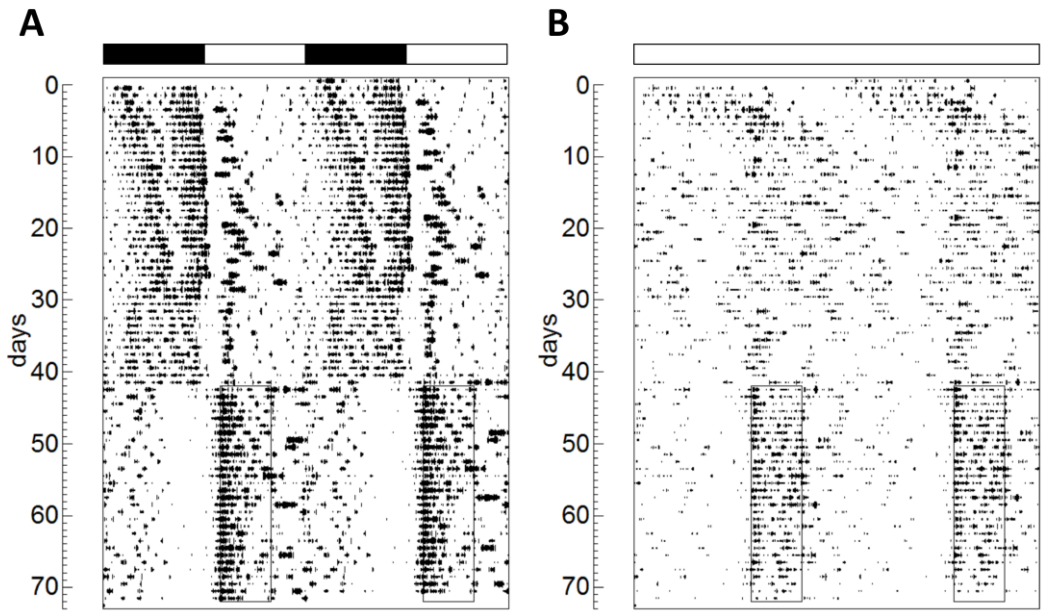

Fig. 1 – Representative double-plotted actograms showing in (A) a rat under LD that shifts the locomotor activity to the time interval when food is given, despite the light/dark cycle and in (B) a arrythimic rat under LL that restores the circadian rhythmicity after food restriction is imposed. Rectangles represent food availability intervals.

Body weight and food consumption

Both LD food-restricted (348.6 ± 5.1) and LL-exposed with *ad libitum* food (353.8 ± 6.1) rats had significantly lower body weight at end of the experiments in comparison with the control group (381.5 ± 9.1) (food restriction: $F_{1,36}$=6.1, p<0.05; light schedule: $F_{1,36}$=3.0758, p=0.088; food restriction x light schedule: $F_{1\ 36}$=4.28, p<0.05). However, LL did not further decreased body weight in food restricted animals (350.9±8.0) (p>0.2). Food restriction also reduced body weight when food was available during the dark phase (360.9 ± 5.7) in comparison with the *ad libitum* group (401.2 ± 6.2) (t=4.79, p<0.001).

Mice submitted to food restriction (43.6 ± 1.1) had no body weight difference in comparison with the ad libitum group at end of week 5 (44.6 ± 1.7) (t=0.5, p>0.2). However, there was a significant effect of food restriction (41.4 ± 0.89 vs. 38.3 ± 0.70, means of the factor food, n=30 per group), but not of treatment at the end of week 4 in the experiment with melatonin administration (food restriction: $F_{1,54}$=6.8, p<0.05; treatment: $F_{2,54}$)=0.17, p>0.2; food restriction x treatment: $F_{2,54}$=0.35, p>0.2). Similar results were found in the experiment with imipramine and agomelatine treatment (data not shown).

Effects of exposure to LL and food restriction on the sucrose preference in rats

There was a significant interaction between LL and food restriction factors ($F_{1,36}$=42.20; p<0.001). *Posthoc* analysis indicated that LL reduced sucrose preference in animals with *ad libitum* food in weeks 4-5 in comparison with the LD *ad libitum* group (both p<0.05) whereas the sucrose preference was reduced in animals with food restriction under regular LD in weeks 2-5 in comparison with the control group (all p<0.05) (Fig. 2). Thus food restriction prevented the depressive-like behaviour induced by LL.

Food restriction did not reduce the sucrose preference when food was available during the dark phase ($F_{1,18}$=1.3546, p>0.2), indicating that food restriction by itself is not enough to promote depressive-like behaviour (Fig. 3A).

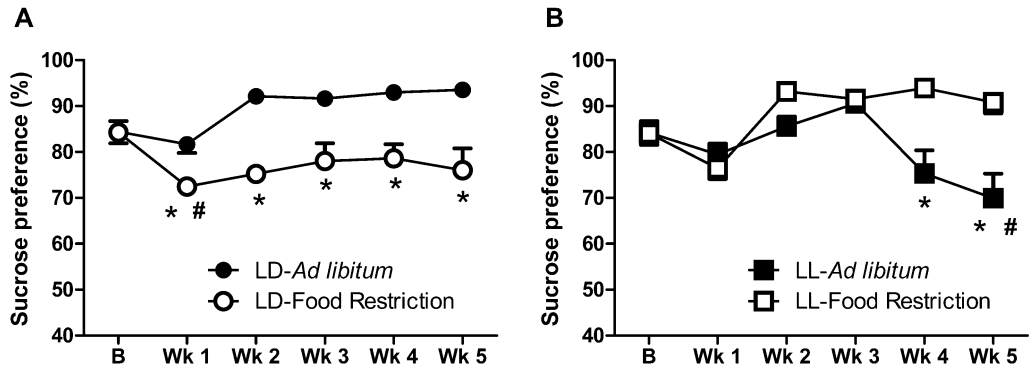

Figure 2 – Sucrose preference in rats under LD (A) or LL (B) with *ad libitum* food or food restriction (food in the light phase). *p<0.05 in comparison with the control group (*LD-ad libitum*). #p<0.05 in comparison with baseline of the same group. Repeated measures two-way ANOVA. N= 10 rats per group.

Synchronization of the food availability with the light/dark cycle has antidepressant effect

After detection of anhedonic-behaviour in food-restricted rats in weeks 2-3 $F_{1,28}$=21.80, p<0.001), the food restricted group was phase advanced 10 h, so that the food availability was in coincidence with the lights off. After this group subdivision, a second analysis including weeks 4-5 indicated that phase advanced group increased the sucrose preference ($F_{2,27}$=5.71, p<0.01) (Fig. 3B), despite the animals still being under food restriction (p<0.05 in comparison with week 4 of the same group and with food restriction-light phase in week 5).

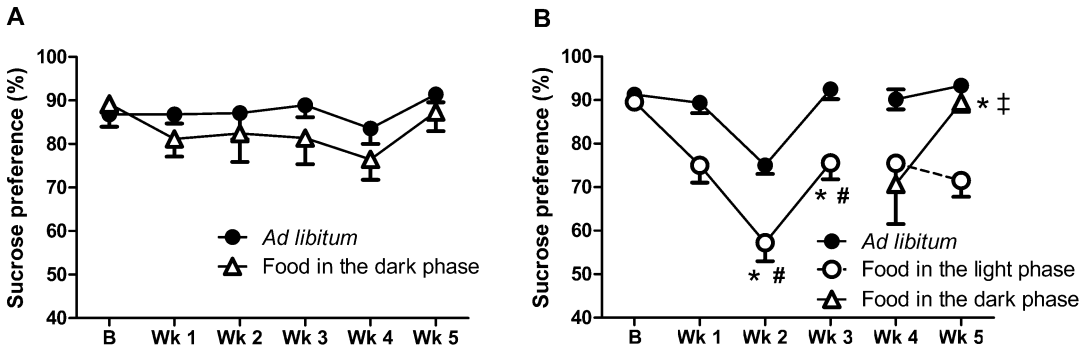

Fig. 3 – (A) Sucrose preference in rats submitted to food restriction (food in the dark phase). (B) Sucrose preference in rats submitted to food restriction (food in the light phase). After week 3, half of the animals under food restriction had their light/dark cycle changed to coincide food availability with the onset of the dark phase. *p<0.05 in comparison with *ad libitum group*). #p<0.05 in comparison with baseline of the same group. ‡p<0.05 in comparison with week 4. Repeated measures ANOVA. N= 10 rats per group (in the first segment of the B graph there were 20 rats in the food restriction group).

Acute antidepressant-like effect of melatonin, imipramine and agomelatine

One-way ANOVA detected treatment effect when the animals were administered imipramine, melatonin or vehicle in the forced swimming test ($F_{3,35}$=5.43; p<0.05). Imipramine (p<0.01) and the high dose of melatonin (10 mg/kg) (p<0.05) showed antidepressant effect in the forced swimming test, but the low dose of melatonin did not (1 µg/kg) (Fig. 4C).

In a second experiment, agomelatine had antidepressant effect ($F_{2,27}$=4.37; p<0.05). *Post-hoc* comparisons indicated significant effect of both doses (p<0.05) (Fig. 4D). None of the drugs changed the locomotor activity in the open field test (Fig. 4A,B)

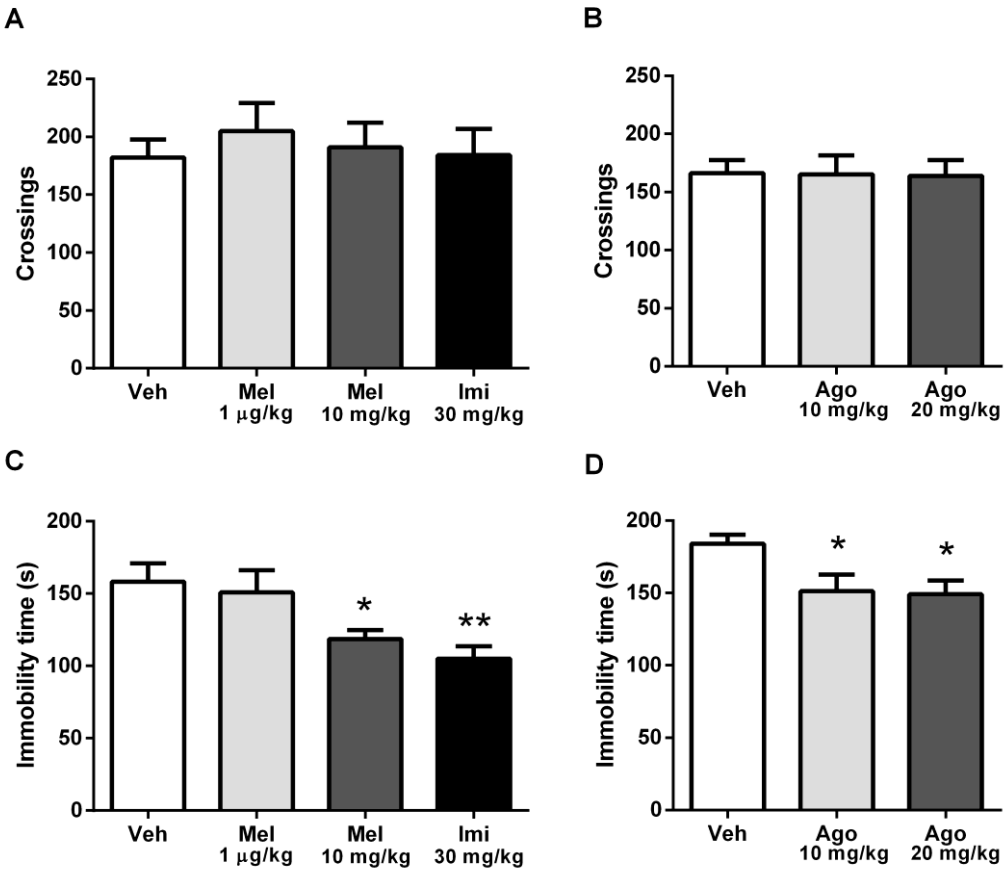

Fig. 4 – Locomotor activity in the open field test (A,B) and antidepressant-like effect in the forced swimming test (C,D) effect of melatonin in the high used dose (10 mg/kg), imipramine and agomelatine in mice. *p<0.05 in comparison with vehicle. **p<0.01 in comparison with vehicle. One-way ANOVA. N=8-10 mice per group.

Food restriction and pharmacological treatment

Food availability only during the light phase was also able to induce depressive-like behaviour in mice ($F_{1,24}$=5.61; p<0.05). A reduction in sucrose preference was observed in weeks 4-5 (p<0.05) (Fig. 5).

Fig. 5 – Sucrose preference in mice with food ad libitum or food restriction (food in the light phase). *p<0.05 in comparison with *ad libitum group*). #p<0.05 in comparison with baseline of the same group. Repeated measures ANOVA. N= 13 animals per group.

Contrary to expected, melatonin treatment in the light phase was neither able to prevent the reduction in sucrose preference in food-restricted animals nor it was able to promote depressive-like behaviour in animals with food *ad libitum* (food restriction: $F_{1,54}$=9.67; p<0.05; treatment: $F_{2,54}$=0.58, p>0.2; food restriction x treatment: F2,54=0.24; p>0.2) (Fig. 6).

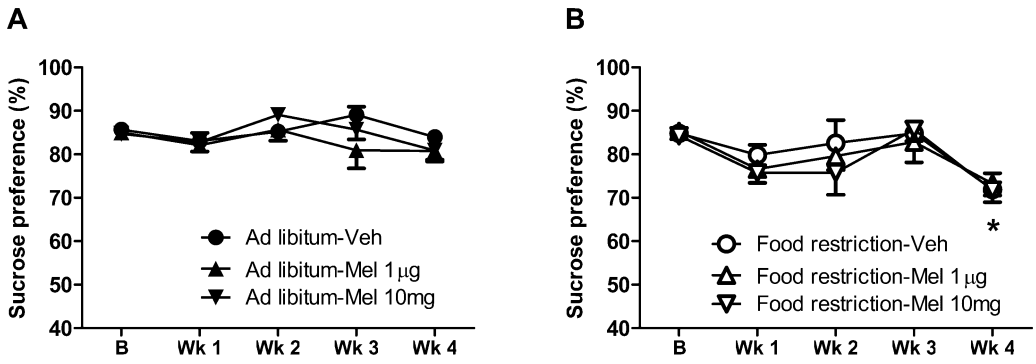

Fig. 6 – Sucrose preference in mice treated with melatonin or vehicle with food *ad libitum* (A) or food restriction (food in the light phase) (B). *p<0.05 for all three groups in comparison with *ad libitum* group. Two-way repeated measures ANOVA. N= 10 animals per group.

Chronic concomitant agomelatine or imipramine treatment also did not prevent the depressive-like effect of food restriction ($F_{2,24}$=0.37, p>0.2). However, there a was significant week effect, indicating a reduction in the sucrose preference ($F_{4,96)}$=3.67, p<0.01) (Fig 7A). Ketamine administration after the sucrose preference test in week 4 in the vehicle-treated group increased the sucrose preference in comparison with the groups treated with imipramine or

agomelatine ($F_{2,24}$)=4.80, p<0.05). *Post-hoc* comparisons indicated that ketamine had increase sucrose preference in comparison with both groups (p<0.05) (Fig. 7B).

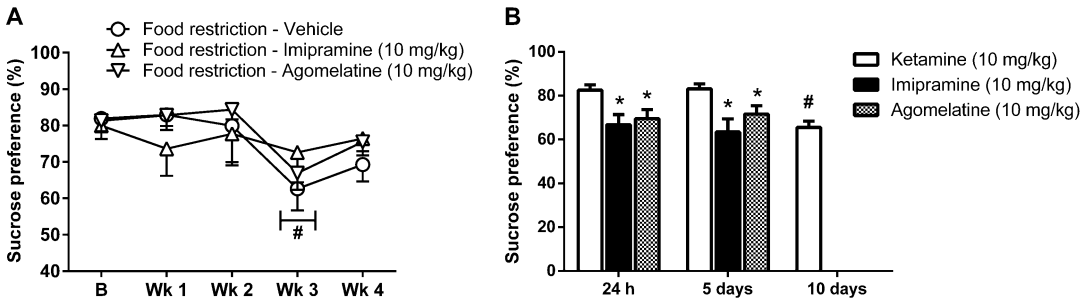

Fig. 7 – Sucrose preference in mice treated with imipramine, agomelatine or vehicle with food restriction (food in the light phase) (A). Sucrose preference 24 h after a single ketamine administration in the vehicle-treated animals (B). *p<0.05 n comparison with the ketamine group. # p<0.05 for the week factor in the repeated measures ANOVA. Two-way repeated measures ANOVA. N=8-10 mice per group.

Discussion

Here we showed that both arrythmicity induced by constant light and desynchronization between the imposed light/dark cycle and feeding schedule are able to promote depressive-like behaviour in rodents (Fig. 2). We also showed that both rescue of the LL-induced arrythmicity with timed food availability and synchronization of food availability with the onset of the dark phase have antidepressant-like effects (Fig. 2B). Pharmacological treatment with imipramine, agomelatine or melatonin did not improve the depressive-like behaviour induced by food restriction (Fig. 6 and Fig 7). However, a single administration of ketamine had antidepressant-like effects in this model (Fig. 7B).

In the current study, LL-induced depressive-like behaviour was reversed by phase advancing the light/dark cycle, so that food availability would coincide with the onset of the dark phase (Fig. 3B). Despite having food available for only 6 hours, animals receiving food during the dark phase did not reduce the sucrose preference in five weeks of restriction. In fact, food restriction, with food being given in the dark phase, has antidepressant-like effects. More specifically, food restriction consisting of 60% of average daily intake for 10 days reduced immobility time in the forced swimming test and prevented the deficits of social

interaction after exposure to the chronic social defeat model, an effect that was shown to be dependent on orexins (Lutter *et al*, 2008). On the other hand, 75% of average daily intake of food provided 2.5 h before the onset of the dark phase induced depressive-like behaviour in mice after 3 weeks (Pankevich *et al*, 2010).

Though agomelatine and the high dose of melatonin used in the chronic treatment had acute antidepressant-like effect in the forced swimming test, neither the high nor the low doses of melatonin were able to prevent the depressive-like behaviour induced by food restriction. Imipramine was administered in a lower dose in comparison to the used in the forced swimming test, although 10 mg/kg is a commonly used dose for chronic treatment in mice (Papp *et al*, 2014). One could hypothesized that exogenous administration of melatonin in a phase where the animal is active, but cannot secrete melatonin due to the tonic inhibition by light, would complete the shift towards the subjective night being set at the food availability interval. We also had initially hypothesized that a low dose of melatonin that reaches plasma concentration closer to physiological levels would be enough to signal the onset of the dark phase and, thus, have antidepressant-like effect. It is important to note that melatonin is quickly metabolized (17-13 min in rats) (Gibbs and Vriend, 1981), and even a single high dose might not be enough to signal the dark phase during the light phase. In other study, two melatonin administrations during the light phase were shown to induce depressive-like behaviour in the diurnal fat sand rats (Ashkenazy *et al*, 2009b). Another possibility is that food restriction might comprise a treatment-resistant model of depression.

In fact, ketamine, used as a dissociative anaesthetic, has rapid antidepressant action in refractory patients (Zarate *et al*, 2006) and in mice submitted to the chronic mild stress model (Autry *et al*, 2011). In this study, ketamine was chosen to be administered in the vehicle group after 4 weeks of food restriction. While the mice kept under antidepressant treatment did not improve the sucrose preference, a single administration of ketamine improved the sucrose preference in the mice that were being treated with vehicle.. This result is consistent with the effect of ketamine in refractory patients. Treatment-resistant mice with depressive-like behaviour induced by the chronic mild stress model have been analysed in terms of proteomics (Bisgaard *et al*, 2012) and

transcriptomics (Christensen *et al*, 2011), although, as far as the authors are aware, the effect of ketamine had not yet been evaluated in treatment-resistant animals. The confirmation of food availability during the light phase only as a model of treatment-resistant depression could be useful as a preclinical model for testing potential antidepressants that might act in a significantly different way than the currently available antidepressants. However, it was not the initial goal of this study and further experiments should be performed to specifically address this subject. For example, more doses or classes of antidepressants could have been tested. Additionally, other schedules of treatment could be performed, such as treatment after removal of the food (ZT8) or at the onset of the dark phase (ZT12).

In conclusion, we showed that maintenance of the circadian rhythmicity by food restriction might have antidepressant-like effects, while desynchronization between food availability and the light dark/cycle has the opposite effect. Stabilization of daily activities is one of the aims of the interpersonal and social rhythm therapy, which is effective in bipolar patients (Frank *et al*, 1997; Inder *et al*, 2014). Therefore, not only sleep and social habits should be encouraged to be regular, but also regular schedule for meals should be kept, both for treatment in patients and also as matter of public health in the prevention of mood disorders.

CAPÍTULO 3

Early onset of depressive-like phenotype caused by dim light at night in the Per3 knockout mouse

Bruno Jacson Martynhak MSc[1, 2], Alexandra L. Hogben PhD[1], Panos Zanos PhD[1], Roberto Andreatini PhD[2], Ian Kitchen PhD[1], Simon N Archer PhD[1], Malcolm von Schantz PhD[1], Alexis Bailey PhD[1], Daan R van der Veen PhD[1]

[1] Faculty of Health and Medical Sciences, University of Surrey, Guildford, Surrey GU2 7XH, UK

[2] Department of Pharmacology, Federal University of Paraná, Curitiba, Brazil

Running title: Depressive phenotype and night-time light exposure in Per3 null mice

Corresponding author

Daan R van der Veen

Faculty of Health and Medical Sciences

University of Surrey

Guildford, Surrey GU2 7XH - UK

E-mail: d.vanderveen@surrey.ac.uk

Telephone: +44 1483 686437

Telefacsimile: +44 1483 686401

Nota: Manuscrito a ser submetido ao periódico Neuropsychopharmacology

Abstract

Night-time light exposure is known to alter mood and cause depressive phenotypes in both humans and various rodent models. Besides a direct effect of light on affect, an indirect pathway has been suggested whereby light at night alters sleep and circadian timing, which in turn leads to the development of mood disorders. To further characterise this indirect effect of light on affect, we explored the development of mood disorders in mice carrying a functional knockout of the *Period3* gene, which is known to associate with altered sleep homeostasis and circadian photoreception. In contrast to WT mice, *Per3* null mice exhibited a rapid onset of depressive-like phenotypes as soon as 1 week after the start of night-time dim light (5 lux) exposure, as measured by a significantly reduced sucrose preference. Analysis of circadian behavioural patterns showed that WT mice respond to night-time light exposure by delaying their nocturnal onset of behavioural activity, and that this response was significantly attenuated in *Per3* null mice. The onset of the depressive-like phenotype in *Per3* null mice associated with increased levels of circulation corticosterone, as well as increased hippocampal expression of TNFα, while hippocampal BDNF expression was increased in both WT and *Per3* null mice. Pharmacological treatment with imipramine prevented the development of a depressive-like phenotype in response to night-time light exposure in *Per3* null mice without altering the differential circadian behavioural responses observed. These results suggest that the shift in circadian timing of activity caused by nocturnal dim light exposure may in fact be beneficial in protecting against developing a depressive-like phenotype, and that the involvement of the circadian system in the aetiology of depressive disorders is upstream of the pharmacological intervention offered through imipramine treatment.

Key words: depressive-like behaviour; dim light at night, *Per3*, circadian rhythms

INTRODUCTION

The availability of electricity in modern industrialised society has led to increased nocturnal light exposure in most countries (Bennie *et al*, 2014). Electrical light exposure during naturally dark periods is associated with delayed sleep onset and reduced time in bed (Knutson, 2014; Peixoto *et al*, 2009). Reduced sleep duration during weekdays can lead to compensatory long sleep episodes at weekends (Korczak *et al*, 2008), which is associated with increased depression scores (Levandovski *et al*, 2011). However, the pathway(s) upon which light acts to cause these deleterious effects on affect are poorly understood (Czeisler, 2013).

Light is perceived through rod and cone photoreception pathways (Altimus *et al*, 2010), as well as through the more recently identified non-image forming pathway involving intrinsically photosensitive retinal ganglion cells (Berson *et al*, 2002). The effect of light perceived through these photoreceptors on mood is hypothesised to involve both a direct pathway - connecting light input to the mood regulation regions such as the medial amygdala and lateral habenula - as well as an indirect pathway which involves light induced changes in the central circadian timing governed by the suprachiasmatic nuclei (SCN) (LeGates *et al*, 2014).

Changes in the light exposure have been associated with mood disorders in humans, with seasonal affective disorder, characterized by depressive episodes during winter and recovery in summer (Mersch *et al*, 1999) as the most clear example. Mood disorders are strongly associated with sleep and circadian rhythm disturbances. For instance, the core body temperature rhythm has been shown to have reduced amplitude (Suzuki *et al*, 2007) or to be even absent in some depressed patients (van Londen *et al*, 2001). The reciprocal relationship between circadian rhythms and mood is further supported by the action of antimanic and antidepressants on the circadian rhythms. Selective serotonin reuptake inhibitors antidepressants are known to phase advance circadian rhythms (Sprouse *et al*, 2006). Lithium has both antimanic and antidepressant activity, inhibits PKC and GSK3-β and phase advances circadian rhythms.

Depressive-like behaviour in animal models can be promoted by changes in the light/dark cycle. Both constant darkness (Gonzalez *et al*, 2008)

and constant light (Fonken *et al*, 2009; Martynhak *et al*, 2011; Tapia-Osorio *et al*, 2013) are able to increase immobility in the forced swimming test or to reduce sucrose preference, which are measures of depressive-like behaviour. Depressive-like behaviour induced by constant light was partially rescued when an opaque tube allowed the mice to escape from the light (Fonken *et al*, 2009), which suggests a role for the direct effect of the light. On the other hand, neonatal exposure to constant light prevents both arrhythmicity and depressive-like behaviour induced by constant light exposure in adulthood (Cambras *et al*, 1998; Martynhak *et al*, 2011), suggesting a role for the indirect effects of light through the disruption of the circadian rhythms. Although constant light conditions are useful as models, they do not represent light exposure in humans.

More naturalistic approaches have been tested, particularly attempts to simulate seasonal affective disorder by reducing the length of the light phase in diurnal animals (Einat *et al*, 2006). More recently, a derivation of the constant light protocol has been used. It is comprised by exposure to dim light (5 lux) at night (dLAN) instead of complete darkness (Bedrosian *et al*, 2011). In contrast to constant light exposure, animals under dLAN are capable to synchronize to a 24-h cycle. Although dLAN is able to trigger depressive-like behaviour, it is unknown whether its mechanism is related to the direct or indirect pathways of light. Prolonged exposure to dLAN promotes neurobiological alterations such as increased hippocampal TNF-α (Bedrosian *et al*, 2013), decreased hippocampal BNDF levels (Fonken and Nelson, 2013), and decreased hippocampal CA1 dendritic arborisation (Bedrosian *et al*, 2011), as also observed in the unpredictable chronic mild stress, a validated animal model of depression.

The role of direct or indirect light can be further disentangled with the use of knockout animals. For example, exposure to a period of 7 h does not impair mood in mice lacking ipRGCs (*Opn4*$^{aDTA/aDTA}$) despite them having intact image forming pathways (LeGates *et al*, 2014). The *Per3*-deficient mice model is of particularly interest. Lack of *Per3* expression leads to reduced response to the circadian effect of constant light in a light intensity-response (van der Veen and Archer, 2010). Additionally, these animals show less masking effect of light (ie, less activity inhibition by light) in an aberrant short light/dark cycle in which both WT and *Per3*$^{-/-}$ exhibit free-running activity (van der Veen *et al*, 2010) and also

display more activity in the light phase when exposed to long photoperiods (16L:8D) (Pereira *et al*, 2014). Despite these differential responses, *Per3*$^{-/-}$ are well synchronised to normal light dark-cycle and respond adequately to light-induced phase shifts (Shearman *et al*, 2000a). These data indicate that the indirect effects of light upon the circadian responses are intact in the *Per3* mice, whereas the direct non-visual effects of light are attenuated.

Thus, we sought to evaluate whether the reduced locomotor response to the direct effects of light in *Per3*-deficient mice would either prevent or facilitate the depressive-like behaviour induced by dLAN. Prevention would suggest a common pathway for the locomotor and affective effects of light, whereas facilitation would suggest independent pathways, as reduced locomotor inhibition of light would increase light exposure, enhancing its negative effects on mood.

MATERIALS AND METHODS

Ethics statement

All experimental procedures received a favourable opinion by the University of Surrey Animal Ethics Committee and were carried out under a U.K. Home Office License and in accordance with the Declaration of Helsinki.

Subjects and housing

All mice used in this study were on a C57BL/6J genetic background. Male mice homozygous for a targeted disruption of the *Per3* gene (Shearman *et al*, 2000a) and their wild-type controls were bred in-house from heterozygous breeding pairs and genotyped (Hasan *et al*, 2011; van der Veen *et al*, 2010). Food and water were available *ad libitum* unless otherwise stated.

For the forced swimming test, mice were housed in groups of 2-5 per cage in a temperature-controlled environment with a 12:12-h light/dark cycle (150 lux, <0.5 lux, lights on: 0700 hours).

For the dim light at night experiments, mice were individually housed in light-tight, sound-attenuated cabinets. Home cage locomotor activity was continuously recorded in 1-min bins through passive infrared detectors connected to a computer (ClockLab, Actimetrics, Wilmette, IL). Individual cage

illumination was supplied by LEDs (NSPW500BS, Nichia Europe BV, Amsterdam, the Netherlands) through frosted glass. Light intensity was set with the sensor at the cage bottom directed toward the light. Animals were kept in 12:12-h light/dark cycle cycle (150 lux, <0.5 lux, lights on: 1000 hours), unless specified.

Forced swimming test

To evaluate whether *Per3*-deficient mice had different depressive-like behaviour compared with WT mice in a baseline condition, the forced swimming test was performed (Porsolt *et al*, 1977). Briefly, animals were handled for three consecutive days previous to the test. Animals were placed in a cylinder of water at 23–24ºC for 6 min in a room at 40 lux. Total immobility and latency to the first immobility episode were measured.

Dim light at night (DLAN)

After two weeks of acclimation, a baseline sucrose preference test was performed and mice were randomly assigned to remain in LD cycle (light: ~150 lux/ dark <0.5 lux) or to change to dim light at night cycle, dLAN (light: ~150 lux/dim light: 5 lux) (Fonken *et al*, 2013). Sucrose preference tests were performed weekly to evaluate depressive-like behaviour. After four weeks in dLAN, mice were killed and trunk blood was collected for corticosterone measurement. Brain was collected and kept in RNAlater (Sigma Aldrich, St Louis, MO) and frozen at -80ºC for posterior dissection and qPCR.

dLAN with imipramine treatment

In order to investigate whether the dLAN-induced depressive-like behaviour is rescued with antidepressant treatment, a second experiment was performed similarly to the first one. In this experiment all mice were placed in dLAN cycle and half of the animals received imipramine through the drinking water to avoid synchronization or masking effects due to daily injections. Imipramine solution was changed twice a week and the concentration was adjusted aiming at a dose of 10-15 mg/kg/day according to liquid consumption. The treatment started at the same day the animals were isolated and placed in the cabinets (2 weeks before changing the cycle to dLAN). During the

habituation to sucrose and the sucrose preference tests, the animals did not receive imipramine. The animals were killed at the end of week 3 at the onset of the light phase.

Sucrose preference test

During the second week of acclimation, the mice were habituated with sucrose solution (2%) for 3 consecutive days. During the first 24 h, only sucrose solution was available and both sucrose and water were offered for the following 48 h.

For the sucrose preference test, bottles of water and sucrose solution were weighed before being placed and after 24 h. The test was performed weekly.

Gene expression using quantitative PCR

The hippocampus, prefrontal cortex and hypothalamus were dissected from the frozen brains. The mRNA was extracted with RNeasy Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions.

The qPCR products were detected using dual-labelled (FAM/BHQ1) hybridization probes specific to each of the cDNAs (Sigma). The set of primers that were used are shown in table 1

Table 1 – List of primers and probes

Target	Forward	Reverse	Probe
Tnf-α	TACTTAGACTTTGCGGAG	AGAGTAAAGGGGTCAGAG	AGGTCTACTTTGGAGTCATTGCTC
Bdnf	GGGTCACAGCGGCAGATAAA	GCCTTTGGATACCGGGACTT	TCTGGCGGGACGGTCACAGTCCTA
Per2	GACGCACACAAGAACTGATAAGG	CTCCGCAGGGCATACTTCA	TCCACCTCCCTGCAGACAAGAAGGC
Arp	GGGATTCGGTCTCTTCGACTAA	GCCTTTATTTCCATCTTTCTCAAATT	CCCGCCAAAGCAACCAAGTCAGC

Corticosterone measurement

Trunk blood from each animal was collected in heparin-coated tubes, immediately placed on ice, centrifuged at 4ºC and plasma was stored at −20°C until processing. Corticosterone was measured by using a rat/mouse corticosterone [^{125}I] radioimmunoassay kit (MP Biomedicals, New York, NY).

The corticosterone levels were determined in technical duplicates, all samples were assessed in a single assay.

Statistics

Sucrose preference tests were analysed by two-way ANOVA with repeated measures, with genotype and either dLAN or imipramine treatment as factors. Corticosterone and gene expression were analysed by two independent repeated measures two-way ANOVA, one with genotype, dLAN and week as factors and other with genotype, pharmacological treatment and week as factors. Therefore, the group of animal exposed to dLAN and treated with tap water was used in both analyses. The analysis was followed by the Newman-Keuls post-hoc test.

Phase of first and second peak of locomotor activity were calculated through the peak and trough method using fitted Fourier curve (3 harmonics) of the z-score normalized activity data from the last two days of each week. Some animals did not show any detectable peak under dLAN, and were therefore excluded from the analysis. The data from both experiments in which the animals were under dLAN and received no imipramine treatement was combined. Thus, a single analysis was performed using PROC MIXED in SAS 9.2 (SAS Institute, Cary, NC, USA) with the factors genotype, light condition and treatment and week. The post-hoc comparison was based on least square means (LSMEANS).

RESULTS

Forced swimming test

There were no differences between genotypes, neither in the total time of immobility ($t=1.09$, $p>0.2$) nor in the latency to the first immobility ($t=0.67$, $p>0.2$) in the forced swimming test (Fig. 1), indicating that *Per3*$^{-/-}$ mice does not show increased depressive-like behaviour under regular conditions.

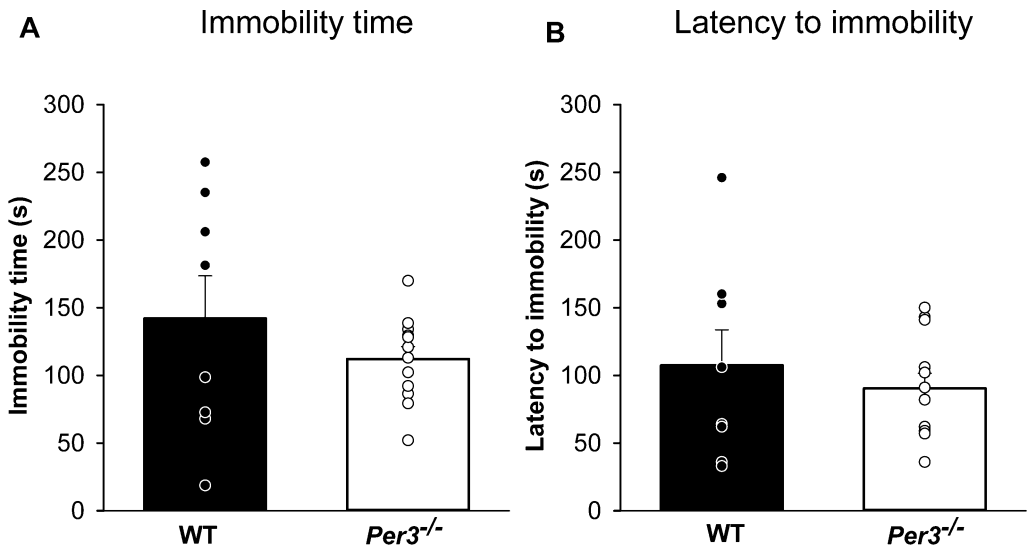

Fig. 1 – Immobility time (A) and latency to immobility (B) in the forced swimming test in WT and *Per3*$^{-/-}$ mice. N=9-12 per group.

Sucrose preference

Dim light at night led to depressive-like behaviour exclusively in *Per3*$^{-/-}$ mice (genotype: $F_{1,27}$=27.90, p<0.001; light schedule: $F_{1,27}$=1.42, p>0.2; genotype x light schedule: $F_{1,27}$=5.19; p<0.05; genotype x light schedule x week: $F_{4,108}$=18.3, p<0.001). *Post hoc* analyses indicated that the *Per3* $^{-/-}$ mice exposed to dLAN reduced their sucrose preference levels in weeks 2 and 3 (p<0.001), whereas the WT counterparts did not (Fig. 2A). The *Per3* $^{-/-}$ mice exposed to dLAN also had significantly lower levels of sucrose preference in these two weeks compared to the other groups (p < 0.001), although their sucrose preference levels restored to baseline levels on week 4 (Fig. 2A).

Imipramine treatment was able to prevent the reduction of sucrose preference in *Per3*$^{-/-}$ mice exposed to dLAN (genotype: $F_{1,26}$=10.0, p<0.01; treatment: $F_{1,26}$=0.9, p>0.2; genotype x treatment: $F_{1,26}$=5.5, p<0.05; genotype x treatment x week: $F_{3,78}$=4.2, p<0.01). *Post hoc* analysis showed that *Per3*$^{-/-}$ mice that received no treatment significantly reduced their sucrose preference on weeks 2 and 3 (p<0.001), whereas imipramine-treated *Per3*$^{-/-}$ mice did not (Fig 2B). Non-treated *Per3*$^{-/-}$ animals also had lower sucrose preference compared with imipramine-treated *Per3*$^{-/-}$ mice (p<0.001). Additionally, WT mice, both treated and non-treated had higher sucrose preference levels than the *Per3*$^{-/-}$ mice that received only tap-water (p<0.05) (Fig. 2B).

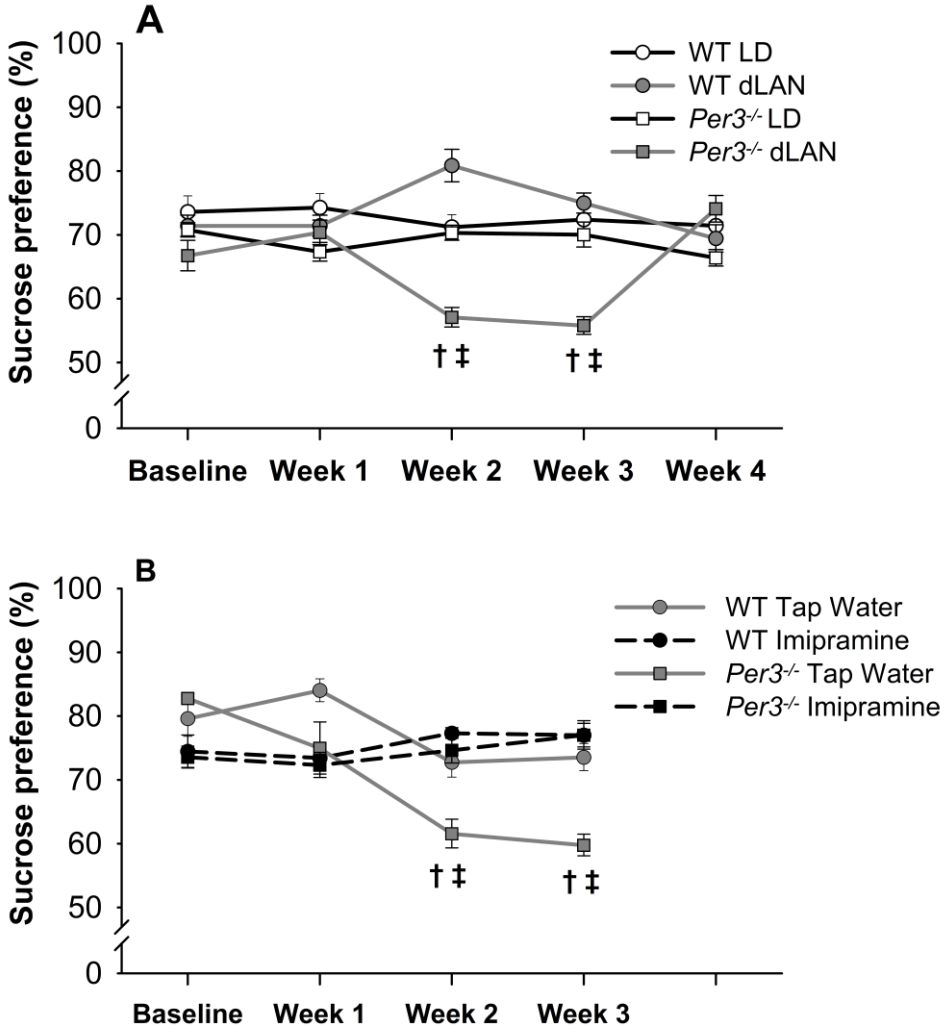

Fig. 2 – Sucrose preference in WT and Per3-/- mice in (A) LD or dim light at night and (B) dim light at night, treated with tap water or imipramine in the drinking water. †p<0.05 in comparison with the control group. ‡p<0.05 in comparison with baseline. N=6-8 animals per group.

Activity

Phase of the first peak of activity was influenced by genotype, dLAN and imipramine treatment (genotype: $F_{1,233}$= 4.59; dLAN: $F_{1,233}$= 25.75; treatment: $F_{1,233}$= 6.34; genotype x dLAN: $F_{1,233}$= 10.47 ; genotype x treatment: $F_{1,233}$= 5.83;p <0.05) (Fig. 3A,3C). dLAN significantly delayed the first peak of activity in weeks 2-3 for *Per3*$^{-/-}$ (p < 0.05) and in weeks 2-4 for WT mice (p<0.001), whereas it phased advanced the peak of activity in week 1 for *Per3*$^{-/-}$ (p<0.001). The delay in phase of activity was more pronounced in WT mice under dLAN compared to *Per3*$^{-/-}$ in weeks 1, 2 and 4 (p<0.001) (Fig 3B). In *Per3*$^{-/-}$ mice, imipramine treatment prevented the phase advance and the dLAN-induced

phase delay in weeks 1 and 2 ($p<0.05$), respectively whereas imipramine treatment in WT mice prevented the phase delay in weeks 3 and 4 ($p < 0.001$).

dLAN phase delayed the second peak of activity similarly in *Per3*$^{-/-}$ and WT mice (genotype: $F_{1,208}=2.53$, $p= 0.11$; light schedule: $F_{1,208}= 10.23$, $p<0.05$), an effect that was partially rescued by imipramine treatment (treatment: $F_{1,208}= 9.62$, $p<0.05$), although *post-hoc* comparisons did not indicate any differences.

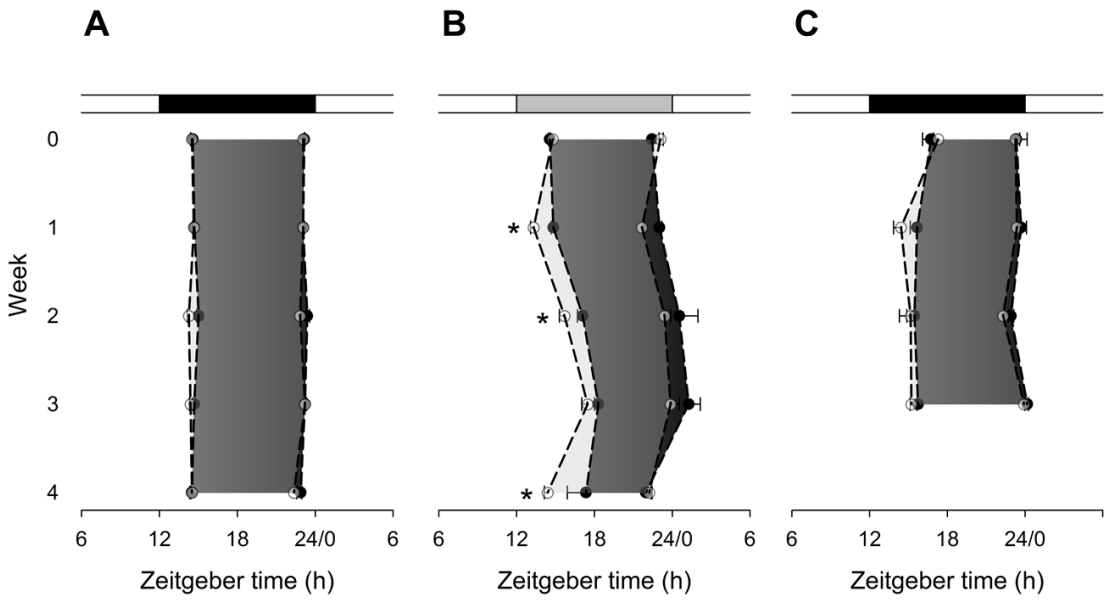

Fig.3 – Phase of activity of the first and second peaks of activity. *p<0.05 in comparison with WT. Gray areas: *Per3*$^{-/-}$ mice only, dark grey ares: both genotypes, black: WT mice. N=6-8 animals per group.

Corticosterone

Plasmatic corticosterone was increased in both genotypes after dLAN exposure (light schedule $F_{1,22}=34.3$, $p<0.001$; genotype: $F_{1,26}=1.3$, $p>0.2$, genotype x light schedule: $F_{1,26}=0.8$; $p>0.2$) (Fig. 4). Moreover, imipramine reduced the corticosterone of both genotypes exposed to dLAN (treatment: $F_{1,26}=13.5$, $p<0.01$; genotype: $F_{1,26}=1.3$; $p>0.2$ neither treatment x genotype ($F_{1,26}=0.6$, $p>0.2$).

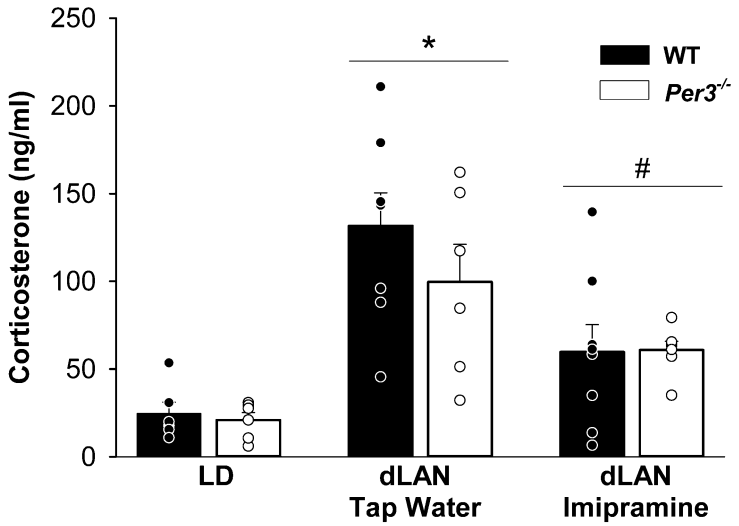

Fig. 4 – Plasmatic corticosterone collected at ZT0 (lights on). *p<0.05 in comparison with LD. #p<0.05 in comparison with dLAN tap water. N=6-8 animals per group

Gene expression

Hippocampal *Tnf-α* expression was increase in *Per3*$^{-/-}$ mice exposed to dLAN (genotype x light schedule: $F_{1,20}$=4.9, p<=0.05) (Fig. 5A). The same pattern was repeated analysing the treatment effect. Imipramine treatment reduced the hippocampal expression of Tnf-α in *Per3*$^{-/-}$ under dLAN (genotype x treatment: $F_{1,18}$=7.6, p=0.05). Thus, the increase in *Tnf-α* expression was observed only in *Per3*$^{-/-}$ mice and reversed by imipramine treatment.

Bdnf expression was increased in both WT and *Per3*$^{-/-}$ after dLAN exposure (light schedule: $F_{1,23}$=8.2, p<0.01; genotype: $F_{1,23}$=1.45, p>0.2; genotype x light schedule: $F_{1,23}$=1.05, p>0.2) (Fig. 5B). Conversely, imipramine treatment also reduced Bdnf expression in both genotypes (treatment: $F_{1,23}$=8,2, p<0.01; genotype: $F_{1,22}$=, p=0.16; genotype x treatment: $F_{1,22}$=0.15, p>0.2) Therefore, similarly to corticosterone, dLAN increased *Bdnf* expression in both genotypes, an effect that was reversed by imipramine treatment.

There were no detectable effects in the pre-frontal expression of *Per2* (Fig. 5C), a central and rhythmically expressed clock gene.

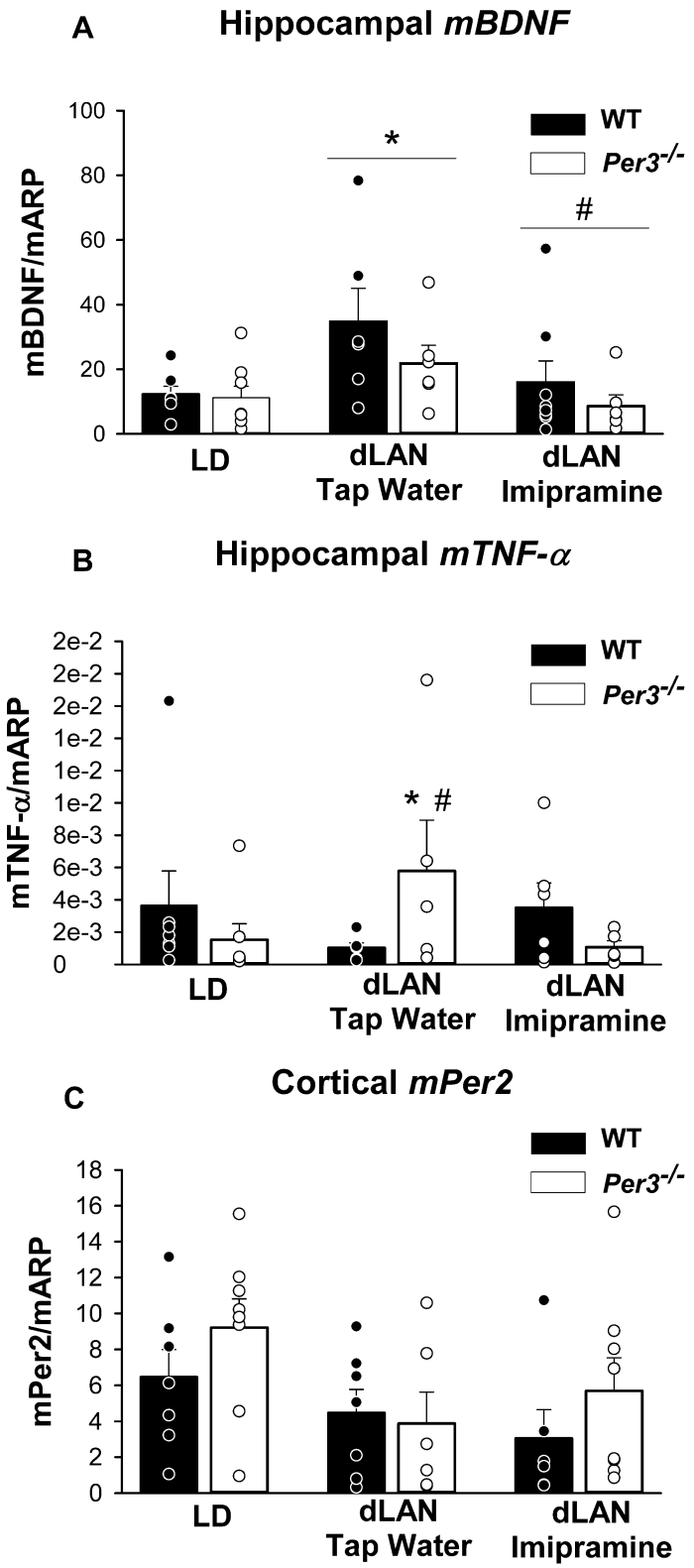

Figure 4 – Expression of hippocampal Bdnf (A), hippocampal Tnf-α expression (B) and pre-frontal Per2 (C). *p<0.05 in comparison with LD. #p<0.05 in comparison with dLAN tap water. N=5-8 per group.

DISCUSSION

The present study sought to investigate potential differential effects of dim light at night in *Per3*$^{-/-}$ mice in comparison to WT. In line with previous data (Pereira *et al*, 2014; van der Veen *et al*, 2010), we showed that *Per3*-deficient mice were less sensitive to the effects of dim light at night exposure on the locomotor circadian activity. Nevertheless, *Per3*-deficient mice were more sensitive to the light-induced effects on depressive-like behaviour. Reduced locomotor response to light can actually increase light exposure due to lessened escape behaviour, enhancing its negative effects on mood. Thus, we support the view for independent pathways for the locomotor and mood regulation in response to the direct effect of light.

Prolonged dLAN exposure increased plasmatic corticosterone and hippocampal *Bdnf* RNA levels measured at the beginning of the light phase, an effect that was also prevented by imipramine treatment in the drinking water. As observed previously with other strains (Bedrosian *et al*, 2012), the depressive-like behaviour was prevented by antidepressant treatment. However, despite the reversal with imipramine treatment, the dLAN-induced increase in corticosterone is not enough to explain the genotype differences in depressive-like behaviour, given that corticosterone was equally increased in both WT and *Per3*$^{-/-}$. Increase in *Bdnf* expression might not reflect increase in activation of its receptor, Trkb. It is known that pro-BDNF might actually lead to opposite effects of BDNF, inducing apoptosis instead of cell survival (Teng *et al*, 2005). An increase in pro-BDNF might explain the increase in hippocampal *Bdnf* expression after exposure to dLAN. However, similarly to corticosterone, this increase in *Bdnf* expression also does not explain the genotype differences, given it was also increased in both genotypes and reduced by imipramine treatment.

Hippocampal *Tnf-α* expression was increased in *Per3*-deficient mice only. Moreover, imipramine treatment was able to rescue *Tnf-α* expression to control levels, which is in line with previous findings (Bedrosian *et al*, 2013). Depression is being associated with a chronic inflammatory process with increased levels of interleukin (IL)-1b, IL-6, TNF-α), prostaglandin E2 and also marked increased oxidative and nitrosative stress (Berk *et al*, 2013). Rats exposed to chronic mild stress show anhedonia-like behaviour and increased

oxidative stress, which are both rescued by antidepressant or anti-inflammatory treatment (Santiago *et al*, 2014).

In a previous study with wild-type mice, 4 weeks of dim light at night was sufficient to trigger depressive-like behaviour (Fonken *et al*, 2013). However, our wild-type animals did not reduce the sucrose preference within this time period. This might be explained by the fact that different strain of mice was used in these experiments. While the mentioned study employed C3H/HeNHsd, mice that have intact melatonin production, we used C57/BL6 mice, which do not produce melatonin under normal conditions (Kasahara *et al*, 2010). Therefore, suppression of melatonin production through the dim light could be more deleterious than for animals that do not usually produce it.

Several stressors, which increase corticosterone, are used to promote depressive-like behaviour in rodents (Willner *et al*, 1987) and dexamethasone administration can also lead to depressive-like behaviour (Casarotto *et al*, 2007). Thus, *Per3*$^{-/-}$ mice might be more sensitive to the corticosterone effects, given that both genotypes had similar increase of plasmatic corticosterone in a circadian phase where it was supposed to be low. Another possibility is that the escape behaviour from light is adaptive, so that WT mice are less exposed to light and consequently more protected from the dim light at night effects on affective states, despite shifting the circadian phase more than *Per3*$^{-/-}$. The escape behaviour comprises the set of behaviours induced by light, such as sleeping, closing the eyelids or burying in the bedding and nesting material.

In conclusion, *Per3*$^{-/-}$ mice had an earlier onset of depressive-like behaviour under dim light at night. Inflammatory process, increased sensibility to corticosterone and enhanced exposure to light are possible factors that might mediate this differential response.

Disclosure

The authors declare no conflict of interest.

CAPÍTULO 4

Circadian fluctuation of reward response and synchronization to reward.

Bruno J Martynhak, Universidade Federal do Paraná, Departamento de Farmacologia. Cel. Francisco H. dos Santos, Paraná, PR, Brazil. brunojm@ymail.com

Nota: Capítulo de livro publicado em:
Ritsner, Michael (Ed.). Anhedonia: A Comprehensive Handbook Volume I. Conceptual Issues And Neurobiological Advances. Springer: Dordrecht, 2014, 352 p. ISBN 978-94-017-8591-4. Chapter 3 p. 51-53.

Abstract

Several physiological processes common to almost all living beings show fluctuations within the 24 hours that compose the Earth's light/dark cycle. Some examples of this in humans are the rise of cortisol levels early in the morning, the secretion on melatonin during the night and the core body temperature maxima and minima occurring during the late afternoon and late at night, respectively. Disruption of the circadian system can be one of the factors leading to depression and anhedonia. Alterations in circadian rhythms found in depressive patients include reduced amplitude of circadian rhythms, elevated temperature at night and early cortisol secretion. One feature of depression is diurnal variation in mood, which is generally worse during the morning and improves throughout the day. Moreover, the hedonic value of reward changes throughout the day and reward can synchronize circadian rhythms, as daily injections of methamphetamine can induce anticipation behavior. In this chapter, we will address the neurobiology of circadian rhythms, diurnal mood variation, fluctuating properties of reward over the 24 hour cycle and, finally, the ability to synchronize circadian rhythms to reward regardless of the imposed light/dark cycle.

Key words: anhedonia, depression, circadian rhythms, diurnal mood variation, reward, food-entrainable oscillator

Abbreviations

-
- 5-HIAA: 5-Hydroxyindoleacetic acid, main metabolite of serotonin
- 5-HT: 5-Hydroxytryptophan or serotonin
- CMS: Chronic mild stress
- CT: Circadian time. CT0 is the onset of the activity
- DA: Dopamine
- DD: Dark:dark cycle or constant darkness
- DMV: Diurnal mood variation
- DOPAC: 3,4-Dihydroxyphenylacetic acid, a dopamine metabolite
- DAT: Dopamine transporter
- DSM - Diagnostic and Statistical Manual of Mental Disorders
- EEG: Electroencephalography
- HVA: Homovanillic acid, a dopamine metabolite
- ICD - International Classification of Diseases
- FAA – Food anticipatory activity
- FEO – Food entrainable oscillator
- GABA - γ-Aminobutyric acid
- LD: Light:dark cycle
- MASCO: Methamphetamine-sensitive circadian oscillator
- Nac: *Nucleus accumbens*
- REM: Rapid eye movement
- SCN: Suprachiasmatic nucleus
- SCNx: Lesion in the suprachiasmatic nucleus
- SSRI: Selective serotonin reuptake inhibitors
- STAR*D: Sequenced Treatment Alternatives to Relieve Depression
- TH: Tyrosine hydroxylase
- VTA: Ventral tegmental area
- ZT: Zeitgeber time. ZT0 is the lights on time

Overview of the neurobiology of the circadian rhythms

Nearly all organisms on the planet show 24 h rhythmicity across multiple levels of their biology, from gene expression to behavior. This rhythmicity persists even in the absence of time cues. In this case, the organism is said to be free-running, displaying an endogenous circadian period. This period is close to the 24 h cycle of the Earth, and it is synchronized every day from exposure to the light/dark cycle. In mammals, light stimulates a photopigment called melanopsin, localized in the ganglion cells of the retina. Light information flows through the retino-hypothalamic tract to a tiny structure called the suprachiasmatic nucleus (SCN) in the hypothalamus. The SCN is responsible for generating both endogenous circadian rhythms and for entraining this rhythm to light information from the environment. The SCN sends projections to many areas of the brain including the paraventricular nucleus and the pineal gland.

Inside every single nucleated cell lies a set of genes that are not only expressed in a circadian manner but also drive the expression of other genes, orchestrating a molecular clock. The molecular clock is composed of core clock genes and clock-controlled genes. Given that each nucleated cell has its own clock, the role of the SCN is to synchronize rhythms within different cells and different tissues. Therefore, the SCN is also known as the master clock or the central pacemaker. The core clock genes show both positive and negative feedback loops of expression. The basic feedback loop is composed of the heterodimer *Clock:Bmal1* that promotes the expression of members of the *Per* and *Cry* gene families, which in turn also form heterodimers. These dimers enter the nucleus and inhibit the action of *Clock:Bmal1*, therefore inhibiting their own expression. The dimers are then degraded, restarting the cycle.

Because the circadian timing system influences a vast array of behavioral and molecular responses, it is not surprising that the properties of the reward system are also influenced by the phase of these rhythms. Additionally, circadian rhythm disruption may be involved in a series of disorders, including depression, which will be a particular focus of this chapter. This chapter will address (1) the diurnal mood variation in both depressed and healthy subjects, (2) the diurnal variation of the reward system in animal

models, and (3) the synchronization of the circadian system to the availability of reward.

Circadian alterations in depression

Major depressive disorder and bipolar disorder have long been associated with circadian rhythm disruption. As far back as the sixteenth century, there have been descriptions of sleep-wake cycle disturbances and its effects on mood (Lemmer, 2009).

Symptoms related to circadian rhythm disruption in major depression include elevated nocturnal body temperature, advanced or delayed and increased cortisol secretion, as well as advanced or delayed and reduced melatonin secretion. Reduced amplitude of circadian rhythms is also common in depressive patients. Flatter diurnal cortisol curves were more likely to occur in participants with severe depression than in those with mild to moderate levels of depression (Hsiao *et al*, 2010), including healthy adolescents that have been through one episode of major depression (Doane *et al*, 2013). Temperature rhythms have been shown to have reduced amplitude (Suzuki *et al*, 2007) or are even absent in some depressed patients (van Londen *et al*, 2001).

The internal synchronization of different rhythms might also be compromised in severely depressed patients. The onset of melatonin secretion and minimum core body temperature is desynchronized in severely depressed patients (Hasler *et al*, 2010). Moreover, the extent of this misalignment is correlated with the severity of the anhedonic state, measured by the BDI-anhedonia score.

Changes in sleep architecture are also observed in depressed patients, including shortened latency to rapid eye movement (REM) sleep and increased overall duration and reduction in slow-wave sleep, all of which contribute to earlier wake from sleep in patients (Monteleone and Maj, 2008). Bipolar patients in the mania phase have reduced need for sleep (Mitchell *et al*, 2008) and show increased melatonin levels (Kennedy *et al*, 1989). Interestingly, one night of sleep deprivation can promote temporary antidepressant effects in depressed patients or manic shift in some bipolar patients (Benedetti, 2012). Sleep deprivation is also effective when the subject is sleep-deprived only for the

second-half of the night when REM sleep is more concentrated in healthy individuals.

It is important to note that remitted patients who fail to restore normal rhythms have increased risk of major depression early relapse (Breslau *et al*, 1996). Moreover, most of current treatments for mood disorders shift or stabilize circadian rhythm (McClung, 2013). In particular, selective serotonin reuptake inhibitors (SSRI) advance the phase of circadian rhythms (Sprouse *et al*, 2006). Animal models have successfully provided further information regarding the relationship between circadian rhythmicity and depression. Some models have attempted to study seasonal depressive disorder in either diurnal (Ashkenazy *et al*, 2009b) or in nocturnal rodents (Prendergast *et al*, 2005). In these studies, reduction of the photoperiod led to depressive-like behavior, such as increased mobility during the forced swimming test and reduction in sucrose preference. Interestingly, melatonin administration during the light phase of the cycle also promoted depressive-like behavior in nocturnal rodents (Ashkenazy *et al*, 2009b). This finding can be interpreted as a reduction of the photoperiod or a conflict between melatonin signaling and information from the light. Other models do not have clear correlations to human life, such as the induction of depressive-like behavior in nocturnal mice after exposure to a photoperiod of 22 hours of light and 2 hours of dark (Becker *et al*, 2010). Even more drastic changes in the photoperiod were analyzed in animal models. Animals exposed to six weeks of constant darkness (DD) displayed an increase in immobility during the forced swimming test and showed damage to monoaminergic neurons (Gonzalez *et al*, 2008). On the other hand, constant light (LL), which is known to lead to arrhythmicity, also induced depressive-like behavior (Fonken *et al*, 2009; Martynhak *et al*, 2011).

In summary, it is clear that changes in the light/dark cycle can lead to depressive-like behavior, including anhedonia, and that depressed patients show circadian rhythm disruption. Additionally, restoring rhythmicity is associated with better treatment outcomes, and the prevention of rhythm disruption can avert the development of depressive-like behavior.

Diurnal variation of mood in depressed patients

Most of the studies described in this session focus on mood in a general sense, not specifically in states of anhedonia. However, as anhedonia is a core symptom of depression, the diurnal variation in mood might be correlated with diurnal variation in the ability to experience pleasure.

Diurnal mood variation (DMV) with early morning worsening is considered a classic symptom of melancholic features in The Diagnostic and Statistical Manual of Mental Disorders IV (DSM-IV) as well as the 10th International Classification of Diseases (ICD) criteria for somatic major depressive disorder (MDD) (Wirz-Justice, 2008). Depression patients exhibit the worst depressive mood during the morning, gradually improving throughout the day. However, patterns of DMV were analyzed in a large cohort of patients composed of individuals in the STAR*D (Sequenced Treatment Alternatives to Relieve Depression). DMV was reported in 22.4% of cases; 31.9% reported morning, 19.5% afternoon and 48.6% evening worsening (Morris *et al*, 2007). Melancholic symptom features were associated with DMV, regardless of timing. Therefore, melancholic depression is in fact not limited to having a worse mood in the morning, but instead to any type of diurnal mood variation.

Diurnal mood variation has also been hypothesized to influence treatment outcome. In a large study comprising 2,875 major depressive patients in a 14-week clinical trial of the selective serotonin reuptake inhibitor (SSRI) citalopram, participants were divided into three groups: those with early morning worsening DMV, those with any form of DMV, and those with no DMV. Though the group with the classic morning worsening DMV had slightly increased responses compared to the no DMV group, remission rates were not different between groups (Morris *et al*, 2009). Another study compared three groups of depressed patients: morning worsening DMV, evening worsening DMV and no DMV. Among patients between 18-24 years old, those with no DMV and those with morning worsening DMV responded better to fluoxetine (SSRI) than to nortriptyline (tryciclic antidepressant that inhibits noradrenaline re-uptake), whereas patients with worse mood during the evening did not. On the other hand, patients older than 24 years demonstrated the opposite results: patients with evening worsening responded better to nortriptyline, whereas the other groups did not show any difference between treatments (Joyce *et al*, 2005).

Additionally, patients with classic morning worsening DMV responded better to the acute antidepressant effects of total sleep deprivation in comparison to those with evening worsening DMV or no DMV (Reinink *et al*, 1990).

In an attempt to unravel the neurobiology of diurnal variation in mood, depressed subjects with a worse mood in the morning had their neuronal activity measured and compared to healthy subjects, both in the morning and in the evening (Germain *et al*, 2007). Positron emission tomography (PET) scans of the regional cerebral metabolic rate of glucose revealed a hypometabolism in frontal cortical areas and hypermetabolism in subcortical and limbic-paralimbic areas in depressed subjects regardless of the time of day. However, evening improvement in mood was associated with greater increases in both parietal and temporal cortical glucose metabolism. Though the increases were greater compared to healthy subjects, these areas were still hypometabolic. Despite the fact that causal relations are hard to establish, one could say that there is at least a common factor underlying both mood expression and glucose metabolism in specific brain areas. Of note, in this study (Germain *et al*, 2007), the healthy subjects also showed DMV, but instead of the classic morning worsening, the healthy subjects showed evening worsening DMV.

One important difference between DMV in healthy and depressed patients is that melancholic patients experience spontaneous mood variations outside of their control, suggestive of helplessness, whereas healthy controls consider mood variation almost exclusively related to their own activities and/or external circumstances (Wefelmeyer and Kuhs, 1996). Naturally, healthy individuals can show some non-clinical depressive features. A group of healthy individuals were divided into two groups for low and high depression according to their score in a depression scale evaluated by the Centre for Epidemiological Studies-Depression Scale (CES-D). The participants had their positive affect evaluated throughout the day using an abbreviated version of the well Positive and Negative Affect Scales (PANAS). It was observed that both low and high depression groups had the same low scores of positive affect during the morning, with both groups improving in the evening (Murray, 2007). However, the evening peak was lower in the high depression group (Murray, 2007), reinforcing the idea that amplitude reduction/rhythm ablation may be underlying the development of depression.

A very interesting study identified individual-level diurnal and seasonal mood rhythms in cultures across the globe, using data from millions of public Twitter messages (Golder and Macy, 2011). The authors found that mood deteriorates as the day progresses. Not surprisingly, they also found that people had increased positive affect on weekends, with the morning peak in mood delayed by 2 hours.

One bias to consider in the study of mood variation throughout the day is the influence of the circadian phase, the time spent awake or both. A protocol termed forced desynchronization was developed to separate the effect of circadian phase and sleep. Using this protocol (a sleep schedule of 30 h, instead of 24 h), the moods of healthy volunteers were evaluated using two visual analog scales administered regularly during waking periods. Analysis indicated an effect of the circadian phase but not of the time spent wake on mood (Boivin *et al*, 1997). Interestingly, a significant interaction was found between the circadian phase and time awake: depending on the circadian phase, mood improved, worsened or did not change according to the duration of prior wakefulness.

Diurnal variation of reward in animal models

Early studies demonstrated that rewarding self-stimulation of the brain varies across the day, peaking during the mid to late dark period (see (Webb *et al*, 2009a) for review). This rhythm has been shown to persist under constant darkness conditions, suggesting circadian modulation. Other studies that used drugs of abuse as a reward also detected circadian fluctuations in drug intake, where the peak usually correlated with the more active phase within the light/dark cycle (Baird and Gauvin, 2000; Roberts *et al*, 2002).

However, intake itself might be increased during the dark phase simply due the use of nocturnal animals, which are more active at night, instead of being related to an intrinsic rhythmicity property of the reward system. Further experiments were performed to address this issue. One study compared consecutive sucrose preference tests in the light and dark phases (Tonissaar *et al*, 2006). Importantly, the sucrose preference is calculated as the percentage of sucrose intake over the total liquid intake, controlling for factors that might influence locomotor activity. The authors observed increased preference in

subjects for sucrose during the dark phase. However, in subsequent tests, sucrose preference during the light phase increased to the same levels as the dark phase (Tonissaar *et al*, 2006). Another study evaluated the influence of the time of day on intravenous self-administration of cocaine in Sprague-Dawley rats (Baird *et al*, 2000). Four selected times of day were chosen for training: ZT01, ZT07, ZT13, and ZT19. ZT refers to *zeitgeber time*, and ZT0 is the time when lights are turned on. Groups that were trained at ZT07 and ZT19 (around the middle of light and dark phase, respectively) appeared to exhibit enhanced sensitivity to the reinforcing properties of low-dose cocaine relative to other groups, independent of locomotor activity and cocaine pharmacokinetics (Baird *et al*, 2000).

Daily rhythms have also been reported for psychomotor stimulant-induced behavioral sensitization and conditioned place preference in mice. Cocaine sensitization and conditioned place preference were shown to be more evident when performed at ZT4 (light phase) than at ZT12 (the onset of dark phase) (Abarca *et al*, 2002). Similarly, in another study, conditioned place preference was more evident at ZT5 than at ZT20. Furthermore, pinealectomy prevented this reduction in conditioned place preference at ZT20, demonstrating a possible protective role for melatonin during the night (Kurtuncu *et al*, 2004). In other words, pinealectomy rendered the animals more sensitive to the rewarding effects of cocaine administered at night. Such a conclusion may sound exquisite given that melatonin would be reducing reward, whereas reward has an active role in the normal and adaptive behavior. The answer might lie in the difference between how natural and drug-associated reward are processed throughout the day.

Natural and drug-based rewards were evaluated at different times of the day using the conditioned place preference paradigm. The chosen natural reward was mating, and amphetamine was used as a drug reward. Diurnal rhythms were observed for both mating and amphetamine-reward. The peak of the mating-based reward occurred in the middle of the dark phase (ZT17), and the peak for the low-dose amphetamine reward occurred between the end of the dark phase (ZT23) and the middle of the light phase (ZT05). Moreover, this rhythm persisted in constant conditions, reaching its peak at CT17 (Webb *et al*, 2009b), where CT refers to circadian time, and CT0 is the onset of activity in

free-running animals. Interestingly, the peak for mating reward showed the opposite results as those observed for drugs of abuse, in which subjects were less likely to develop place preference during the dark phase (Abarca *et al*, 2002; Kurtuncu *et al*, 2004). In fact, as aforementioned, the increased intake of drugs of abuse is usually during the active phase, but the mentioned studies found it easier to induce place preference in the less active phase. Therefore, there seems to be a difference between the absolute intake of reward, association between intake and reward and, finally, the type of reward. One could hypothesize that an organism is ready to receive small rewards during the active phase (natural rewards) but would be more sensitive to excessive activation of the reward system during the inactive phase.

Given the central role of the dopaminergic system in reward, many studies have looked at the circadian fluctuation of dopamine and its metabolites in the mesolimbic reward system (Webb *et al*, 2009a). Most of the components of the dopaminergic system have been shown to express some degree of diurnal variation.

Using microdialysis, the extracellular concentrations of dopamine (DA) and the metabolites DOPAC and HVA were evaluated both in the striatum and nucleus accumbens in Wistar rats over a 30 h period (Castaneda *et al*, 2004). Additionally, glutamate, gamma-aminobutyric acid (GABA), serotonin (5-HT) and its metabolite 5-HIAA were also measured. When animals were exposed to a regular light/dark schedule (12:12), the authors observed a clear circadian rhythm for DOPAC, HVA as well as glutamate and GABA in both the striatum and nucleus accumbens (NAc). Though dopamine was also reported to follow a circadian rhythm, the peak occurred only during the first measurements of the 30 h experiment and was not observed 24 h later. Therefore, the dopamine peak was most likely only due to arousal. However, when animals were exposed to constant light during sample collection, the rhythm of dopamine concentration was more evident in both the VTA and NAc (Castaneda *et al*, 2004). Additionally, dopamine clearance has been shown to vary diurnally in the NAc and medial prefrontal cortex (Sleipness *et al*, 2008). Using rotating disk electrode voltammetry and adding a fixed concentration of dopamine, the authors observed the highest clearance at ZT4 (Sleipness *et al*, 2008).

The dopamine transporter (DAT) protein levels and the rate-limiting enzyme in DA synthesis, tyrosine hydroxylase (TH) is also expressed with diurnal variation (Sleipness *et al*, 2007). Western blots analysis was performed at ZT4 and ZT20 in the NAc and caudate, both in sham-operated and SCN-lesioned (SCNx) rats (Sleipness *et al*, 2007). In the NAc, both DAT and TH expression were higher at ZT20 than at ZT4. However, SCNx blunted the difference in DAT expression and increased the peak of TH. Caudate TH expression was slightly elevated at ZT20 in sham-operated but not in SCNx rats. These results indicate that most of the diurnal variation in the dopaminergic system is dependent on the activity in the master clock located in the SCN.

A population of neurons in the VTA was found to rhythmically fire selectively during the active phase of the rat and that the SCN indirectly projects to VTA via the medial preoptic nucleus (Luo and Aston-Jones, 2009). Additionally, rhythmic expression of cFos was observed in the nucleus accumbens (NAc) core and shell in the medial prefrontal cortex and in TH-IR and non-TH-IR cells in the ventral tegmental area (VTA), with peak expression during the late night and nadirs during the late day (Baltazar *et al*, 2013).

Circadian fluctuation in anhedonia has also been described. The chronic mild stress protocol (CMS) was developed by Paul Willner's group as an animal model of depression with face, predictive and construct validity (Willner *et al*, 1987). The CMS is comprised of a series of unpredictable mild stressors that within weeks leads animals to anhedonic-like behavior, observed by reduction of intake or preference for sucrose solution, which is rescued with chronic antidepressant treatment. However, despite successful use of the model in other laboratories, Paul Willner's group experienced difficulties in replicating their own results. One of the differences they noticed was that in most of the studies from other groups, sucrose preference occurred during the end of the light phase, instead of the beginning of the light phase (D'Aquila *et al*, 1997). Therefore, it was tested whether animals submitted to the CMS would show anhedonia only when tested close to the dark phase. The animals tested at the beginning of dark phase displayed reduced sucrose intake and consumption, while those tested at the light phase did not (D'Aquila *et al*, 1997), suggesting a diurnal variation in sucrose reward in an animal model of depression.

In addition to decreasing responsiveness to reward, CMS also causes the appearance of many other symptoms of major depressive disorder. Behavioral changes in animals exposed to CMS include decreases in sexual and aggressive behaviors (D'Aquila *et al*, 1994). These phenotypes were observed during the dark phase of the light-dark cycle, which is the active period for the rat (Gorka *et al*, 1996). EEG measurement of active waking is also decreased during the dark phase (Cheeta *et al*, 1997), and a variety of sleep disorders characteristic of depression, including decreased REM sleep latency, an increased number of REM sleep episodes, and more fragmented sleep patterns were also observed (Cheeta *et al*, 1997).

Synchronization to reward

Not only does the response to reward vary throughout the 24 h cycle, but the availability of reward can also set the internal time. When food is available during a restricted and predictable time of the day, mammals exhibit food-anticipatory activity (FAA), an increase in locomotor activity preceding the presentation of food. If food is presented during the light phase, animals shift their activity phase to the time of food availability, despite the phases of the light/dark cycle. Although the participation of the dopaminergic system may not be enough to explain this behavior, it is becoming clear that dopamine plays a central role in the FAA.

When mice received either a D1 or D2 dopaminergic receptor antagonist, the expression of FAA was significantly reduced (Liu *et al*, 2012). Interestingly, the co-administration of high doses of antagonists for both receptors showed a synergic effect, promoting a more robust reduction of the FAA (Liu *et al*, 2012) and suggesting that the D1 and D2 receptors contribute in different ways to the expression of FAA. Consistently, the levels of dopamine and its metabolites in the striatum and midbrain were significantly increased during FAA (Liu *et al*, 2012), which is in agreement with the phasic release of dopamine after exposure to a conditioned stimulus associated with reward. In this case, an internal oscillator would function as the conditioned stimulus, a neutral time point that has been now associated with reward. This theoretical oscillator has been termed the food-entrainable oscillator (FEO), and its discrete localization

is not clear yet. Studies point to the dorsomedial nucleus of the hypothalamus; although the SCN may not be necessary, it plays a role in the FAA.

FAA can occur when meals are provided at different intervals other than every 24 h. The availability of food 2 or 4 times a day, every 12 h or 8 h, is able to induce FAA (Luby *et al*, 2012). When food is provided in intervals of 30 minutes, mice can anticipate up to 6 meal times. Interestingly, to a lesser extent, mice can anticipate food availability given every 18 h (Luby *et al*, 2012).

Considering that the protocol to promote FAA requires food restriction, one could assume that metabolic pathways might be more important than reward to this anticipatory activity. Notwithstanding, it has been shown that palatable daily meals can also promote FAA in rats fed ad-libitum (Mistlberger and Rusak, 1987). Mistlberger & Rusak also showed that to promote FAA in free-fed animals, the palatable food needs to nutrient-rich and to have significant size, as 4 g of palatable food was insufficient, yet 2 h of free availability promoted FAA (Mistlberger *et al*, 1987). However, a daily 5 g dose of a chocolate bar is sufficient to induce FAA in rats (Mendoza *et al*, 2005a). Moreover, the palatable food also shifts the phase within the SCN, specially the dorsal area, observed by cFos expression. Additionally, cFos expression is also induced in the NAc (Mendoza *et al*, 2005b), reinforcing the role of the reward system in FAA and FEO.

This anticipatory activity is not restricted to food, as drugs of abuse can also promote anticipatory activity. Animals can anticipate the administration of drugs such as cocaine (White *et al*, 2000), nicotine (Gillman *et al*, 2008), methamphetamine (Kosobud *et al*, 1998) and fentanyl (Gillman *et al*, 2009). Administration of cocaine for 7 days can induce anticipatory increases of temperature ranging from 2 to 10 days following withdrawal (Jansen *et al*, 2012). Additionally, this anticipatory activity remains intact in SNCx rats, reinforcing the idea that the core of this oscillator involves areas other than the SCN. These data pose the question whether the FEO is also responsible for entrainment to drugs of abuse. To answer this question, rats were placed on a 3-h daily restricted feeding regimen (every 24 h) followed by daily cocaine injections (every 25 h). Though both procedures lead to increases in temperature and activity, the 24 h rhythm induced by restricted feeding predominated over the cocaine rhythm. During free-running and drug

withdrawal, the authors detected two free-running periods, one close to the feeding period and other close to the cocaine administration period. One could speculate that FEO and the drug-related oscillator are comprised of two different oscillators (Jansen *et al*, 2012). However, in the same way that the SCN can be uncoupled in aberrant light/dark cycles, (de la Iglesia *et al*, 2004), the FEO oscillator could also be uncoupled to mediate different rewards given at different times or periods.

As mentioned above, daily methamphetamine can also trigger anticipatory activity in rodents (Kosobud *et al*, 1998). However, the most intriguing aspect involving circadian rhythms related to methamphetamine is its ability to generate a substantial increase in the free-running period when administered via drinking water. As the methamphetamine anticipatory activity seems very distinguish from the FAA, it has been theorized as being controlled by the methamphetamine-sensitive circadian oscillator (MASCO). The hyperlocomotor effect promoted by administration of psychostimulants such as methamphetamine or methylphenidate can be used to model manic-like behavior in rodents (Pereira *et al*, 2011). Lithium is a well-known mood stabilizer, and it is able to prevent psychostimulant-induced hyperlocomotion (Sabioni *et al*, 2008). Curiously, lithium also lengthens the circadian period and the combination of lithium and methamphetamine leads to a dramatic increase in the circadian period (Mohawk *et al*, 2009). The MASCO is mostly likely located extra-SCN. Arrhythmic SNCx mice show restored intrinsic rhythm after treatment with methamphetamine in drinking water (Tataroglu *et al*, 2006). Whether this reward system comprises the MASCO is not known; however, understanding it could prove helpful in the treatment of addiction.

Conclusions and future directions

Anhedonia is a core symptom of depression. In this chapter, we reviewed how mood varies throughout the day in both healthy and depressed individuals. Anhedonia varies according to the circadian time in animal models of depression, and the reinforcing effects of reward are also influenced by circadian rhythms. Mood variation in depressed patients can be viewed as a general reduction in amplitude of the circadian rhythms. The lower peak of mood and ability to feel pleasure could explain very complex disorders, such as severe depression and bipolar disorder.

The differential sensitivity to reward, along with the difference between natural rewards and drugs of abuse, could be involved in the initial steps leading to addiction. Moreover, the synchronization to reward could explain increased cravings for the drug at specific times. It is known that environmental cues can lead to craving. Therefore, it is not surprising that time cues can also be involved in facilitating drug craving. Strengthening entrainment to the light/dark cycle over periods of drug abuse could be an additional tool to improve drug addiction treatment.

CAPÍTULO 5

Stretch, Shrink, and Shatter the Rhythms: the intrinsic circadian period in mania and depression

Bruno Jacson Martynhak[1], Marcela Pereira[2], Camila Pasquini de Souza[1], Roberto Andreatini[1]

1. Pharmacology Department, Federal University of Paraná, Curitiba, Brazil

2. Department of Clinical Neuroscience, Center for Molecular Medicine, Karolinska Institutet, Stockholm, Sweden.

Corresponding author: Bruno Jacson Martynhak
Email: brunojm@ymail.com

Nota: Ensaio/revisão a ser submetido

ABSTRACT

Disturbances in the circadian rhythms have long been associated with depression and mania. Animal models of mania and depression exhibit differential effects upon the intrinsic circadian period and the same occurs with antidepressants and mood stabilizers treatment. The intrinsic circadian period is expressed when there are no time clues or when the light/dark cycle length is beyond the capacity of synchronization. In summary, while there is no clear association between the circadian period and mania, depressive-like behaviour is generally associated either with lengthening of the circadian period or with arrythmicity, and the improvement of depressive-like behaviour is associated with shortening of the circadian period. Thus, this review is an attempt to summarize data regarding these correlations and find a putative role of the circadian intrinsic period in mood regulation, particularly concerning the switch from depression to mania.

Keywords: depression, mania, bipolar disorder, endogenous circadian period, antidepressants, mood stabilizers

Introduction

Major depressive disorder and bipolar disorder have long been associated with disturbances in the circadian rhythms. Since the XVI century there have been descriptions of sleep-wake cycle disturbances and mood (Lemmer, 2009). Clinical circadian features of major depression include elevated nocturnal body temperature, phase-advanced and increased cortisol secretion, as well as phase-advanced and reduced melatonin secretion. Changes in sleep architecture are also observed, including shortened latency of rapid eye movement (REM) sleep and reduction in slow-wave sleep, all of them contributing to an earlier awakening (Monteleone *et al*, 2008). On the other hand bipolar patients in the mania phase have reduced need for sleep (Mitchell *et al*, 2008) and increased melatonin secretion (Kennedy *et al*, 1989).

Animal models have provided further information regarding the relation between circadian rhythmicity and depression. Some models aim to study the seasonal depressive disorder, both in diurnal (Ashkenazy-Frolinger *et al*, 2010; Ashkenazy *et al*, 2009a, b) and nocturnal rodents (Prendergast and Kay, 2008; Prendergast *et al*, 2005). In these studies, reduction of the photoperiod led to depressive-like behaviour, e.g., enhanced immobility in the forced swimming test and reduction in sucrose preference. Interestingly, melatonin administration during the light phase of the cycle also promoted depressive-like behaviour (Ashkenazy *et al*, 2009b), which can be interpreted either as a reduction of the photoperiod or a conflict between the melatonin signalling and the information from the light.

Other animal models do not translate directly to the human life, such as the induction of depressive-like behaviour after exposition to a photoperiod of 22 hours of light and 2 hours of dark (Becker *et al*, 2010). Rats exposed to six weeks of constant darkness (DD) had increased immobility time in the forced swimming test and had damaged monoaminergic neurons (Gonzalez *et al*, 2008). Additionally, DD also increased the levels of pro-inflammatory cytokines, such as IL-1 and IL-6 that are commonly present in high plasmatic concentrations in depressive patients (Kubera *et al*, 2004; Monje *et al*, 2011). Similarly to DD, prolonged exposure to constant light (LL) environment also induced depressive-like behaviour (Fonken *et al*, 2009; Martynhak *et al*, 2011).

These manipulations of the light/dark cycles might have an impact in the endogenous circadian rhythm. The circadian rhythms are exhibited even when free

from environmental clues. When rodents are placed under constant darkness, they enter in 'free-running', in which they express their endogenous (or intrinsic) circadian period. This intrinsic circadian period is close to 24h and reflects how much the animal has to adjust every day to keep synchronized to our 24h earthly light/dark cycle.

Despite the accumulated knowledge about the relation between circadian rhythms and mood, few conclusions on how circadian rhythm disruption leads to depression or mania have been drawn. Thus, the objective of this review is to summarize the current literature and provide a model for the interaction between the intrinsic circadian period and mood disorders.

Stretching and Shrinking the Rhythms

Depression

The selective serotonin reuptake inhibitor (SSRI) antidepressant fluoxetine shortens the intrinsic circadian period evaluated by wheel running activity in mice (Possidente *et al*, 1992). Additionally, the antidepressants moclobemide, an inhibitor of monoamine oxidase A, and desipramine, a selective inhibitor of noradrenaline reuptake, also shorten the circadian period in rats (Wollnik, 1992). *In vitro* studies point to same direction, as SSRIs shorten the circadian period both in fibroblasts and in SCN slices (Nomura *et al*, 2008). Thus, antidepressant activity is associated with shortening of circadian period. However, imipramine had no effect on circadian rhythms neither in golden hamsters (Refinetti and Menaker, 1993) nor in rats (Nagayama, 1996). Also, electroconvulsive shock, which has rapid antidepressant effects, did not alter the circadian period, although it was applied to animals that were not previously submitted to a model of depression (Angles-Pujolras *et al*, 2009).

If depression is related to changes in the circadian period, besides reduction of the circadian period by antidepressants, the induction of depressive-like behaviour in animal models should either increase its length or shatter its rhythms (see the proposed model in Fig. 1). Although the SCN is the most important regulator of oscillations in the circadian rhythm, the shape and amplitude of this rhythm are not solely determined by this structure and models of stress are known to affect the output and expression of the clock. In particular, studies have shown that models of social defeat are able to cause severe disruptions of body temperature, heart rate and

locomotor activity rhythms (Meerlo *et al*, 1997). Still, when socially defeated animals returned to their endogenous rhythm, they did so in phase with the same free running period of control animals, which indicates that these animals were able to keep their clock in its normal pace. In general, these studies have shown that even though animals subjected to stressful models and tests such as social defeat and forced swimming had suppression of their activity and rhythm, once these circadian rhythm patterns returned to normal, the onset and offset of activity tended to occur on the expected time. It is believed that if this is so, it's because the clock has kept its normal pace and did not suffer big changes in period or acute phase shifts throughout the exposure to those stressors (Meerlo *et al*, 2002).

Mice selectively bred for high anxiety (HAB) - and co-segregating depression-like behaviour showed a lengthened circadian period compared to the control strain (Griesauer *et al*, 2014). Neonatal desipramine treatment (a model of depression) also lengthens the free-running circadian drinking activity rhythm in rats (Rosenwasser and Hayes, 1994). However, the Flinders sensitive line (a strain of rats more prone to develop depressive-like behavior) has a shorter circadian period compared to its control strain (Shiromani and Overstreet, 1994). In line with the hypothesis, olfactory bulbectomy (an animal model of depression with increased locomotor activity) lengthens the circadian period in mice (Possidente *et al*, 1996).

The learned helplessness protocol, in which animals are submitted to repeated sessions of inescapable foot shocks, also promoted the lengthening of circadian period. Moreover, it was observed an additional and interesting correlation: the further the circadian period lengthened, the better was the coping with the stressor, evaluated as escape of the shock when the opportunity was given (Stewart *et al*, 1990). The increase in circadian period and better coping resembles the adaptive role of increasing REM sleep after an acute stress (Meerlo *et al*, 1997; Tang *et al*, 2005), revised recently (Suchecki *et al*, 2012). Although increase of REM sleep may be adaptive, it is usually observed an increased amount of REM sleep in depressed patients (Jones *et al*, 1987). Moreover, SSRIs have suppressive effect in REM sleep (Gillin *et al*, 1978; Mayers and Baldwin, 2005). Either this increase in REM sleep could be harmful or it could not be enough to cope with the stressors. Given that REM sleep deprivation has antidepressant effects in humans (Benedetti and Colombo, 2011) and animal models (Lopez-Rodriguez *et al*, 2004), shortening the free-running period could also have antidepressant activity, or, at least, to be predictive for antidepressant activity.

Studies report that one night of sleep deprivation followed by a night of sleep phase advance causes a marked and rapid though transient mood improvement in depressive patients. This therapy would act as a reboot of the circadian rhythm and promote synchronization between internal clocks, light/dark cycle and sleep architecture (Benedetti *et al*, 2007; Echizenya *et al*, 2013; Riemann *et al*, 1999). Curiously, sleep deprivation is also an animal model to induce manic-like behaviour and reduced need for sleep can be a predictor for switch to mania in bipolar patients (Colombo *et al*, 1999; Gessa *et al*, 1995; Wehr *et al*, 1987; Wirz-Justice, 2003).

Additionally, in humans, the preference for late sleep habits (eveningness) is associated with depressive symptoms in both diagnosed patients and healthy subjects (Gaspar-Barba *et al*, 2009; Kitamura *et al*, 2010). Eveningness is also associated with increased intrinsic endogenous rhythms in healthy subjects (Duffy *et al*, 2001). Therefore, there is a good relation between a trend to increase the circadian period and the development of depression.

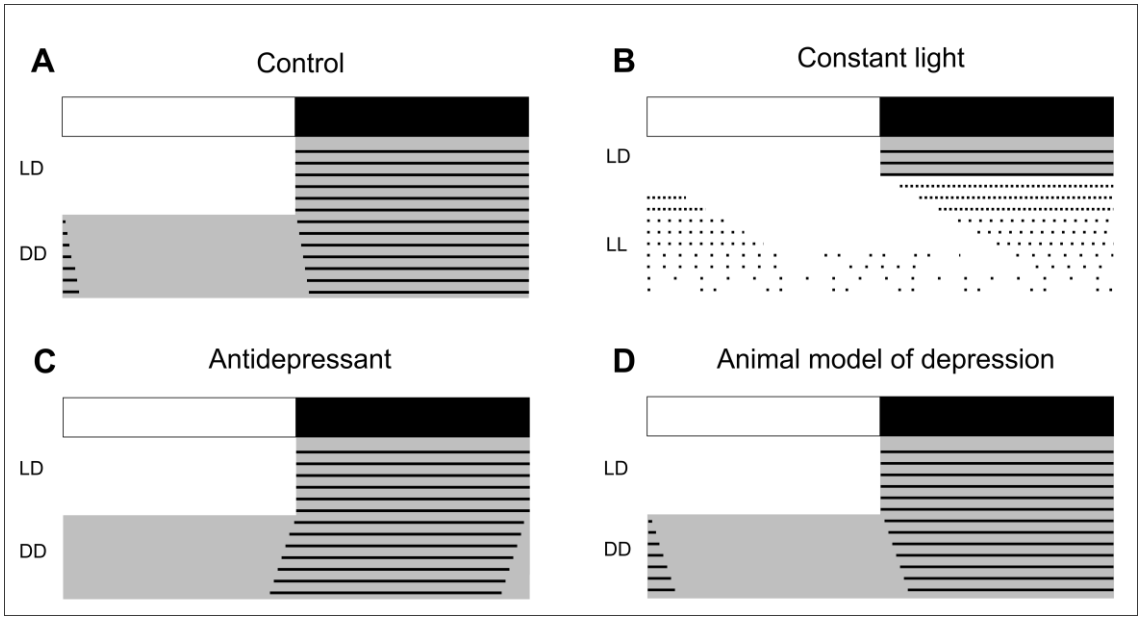

Fig. 1 – Hypothetical actograms representing the relation between the intrinsic circadian period and the response to antidepressant treatment or to an animal model of depression (A) control animal, (B) constant light, “shattering”, ie, turning the animal arrythimic (C) SSRI treatment, “shrinking”, ie, shortening the intrinsic circadian period (D) animal model of depression, “stretching”, ie, lengthening the intrinsic circadian period. LD: light dark, DD: constant dark, LL: constant light, SSRI: selective serotonin reuptake inhibitors. Gray areas represent the dark phase. Lines and dots represent locomotor activity.

Mania

Considering that manic-like behaviour is often opposite to depressive-behaviour, one could expect that a rodent model of mania would have shortened circadian period. However, animal models of mania are scarcer in comparison with depression models and many of these models only show partial validity (Kara and Einat, 2013). Two strains of mice that present several features of mania are the *ClockΔ19* mutant mice (Roybal *et al*, 2007) and the neuron-specific Na^+,K^+-ATPase α3 mutant (Kirshenbaum *et al*, 2011). These mice show hyperactivity, increased risk-taking behaviour and increased hedonic response. Moreover, lithium treatment was effective in reversing these behaviours. However, contrary to the hypothesis, while the *Clock* knockout mice have shortened circadian endogenous period (Debruyne *et al*, 2006), the *ClockΔ19* mutant mice have lengthened circadian period (King *et al*, 1997; Vitaterna *et al*, 1994), similarly to the Na^+,K^+-ATPase α3 mutant mice (Kirshenbaum *et al*, 2011) (Fig. 2).

Interestingly, the knockdown of *Clock* specifically in the ventral tegmental area (VTA) promotes a mixed model of mania and depression (Mukherjee *et al*, 2010), which is a common feature in bipolar patients. Furthermore, the circadian period of these animals is shortened, similarly to the *Clock* knockout (Fig. 2). Unfortunately, there is no data about the behaviour of *Clock* knockout in mania and depression paradigms. It is also unknown whether lithium treatment in the *Clock*Δ19 mice would promote further lengthening of the circadian period.

The mood stabilizer lithium phase delays the rhythms and lengthens the circadian period (Iwahana *et al*, 2004), an action completely opposed to antidepressants. It should be noted that lithium has both antimanic and antidepressant effects, although lithium is less effective in the treatment of depression when compared to SSRIs. Moreover, both lithium and antidepressants have similar suppressive effects on the REM sleep (Gillin *et al*, 1978; Mayers *et al*, 2005).

Acute administration of psychostimulants is a widely used model of mania for screening of potential antimanic drugs (Barbosa *et al*, 2011; Kara *et al*, 2013; Pereira *et al*, 2011; Sabioni *et al*, 2008; Valvassori *et al*, 2014; Valvassori *et al*, 2013). However, similarly to lithium, methamphetamine (MAP) in the drinking water also increases the circadian period (Tataroglu *et al*, 2006) and promotes a rhythmicity in animals with lesion in the suprachiasmatic nuclei (SNC) and , therefore, do not present rhythmicity (Mohawk *et al*, 2009). Other effects of MAP are potentiated by lithium, such as stereotypy (Ozawa and Miyauchi, 1977), an effect that may be caused by the ability of

lithium to prolong the half-life of MAP in the rodent brain (Miyauchi *et al*, 1981). Nonetheless, lithium inhibits some of MAP's toxic effects, which is due to the high activation of dopaminergic system and present during manic phase, e.g. oxidative stress imbalance (Frey *et al*, 2006; Youdim and Arraf, 2004). Combination of lithium and MAP treatment was also studied, showing that MAP increased circadian period, whereas lithium did not. However, the combination of these drugs led to a further increase in the circadian period (Mohawk *et al*, 2009).

The MAP effect on the circadian rhythms is in line with the behaviour showed by the ClockΔ19 and Na^+,K^+-ATPase α3 sodium pump mutant mice. However, a single day of withdrawal after six days of continuous amphetamine administration promotes depressive-like behaviour (Cryan *et al*, 2003), which is consistent with lengthening the period and development of depressive-like behaviour (Fig. 2).

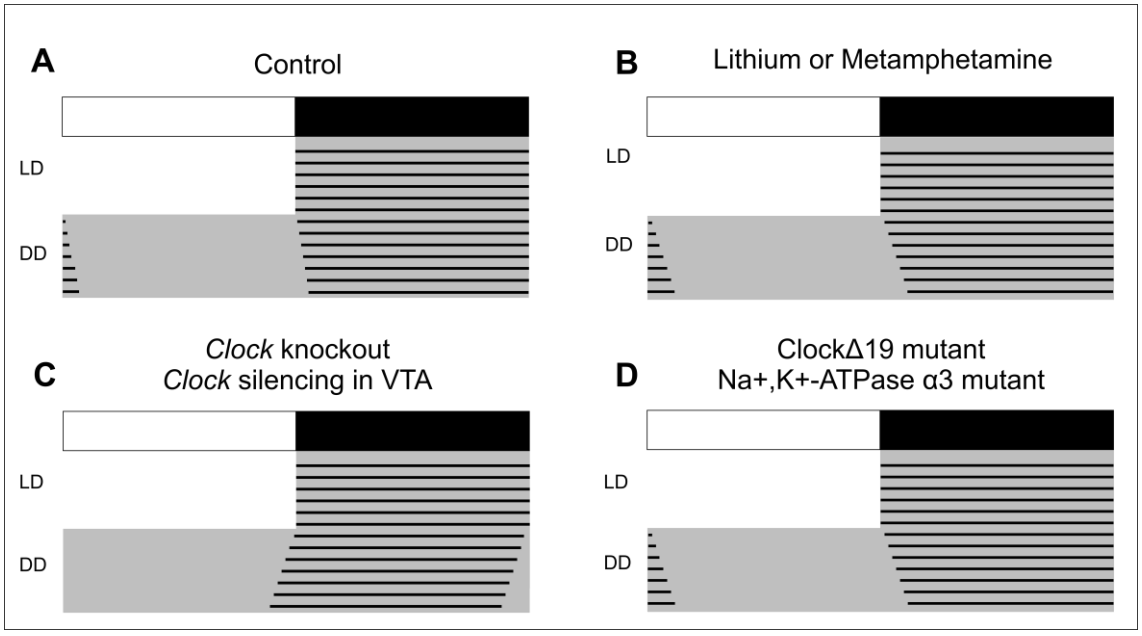

Fig. 2 – Hypothetical actograms representing the relation between the intrinsic circadian period and mania. (A) control animal (B) lithium or metamphetamine treatment, both lengthen the circadian period (C) Both Clock knockout and inhibition of Clock by RNAi shorten the circadian period (D) Two genetic animal models of mania have lengthened circadian period. LD: light dark, DD: constant dark, VTA: ventral tegmental area. Gray areas represent the dark phase. Lines and dots represent locomotor activity.

MAP is also known to activate the glycogen synthase kinase-3 β enzyme (GSK3-β) (Mohawk *et al*, 2009), whereas lithium inhibits it (Enman and Unterwald, 2012). GSK3- β is a serine/threonine kinase involved in energy metabolism,

neurodevelopment, and circadian regulation. GSK3-β has been implicated in the pathophysiology of mania, as its overexpression induces manic-like behaviours such as hypophagia, increased locomotor activity and increased acoustic startle response (Prickaerts *et al*, 2006). The pathway Akt/GSK3-β is reported to be disrupted when the levels of dopamine are high and it results in elevation of GSK3-β levels (Beaulieu *et al*, 2009; Enman *et al*, 2012). GSK3-β seems to have a direct role in the activation of genes related with circadian rhythms, as it phosphorylates the clock genes *Rev-erbα* and *Per2* (Mohawk *et al*, 2009).

However, the effects of inhibiting the GSK3-β upon the circadian period are not clear. One could expect that GSK3-β inhibition would lengthen the circadian period, similarly to lithium. Indeed, knockout of one allele of *GSK3-β* in mice was reported to either lengthen the circadian period (Lavoie *et al*, 2013) or at least to enhance the circadian effects of MAP(O'Brien *et al*, 2004). Results in Drosophila point to same direction. Overexpression of GSK3-β shortens the circadian period (Martinek *et al*, 2001), an effect compensated by lithium treatment (Dokucu *et al*, 2005).

In contrast, in vitro studies point to another direction. In fibroblasts, both inhibition of GSK3-β (Li *et al*, 2012) and inhibition of *GSK3-β* by small-interfering RNA (siRNA) shortened the circadian period (Hirota *et al*, 2008). Accordingly, knock-in mice that have both isoforms, GSK3-α and GSK3-β, constitutively active, have lengthened circadian period (Paul *et al*, 2012), which is in line with the effect of GSK3-β activation induced by MAP. However, this effect only appears in mixed crossed background mice (C57BL/6 x Balb/c), but not in backcrossed C57BL/6 and neither when a single isoform is constitutively active. Lithium lengthens the rhythms and this paradox could be explained by the presence of additional targets of lithium within the clock network, like the possible direct increase in *Per2* transcription (Li *et al*, 2012).

Contrary to lithium, valproic acid, another mood stabilizer, shortens the circadian period in rats (Rietveld and van Schravendijk, 1987) and in fibroblasts (Chansard *et al*, 2007), but it has no circadian effects in Syrian hamsters (Klemfuss and Kripke, 1995). In Drosophila, however, sodium valproate lengthens the circadian period (Dokucu *et al*, 2005).

Thus, it appears that lithium increases the length of circadian rhythms while the results with valproate is not clear.

Shattering the Rythms

The unpredictable chronic mild stress (UCMS) is a highly validated animal model of depression. Animals are exposed to a series of stressors including changes in the light/dark cycle. Exposure to UCMS reduces the amplitude of the temperature and cortisol rhythms (Ushijima *et al*, 2006). The amplitude of the circadian locomotor activity under normal light/dark cycle is also blunted, particularly due to a reduction of activity in the dark phase (Gorka *et al*, 1996), as similarly to the learned helplessness protocol (Kant *et al*, 1991). However, it is difficult to differentiate between the actual circadian alterations and the masking effects of the stressors (e.g., inhibition of activity by light). As far as the authors are aware, there is no evaluation of the intrinsic circadian period after the UCMS.

Prolonged exposure to LL is known to promote arrythmicity in the circadian locomotor rhythms (Honma *et al*, 1978) (Fig. 1). This lack of rhythmicity is prevented when the animals are previously exposed to LL during lactation (Cambras *et al*, 1997; Cambras *et al*, 1998). In order to further explore the role of arrythmicity, our group tested whether rats exposed to LL would be protected from LL-induced depressive-like behaviour. In fact, neonatal-LL animals did not reduce sucrose preference under LL, whereas the rats raised under standard light/dark cycle showed anhedonic-like behaviour (Martynhak *et al*, 2011). Additionally, the anhedonia-like behaviour was reversed with imipramine treatment (Martynhak *et al*, 2011). Therefore, we concluded that LL is able to induce depressive-like behaviour by shattering the rhythms, despite the direct effect of light in LL *per se*.

Disruption of the circadian rhythms are also promoted by overexpression of the class IIa histone deacetylase 5 (HDAC5) in mouse fibroblasts (Fogg *et al*, 2014). HDACs are a class of enzymes that have an important role in gene expression. Studies have shown that hippocampal HDAC5 expression is increased after social defeat stress and its reduction is associated with resilience or improvement of depressive-like behaviour (Sun *et al*, 2013). Moreover, in line with the shrinking pattern, mutation of HDAC4 in Drosophila shortens the locomotor intrinsic circadian period (Fogg *et al*, 2014).

Another method to promote arrythmicity is by lesion the suprachiasmatic nuclei (Stetson and Watson-Whitmyre, 1976). Therefore, one could expect that lesion of the suprachiasmatic nuclei would lead to depressive-like behaviour. However, one study reported that lesion in the suprachiasmatic nuclei reduced the immobility time in the forced swimming test (Tataroglu *et al*, 2004). Additionally, a decrease in the immobility

time in black-Swiss mice has been associated manic-like behaviour (Flaisher-Grinberg and Einat, 2009). Thus, the reduced immobility time in this test due to lesions in the suprachiasmatic nuclei could be viewed as a manic-like behaviour, contrasting with the hypothesis of arrythmicity and depression.

Circadian locomotor arrythmicity is also observed in the *Per2* mutant mice. Under constant darkness these animals initially have short circadian period and then gradually become arrhythmic (Zheng *et al*, 1999). During a conventional light/dark cycle, the *Per2* mutant mice show decreased monoamine oxidase A in the mesolimbic system and reduced immobility time in the forced swimming test (Hampp *et al*, 2008), which could be interpreted as a manic-like phenotype. Unfortunately, the behaviour of the *Per2* mutant mice in the forced swimming test under constant darkness (i.e., arrythmicity) has not yet been evaluated. Nevertheless, the forced swimming test is highly dependent on locomotor activity and bouts of ultradian activity could bias these studies. For example, the *Per2* mutant mice have increased locomotor activity in the afternoon, a trait of phase advance and short circadian period that could bias the behavioural tests, which were performed in the afternoon (Hampp *et al*, 2008). The sucrose/saccharin preference test is more suitable in these cases, due to less influence of locomotor activity and circadian phase differences due to the possibility to run the test through the 24h of the light/dark cycle (Martynhak *et al*, 2011).

Even though animals under constant darkness still express their endogenous circadian period, it could be observed the induction of depressive-like behaviour (Gonzalez *et al*, 2008). However, the direct stressful effect of light deprivation *per se*, rather than indirectly through the circadian system, was not evaluated. This could be done by promoting synchronization to a photoskeletal period, with two pulses of light, which is known to synchronize the rhythms to 24h. Similarly, in another study, mice were submitted to a period of 7 h: 3.5 h light and 3.5 h dark (LeGates *et al*, 2012). The animals were not able to synchronize to this aberrant cycle and thus, to express their free-running period. In spite of the rhythmicity, the mice showed reduced immobility in the forced swimming test, reduced sucrose preference and increased corticosterone secretion, indicating that the direct effect of light can also participate in the regulation of the depressive-like behaviour (LeGates *et al*, 2012). Therefore, changes in the light/dark cycle might exert its effects not only through the circadian system, but due to the direct effect of light (LeGates *et al*, 2014).

Conclusion

Although not all data presented here point towards the same direction, in general, development of depressive-like behaviour is associated either with arrythmicity or lengthening of the circadian period whereas improvement is associated with shortening of the circadian period. As for mania, one could expect the opposite of depression, i.e., shortening of the circadian period. However, the data regarding this association between both is not as clear as for depression, maybe due to the lesser number of animal models of mania with high construct validity or to lack of an actual relationship or predictive value between the circadian period and mania.

One interesting approach is the forced desynchronization protocol, the exposure to a 22h LD cycle, which is close to the limit of entrainment to a imposed light/dark cycle (de la Iglesia *et al*, 2004). Under these conditions, two locomotor rhythms with different period lengths are expressed simultaneously. One rhythm is entrained by the 22h LD cycle, whereas the other free-runs. The change between short and long periods would ideally result in shifts between mania and depression or in mixed states.

Manipulation of the length of the circadian cycle to different periods other than the usual 24h might be helpful to provide insights to a model of bipolar disorder in which both depressive and manic phases are present within the same animal. Ideally, chronic exposure to a lengthened cycle would promote depressive-like behaviour, whereas the shortening the cycle would promote antidepressant or manic-like effect.

Acknowledgments

The authors thank Sâmia R. Joca and Janaína M. Zanoveli for critically reading the manuscript.

CONSIDERAÇÕES FINAIS

O presente estudo buscou investigar a relação entre ritimicidade circadiana e comportamento tipo-depressivo. Estratégias que visam a prevenção da perda da ritimicidade circadiana se mostraram efetivas em também prevenir o comportamento tipo depressivo em modelos animais. A forma utilizada para promover a perda da ritimicidade circadiana neste estudo foi a exposição ao claro constante (LL).

Os dados aqui apresentados sugerem que o efeito tipo-depressivo induzido pelo LL deve-se à perda da ritimicidade. Porém, os grupos de animais protegidos da anedonia induzida pelo LL não foram mantidos em LL por um tempo mais prolongado. Desta forma, não é possível afirmar se a exposição prolongada ao LL não iria eventualmente levar também ao comportamento tipo-depressivo mesmo em animais que mantém a expressão da ritimicidade circadiana. Outros protocolos de alteração do ciclo claro/escuro também promovem comportamento-tipo depressivo em modelos animais, ainda que a ritimicidade seja mantida, como a exposição ao escuro constante (Gonzalez *et al*, 2008), ou a um ciclo claro/escuro de 7 h (3,5 h : 3,5 h) ao invés das 24 h usuais (LeGates *et al*, 2012). Este último trabalho se destaca por mostrar o papel direto da luz na regulação do comportamento tipo-depressivo através das células ganglionares que expressam o fotorreceptor melanopsina.

Para avaliar a participação da via indireta (via ritmos circadianos) ou direta da luz no comportamento tipo-depressivo, o modelo da exposição à luz de baixa intensidade à noite (dLAN) foi utilizado em camundongos nocaute para o gene-relógio *Per3* (*Per3*$^{-/-}$). Apesar de se sincronizarem normalmente ao ciclo claro/escuro convencional e responderem adequadamente a avanços e atrasos de fase induzidos por pulsos de luz, estes animais apresentam reduzida sensibilidade ao efeito direto da luz em inibir a atividade motora (van der Veen *et al*, 2010), efeito conhecido como mascaramento. Diferenças na resposta de mascaramento a longo prazo podem afetar também a ritimicidade circadiana. Por exemplo, os camundongos *Per3*$^{-/-}$ apresentam menor aumento da duração do período circadiano endógeno quando expostos ao LL (van der Veen *et al*, 2010). Nossos dados mostraram que os camundongos *Per3*$^{-/-}$ se

foram mais sensíveis ao efeito tipo-depressivo do dLAN. Hipotetizamos que ao buscar menos escape da luz que os animais selvagem - através do sono ou comportamento de se enterrar no cepilho - os camundongos *Per3*$^{-/-}$ são mais expostos à luz. Por usa vez, esta maior exposição direta à luz pode antecipar os efeitos no comportamento tipo-depressivo. Estes dados estão de acordo com resultados da literatura, em que a disponibilidade de um tubo opaco preveniu parcialmente o comportamento tipo-depressivo induzido pelo LL (Fonken *et al*, 2009). Desta forma, nossos dados apontam tanto para o papel direto quanto indireto da luz na regulação do comportamento tipo-depressivo em modelos animais.

Importante ressaltar que todos os procedimentos realizados foram destinados a prevenir o comportamento tipo-depressivo: prevenção à perda da ritimicidade, pré-tratamento com imipramina antes da exposição ao dLAN, co-tratamento com imipramina, melatonina e agomelatina juntamente com o início da restrição, bem como restrição alimentar juntamente com o início da exposição ao LL. A administração aguda de quetamina constituiu o único momento em que os animais foram tratados somente após detecção da baixa preferência por sacarose. Visto que tanto o tratamento com imipramina ou agomelatina não foram eficazes em prevenir a redução da preferência por sacarose induzida por restrição alimentar, este modelo pode vir a ser utilizado como um modelo resistente ao tratamento. Entretanto, mais experimentos com mais dose, drogas e esquemas de administração são necessários. A quetamina, que apresenta ação antidepressiva em pacientes depressivos refratários ao tratamento (Zarate *et al*, 2006) foi eficaz em aumentar a preferência por sacarose em animais sob restrição alimentar, reforçando que o efeito na preferência por sacarose representa um comportamento-tipo depressivo.

Os resultados deste estudo apontam para a importância da manutenção da ritimicidade circadiana e sincronização interna para a prevenção do comportamento tipo-depressivo. A luz pode regular o comportamento tanto de forma indireta, através do controle da ritimicidade circadiana, quanto exercer uma influência direta sobre o comportamento. Dado ambas as formas de ação da luz no controle do humor, este estudo não permite afirmar que a perda da ritimicidade circadiana induz comportamento-tipo depressivo, visto que o

mesmo também é induzido por ação direta da luz. Assim, a perda da ritimicidade circadiana é ou suficiente para promover o comportamento tipo-depressivo ou atua como facilitador dos efeitos diretos da luz. A indução de arritimicidade através da lesão dos núcleos supraquiasmáticos poderia ser utilizada para controlar o efeito direto da luz sobre o comportamento tipo-depressivo.

Em conclusão, sugere-se que a estabilidade da ritimicidade circadiana, via controle do ciclo claro/escuro e horários regulares de alimentação, bem como redução da poluição visual noturna podem ser fatores protetores para o desenvolvimento da depressão.

REFERÊNCIAS

Abarca C, Albrecht U, Spanagel R (2002). Cocaine sensitization and reward are under the influence of circadian genes and rhythm. *Proc Natl Acad Sci U S A* **99**(13): 9026-9030.

Altimus CM, Guler AD, Alam NM, Arman AC, Prusky GT, Sampath AP*, et al* (2010). Rod photoreceptors drive circadian photoentrainment across a wide range of light intensities. *Nat Neurosci* **13**(9): 1107-1112.

Angles-Pujolras M, Diez-Noguera A, Soria V, Urretavizcaya M, Menchon JM, Cambras T (2009). Electroconvulsive shock alters the rat overt rhythms of motor activity and temperature without altering the circadian pacemaker. *Behav Brain Res* **196**(1): 37-43.

Antle MC, Silver R (2005). Orchestrating time: arrangements of the brain circadian clock. *Trends Neurosci* **28**(3): 145-151.

Archer SN, Robilliard DL, Skene DJ, Smits M, Williams A, Arendt J*, et al* (2003). A length polymorphism in the circadian clock gene Per3 is linked to delayed sleep phase syndrome and extreme diurnal preference. *Sleep* **26**(4): 413-415.

Artioli P, Lorenzi C, Pirovano A, Serretti A, Benedetti F, Catalano M*, et al* (2007). How do genes exert their role? Period 3 gene variants and possible influences on mood disorder phenotypes. *Eur Neuropsychopharmacol* **17**(9): 587-594.

Ashkenazy-Frolinger T, Kronfeld-Schor N, Juetten J, Einat H (2010). It is darkness and not light: Depression-like behaviors of diurnal unstriped Nile grass rats maintained under a short photoperiod schedule. *J Neurosci Methods* **186**(2): 165-170.

Ashkenazy T, Einat H, Kronfeld-Schor N (2009a). Effects of bright light treatment on depression- and anxiety-like behaviors of diurnal rodents maintained on a short daylight schedule. *Behav Brain Res* **201**(2): 343-346.

Ashkenazy T, Einat H, Kronfeld-Schor N (2009b). We are in the dark here: induction of depression- and anxiety-like behaviours in the diurnal fat sand rat, by short daylight or melatonin injections. *Int J Neuropsychopharmacol* **12**(1): 83-93.

Autry AE, Adachi M, Nosyreva E, Na ES, Los MF, Cheng PF*, et al* (2011). NMDA receptor blockade at rest triggers rapid behavioural antidepressant responses. *Nature* **475**(7354): 91-95.

Baird TJ, Gauvin D (2000). Characterization of cocaine self-administration and pharmacokinetics as a function of time of day in the rat. *Pharmacol Biochem Behav* **65**(2): 289-299.

Baltazar RM, Coolen LM, Webb IC (2013). Diurnal rhythms in neural activation in the mesolimbic reward system: critical role of the medial prefrontal cortex. *Eur J Neurosci*.

Barbosa FJ, Hesse B, de Almeida RB, Baretta IP, Boerngen-Lacerda R, Andreatini R (2011). Magnesium sulfate and sodium valproate block methylphenidate-induced hyperlocomotion, an animal model of mania. *Pharmacol Rep* **63**(1): 64-70.

Beaulieu JM, Gainetdinov RR, Caron MG (2009). Akt/GSK3 signaling in the action of psychotropic drugs. *Annu Rev Pharmacol Toxicol* **49**: 327-347.

Becker A, Bilkei-Gorzo A, Michel K, Zimmer A (2010). Exposure of mice to long-light: a new animal model to study depression. *Eur Neuropsychopharmacol* **20**(11): 802-812.

Bedrosian TA, Fonken LK, Walton JC, Haim A, Nelson RJ (2011). Dim light at night provokes depression-like behaviors and reduces CA1 dendritic spine density in female hamsters. *Psychoneuroendocrinology* **36**(7): 1062-1069.

Bedrosian TA, Weil ZM, Nelson RJ (2012). Chronic citalopram treatment ameliorates depressive behavior associated with light at night. *Behav Neurosci* **126**(5): 654-658.

Bedrosian TA, Weil ZM, Nelson RJ (2013). Chronic dim light at night provokes reversible depression-like phenotype: possible role for TNF. *Mol Psychiatry* **18**(8): 930-936.

Belzung C (2014). Innovative drugs to treat depression: did animal models fail to be predictive or did clinical trials fail to detect effects? *Neuropsychopharmacology* **39**(5): 1041-1051.

Benedetti F (2012). Antidepressant chronotherapeutics for bipolar depression. *Dialogues Clin Neurosci* **14**(4): 401-411.

Benedetti F, Barbini B, Colombo C, Smeraldi E (2007). Chronotherapeutics in a psychiatric ward. *Sleep Med Rev* **11**(6): 509-522.

Benedetti F, Colombo C (2011). Sleep deprivation in mood disorders. *Neuropsychobiology* **64**(3): 141-151.

Bennie J, Davies TW, Duffy JP, Inger R, Gaston KJ (2014). Contrasting trends in light pollution across Europe based on satellite observed night time lights. *Sci Rep* **4**: 3789.

Berk M, Williams LJ, Jacka FN, O'Neil A, Pasco JA, Moylan S*, et al* (2013). So depression is an inflammatory disease, but where does the inflammation come from? *BMC Med* **11**: 200.

Berson DM, Dunn FA, Takao M (2002). Phototransduction by retinal ganglion cells that set the circadian clock. *Science* **295**(5557): 1070-1073.

Berton O, Nestler EJ (2006). New approaches to antidepressant drug discovery: beyond monoamines. *Nat Rev Neurosci* **7**(2): 137-151.

Bisgaard CF, Bak S, Christensen T, Jensen ON, Enghild JJ, Wiborg O (2012). Vesicular signalling and immune modulation as hedonic fingerprints: proteomic profiling in the chronic mild stress depression model. *J Psychopharmacol* **26**(12): 1569-1583.

Boivin DB, Czeisler CA, Dijk DJ, Duffy JF, Folkard S, Minors DS*, et al* (1997). Complex interaction of the sleep-wake cycle and circadian phase modulates mood in healthy subjects. *Arch Gen Psychiatry* **54**(2): 145-152.

Booker JM, Hellekson CJ, Putilov AA, Danilenko KV (1991). Seasonal depression and sleep disturbances in Alaska and Siberia: a pilot study. *Arctic Med Res* **Suppl**: 281-284.

Bosker FJ, Hartman CA, Nolte IM, Prins BP, Terpstra P, Posthuma D*, et al* (2011). Poor replication of candidate genes for major depressive disorder using genome-wide association data. *Mol Psychiatry* **16**(5): 516-532.

Breslau N, Roth T, Rosenthal L, Andreski P (1996). Sleep disturbance and psychiatric disorders: a longitudinal epidemiological study of young adults. *Biol Psychiatry* **39**(6): 411-418.

Cabello M, Mellor-Marsa B, Sabariego C, Cieza A, Bickenbach J, Ayuso-Mateos JL (2012). Psychosocial features of depression: a systematic literature review. *J Affect Disord* **141**(1): 22-33.

Caci H, Bouchez J, Bayle FJ (2009). Inattentive symptoms of ADHD are related to evening orientation. *J Atten Disord* **13**(1): 36-41.

Cambras T, Canal MM, Torres A, Vilaplana J, Diez-Noguera A (1997). Manifestation of circadian rhythm under constant light depends on lighting conditions during lactation. *Am J Physiol* **272**(4 Pt 2): R1039-1046.

Cambras T, Castejon L, Diez-Noguera A (2011). Social interaction and sex differences influence rat temperature circadian rhythm under LD cycles and constant light. *Physiol Behav* **103**(3-4): 365-371.

Cambras T, Castejon L, Diez-Noguera A (2012). Social interaction with a rhythmic rat enhances the circadian pattern of the motor activity and temperature of LL-induced arrhythmic rats. *Physiol Behav* **105**(3): 835-840.

Cambras T, Vilaplana J, Torres A, Canal MM, Casamitjana N, Campuzano A*, et al* (1998). Constant bright light (LL) during lactation in rats prevents arrhythmicity due to LL. *Physiol Behav* **63**(5): 875-882.

Canal-Corretger MM, Cambras T, Vilaplana J, Diez-Noguera A (2000). Bright light during lactation alters the functioning of the circadian system of adult rats. *Am J Physiol Regul Integr Comp Physiol* **278**(1): R201-208.

Canal-Corretger MM, Vilaplana J, Cambras T, Diez-Noguera A (2001). Functioning of the rat circadian system is modified by light applied in critical postnatal days. *Am J Physiol Regul Integr Comp Physiol* **280**(4): R1023-1030.

Carlezon WA, Jr., Duman RS, Nestler EJ (2005). The many faces of CREB. *Trends Neurosci* **28**(8): 436-445.

Casarotto PC, Andreatini R (2007). Repeated paroxetine treatment reverses anhedonia induced in rats by chronic mild stress or dexamethasone. *Eur Neuropsychopharmacol* **17**(11): 735-742.

Cassone VM, Chesworth MJ, Armstrong SM (1986). Dose-dependent entrainment of rat circadian rhythms by daily injection of melatonin. *J Biol Rhythms* **1**(3): 219-229.

Castaneda TR, de Prado BM, Prieto D, Mora F (2004). Circadian rhythms of dopamine, glutamate and GABA in the striatum and nucleus accumbens of the awake rat: modulation by light. *J Pineal Res* **36**(3): 177-185.

Chan JW, Lam SP, Li SX, Yu MW, Chan NY, Zhang J*, et al* (2014). Eveningness and insomnia: independent risk factors of nonremission in major depressive disorder. *Sleep* **37**(5): 911-917.

Chansard M, Molyneux P, Nomura K, Harrington ME, Fukuhara C (2007). c-Jun N-terminal kinase inhibitor SP600125 modulates the period of mammalian circadian rhythms. *Neuroscience* **145**(3): 812-823.

Cheeta S, Ruigt G, van Proosdij J, Willner P (1997). Changes in sleep architecture following chronic mild stress. *Biol Psychiatry* **41**(4): 419-427.

Christensen T, Bisgaard CF, Wiborg O (2011). Biomarkers of anhedonic-like behavior, antidepressant drug refraction, and stress resilience in a rat model of depression. *Neuroscience* **196**: 66-79.

Colombo C, Benedetti F, Barbini B, Campori E, Smeraldi E (1999). Rate of switch from depression into mania after therapeutic sleep deprivation in bipolar depression. *Psychiatry Res* **86**(3): 267-270.

Cryan JF, Hoyer D, Markou A (2003). Withdrawal from chronic amphetamine induces depressive-like behavioral effects in rodents. *Biol Psychiatry* **54**(1): 49-58.

Czeisler CA (2013). Perspective: casting light on sleep deficiency. *Nature* **497**(7450): S13.

D'Aquila PS, Brain P, Willner P (1994). Effects of chronic mild stress on performance in behavioural tests relevant to anxiety and depression. *Physiol Behav* **56**(5): 861-867.

D'Aquila PS, Newton J, Willner P (1997). Diurnal variation in the effect of chronic mild stress on sucrose intake and preference. *Physiol Behav* **62**(2): 421-426.

de la Iglesia HO, Cambras T, Schwartz WJ, Diez-Noguera A (2004). Forced desynchronization of dual circadian oscillators within the rat suprachiasmatic nucleus. *Curr Biol* **14**(9): 796-800.

Debruyne JP, Noton E, Lambert CM, Maywood ES, Weaver DR, Reppert SM (2006). A clock shock: mouse CLOCK is not required for circadian oscillator function. *Neuron* **50**(3): 465-477.

Detke MJ, Rickels M, Lucki I (1995). Active behaviors in the rat forced swimming test differentially produced by serotonergic and noradrenergic antidepressants. *Psychopharmacology (Berl)* **121**(1): 66-72.

Doane LD, Mineka S, Zinbarg RE, Craske M, Griffith JW, Adam EK (2013). Are flatter diurnal cortisol rhythms associated with major depression and anxiety disorders in late adolescence? The role of life stress and daily negative emotion. *Dev Psychopathol* **25**(3): 629-642.

Dokucu ME, Yu L, Taghert PH (2005). Lithium- and valproate-induced alterations in circadian locomotor behavior in Drosophila. *Neuropsychopharmacology* **30**(12): 2216-2224.

Duffy JF, Rimmer DW, Czeisler CA (2001). Association of intrinsic circadian period with morningness-eveningness, usual wake time, and circadian phase. *Behav Neurosci* **115**(4): 895-899.

Eastman C, Rechtschaffen A (1983). Circadian temperature and wake rhythms of rats exposed to prolonged continuous illumination. *Physiol Behav* **31**(4): 417-427.

Echizenya M, Suda H, Takeshima M, Inomata Y, Shimizu T (2013). Total sleep deprivation followed by sleep phase advance and bright light therapy in drug-resistant mood disorders. *J Affect Disord* **144**(1-2): 28-33.

Ehlers CL, Frank E, Kupfer DJ (1988). Social zeitgebers and biological rhythms. A unified approach to understanding the etiology of depression. *Arch Gen Psychiatry* **45**(10): 948-952.

Einat H, Kronfeld-Schor N, Eilam D (2006). Sand rats see the light: short photoperiod induces a depression-like response in a diurnal rodent. *Behav Brain Res* **173**(1): 153-157.

Enman NM, Unterwald EM (2012). Inhibition of GSK3 attenuates amphetamine-induced hyperactivity and sensitization in the mouse. *Behav Brain Res* **231**(1): 217-225.

Feinstein C, Kaminer Y, Barrett RP, Tylenda B (1988). The assessment of mood and affect in developmentally disabled children and adolescents: the Emotional Disorders Rating Scale. *Res Dev Disabil* **9**(2): 109-121.

Ferrari AJ, Charlson FJ, Norman RE, Patten SB, Freedman G, Murray CJ*, et al* (2013). Burden of depressive disorders by country, sex, age, and year: findings from the global burden of disease study 2010. *PLoS Med* **10**(11): e1001547.

Flaisher-Grinberg S, Einat H (2009). A possible utilization of the mice forced swim test for modeling manic-like increase in vigor and goal-directed behavior. *J Pharmacol Toxicol Methods* **59**(3): 141-145.

Fogg PC, O'Neill JS, Dobrzycki T, Calvert S, Lord EC, McIntosh RL*, et al* (2014). Class IIa histone deacetylases are conserved regulators of circadian function. *J Biol Chem*.

Fonken LK, Finy MS, Walton JC, Weil ZM, Workman JL, Ross J*, et al* (2009). Influence of light at night on murine anxiety- and depressive-like responses. *Behav Brain Res* **205**(2): 349-354.

Fonken LK, Kitsmiller E, Smale L, Nelson RJ (2012). Dim nighttime light impairs cognition and provokes depressive-like responses in a diurnal rodent. *J Biol Rhythms* **27**(4): 319-327.

Fonken LK, Nelson RJ (2013). Dim light at night increases depressive-like responses in male C3H/HeNHsd mice. *Behav Brain Res* **243**: 74-78.

Foster RG, Wulff K (2005). The rhythm of rest and excess. *Nat Rev Neurosci* **6**(5): 407-414.

Frank E (2007). Interpersonal and social rhythm therapy: a means of improving depression and preventing relapse in bipolar disorder. *J Clin Psychol* **63**(5): 463-473.

Frank E, Hlastala S, Ritenour A, Houck P, Tu XM, Monk TH*, et al* (1997). Inducing lifestyle regularity in recovering bipolar disorder patients: results from the maintenance therapies in bipolar disorder protocol. *Biol Psychiatry* **41**(12): 1165-1173.

Frey BN, Valvassori SS, Reus GZ, Martins MR, Petronilho FC, Bardini K*, et al* (2006). Effects of lithium and valproate on amphetamine-induced oxidative stress generation in an animal model of mania. *J Psychiatry Neurosci* **31**(5): 326-332.

Fujioka A, Fujioka T, Tsuruta R, Izumi T, Kasaoka S, Maekawa T (2011). Effects of a constant light environment on hippocampal neurogenesis and memory in mice. *Neurosci Lett* **488**(1): 41-44.

Gaspar-Barba E, Calati R, Cruz-Fuentes CS, Ontiveros-Uribe MP, Natale V, De Ronchi D*, et al* (2009). Depressive symptomatology is influenced by chronotypes. *J Affect Disord* **119**(1-3): 100-106.

Germain A, Nofzinger EA, Meltzer CC, Wood A, Kupfer DJ, Moore RY*, et al* (2007). Diurnal variation in regional brain glucose metabolism in depression. *Biol Psychiatry* **62**(5): 438-445.

Gessa GL, Pani L, Fadda P, Fratta W (1995). Sleep deprivation in the rat: an animal model of mania. *Eur Neuropsychopharmacol* **5 Suppl**: 89-93.

Gibbs FP, Vriend J (1981). The half-life of melatonin elimination from rat plasma. *Endocrinology* **109**(5): 1796-1798.

Gillin JC, Wyatt RJ, Fram D, Snyder F (1978). The relationship between changes in REM sleep and clinical improvement in depressed patients treated with amitriptyline. *Psychopharmacology (Berl)* **59**(3): 267-272.

Gillman AG, Kosobud AE, Timberlake W (2008). Pre- and post-nicotine circadian activity rhythms can be differentiated by a paired environmental cue. *Physiol Behav* **93**(1-2): 337-350.

Gillman AG, Leffel JK, 2nd, Kosobud AE, Timberlake W (2009). Fentanyl, but not haloperidol, entrains persisting circadian activity episodes when administered at 24- and 31-h intervals. *Behav Brain Res* **205**(1): 102-114.

Glassman AH, Roose SP, Bigger JT, Jr. (1993). The safety of tricyclic antidepressants in cardiac patients. Risk-benefit reconsidered. *JAMA* **269**(20): 2673-2675.

Golden RN, Gaynes BN, Ekstrom RD, Hamer RM, Jacobsen FM, Suppes T*, et al* (2005). The efficacy of light therapy in the treatment of mood disorders: a review and meta-analysis of the evidence. *Am J Psychiatry* **162**(4): 656-662.

Golder SA, Macy MW (2011). Diurnal and seasonal mood vary with work, sleep, and daylength across diverse cultures. *Science* **333**(6051): 1878-1881.

Golombek DA, Rosenstein RE (2010). Physiology of circadian entrainment. *Physiol Rev* **90**(3): 1063-1102.

Gonzalez MM, Aston-Jones G (2008). Light deprivation damages monoamine neurons and produces a depressive behavioral phenotype in rats. *Proc Natl Acad Sci U S A* **105**(12): 4898-4903.

Gordijn MC, Beersma DG, Bouhuys AL, Reinink E, Van den Hoofdakker RH (1994). A longitudinal study of diurnal mood variation in depression; characteristics and significance. *J Affect Disord* **31**(4): 261-273.

Gorka Z, Moryl E, Papp M (1996). Effect of chronic mild stress on circadian rhythms in the locomotor activity in rats. *Pharmacol Biochem Behav* **54**(1): 229-234.

Grassi Zucconi G, Cipriani S, Balgkouranidou I, Scattoni R (2006). 'One night' sleep deprivation stimulates hippocampal neurogenesis. *Brain Res Bull* **69**(4): 375-381.

Griesauer I, Diao W, Ronovsky M, Elbau I, Sartori S, Singewald N*, et al* (2014). Circadian abnormalities in a mouse model of high trait anxiety and depression. *Ann Med*.

Hampp G, Ripperger JA, Houben T, Schmutz I, Blex C, Perreau-Lenz S*, et al* (2008). Regulation of monoamine oxidase A by circadian-clock components implies clock influence on mood. *Curr Biol* **18**(9): 678-683.

Hasan S, van der Veen DR, Winsky-Sommerer R, Dijk DJ, Archer SN (2011). Altered sleep and behavioral activity phenotypes in PER3-deficient mice. *Am J Physiol Regul Integr Comp Physiol* **301**(6): R1821-1830.

Hasler BP, Buysse DJ, Kupfer DJ, Germain A (2010). Phase relationships between core body temperature, melatonin, and sleep are associated with depression severity: further evidence for circadian misalignment in non-seasonal depression. *Psychiatry Res* **178**(1): 205-207.

Hirota T, Lewis WG, Liu AC, Lee JW, Schultz PG, Kay SA (2008). A chemical biology approach reveals period shortening of the mammalian circadian clock by specific inhibition of GSK-3beta. *Proc Natl Acad Sci U S A* **105**(52): 20746-20751.

Honma KI, Hiroshige T (1978). Endogenous ultradian rhythms in rats exposed to prolonged continuous light. *Am J Physiol* **235**(5): R250-256.

Hsiao FH, Yang TT, Ho RT, Jow GM, Ng SM, Chan CL*, et al* (2010). The self-perceived symptom distress and health-related conditions associated with morning to evening diurnal cortisol patterns in outpatients with major depressive disorder. *Psychoneuroendocrinology* **35**(4): 503-515.

Inder ML, Crowe MT, Luty SE, Carter JD, Moor S, Frampton CM*, et al* (2014). Randomized, controlled trial of Interpersonal and Social Rhythm Therapy for young people with bipolar disorder. *Bipolar Disord*.

Iwahana E, Akiyama M, Miyakawa K, Uchida A, Kasahara J, Fukunaga K*, et al* (2004). Effect of lithium on the circadian rhythms of locomotor activity and glycogen synthase kinase-3 protein expression in the mouse suprachiasmatic nuclei. *Eur J Neurosci* **19**(8): 2281-2287.

Jansen HT, Sergeeva A, Stark G, Sorg BA (2012). Circadian discrimination of reward: evidence for simultaneous yet separable food- and drug-entrained rhythms in the rat. *Chronobiol Int* **29**(4): 454-468.

Jones D, Gershon S, Sitaram N, Keshavan M (1987). Sleep and depression. *Psychopathology* **20 Suppl 1**: 20-31.

Joyce PR, Porter RJ, Mulder RT, Luty SE, McKenzie JM, Miller AL*, et al* (2005). Reversed diurnal variation in depression: associations with a differential antidepressant response, tryptophan: large neutral amino acid ratio and serotonin transporter polymorphisms. *Psychol Med* **35**(4): 511-517.

Justel N, Ruetti E, Mustaca AE, Papini MR (2012). Effects of pretraining treatment with testosterone on successive and anticipatory negative contrast. *Physiol Behav* **105**(4): 933-937.

Kant GJ, Bauman RA, Pastel RH, Myatt CA, Closser-Gomez E, D'Angelo CP (1991). Effects of controllable vs. uncontrollable stress on circadian temperature rhythms. *Physiol Behav* **49**(3): 625-630.

Kara NZ, Einat H (2013). Rodent models for mania: practical approaches. *Cell Tissue Res* **354**(1): 191-201.

Kasahara T, Abe K, Mekada K, Yoshiki A, Kato T (2010). Genetic variation of melatonin productivity in laboratory mice under domestication. *Proc Natl Acad Sci U S A* **107**(14): 6412-6417.

Katz RJ, Roth KA, Carroll BJ (1981). Acute and chronic stress effects on open field activity in the rat: implications for a model of depression. *Neurosci Biobehav Rev* **5**(2): 247-251.

Kennedy SH, Tighe S, McVey G, Brown GM (1989). Melatonin and cortisol "switches" during mania, depression, and euthymia in a drug-free bipolar patient. *J Nerv Ment Dis* **177**(5): 300-303.

King DP, Zhao Y, Sangoram AM, Wilsbacher LD, Tanaka M, Antoch MP*, et al* (1997). Positional cloning of the mouse circadian clock gene. *Cell* **89**(4): 641-653.

Kirshenbaum GS, Clapcote SJ, Duffy S, Burgess CR, Petersen J, Jarowek KJ*, et al* (2011). Mania-like behavior induced by genetic dysfunction of the neuron-specific Na+,K+-ATPase alpha3 sodium pump. *Proc Natl Acad Sci U S A* **108**(44): 18144-18149.

Kitamura S, Hida A, Watanabe M, Enomoto M, Aritake-Okada S, Moriguchi Y*, et al* (2010). Evening preference is related to the incidence of depressive states independent of sleep-wake conditions. *Chronobiol Int* **27**(9-10): 1797-1812.

Klemfuss H, Kripke DF (1995). Antimanic drugs stabilize hamster circadian rhythms. *Psychiatry Res* **57**(3): 215-222.

Knutson KL (2014). Sleep duration, quality, and timing and their associations with age in a community without electricity in Haiti. *Am J Hum Biol* **26**(1): 80-86.

Ko CH, Takahashi JS (2006). Molecular components of the mammalian circadian clock. *Hum Mol Genet* **15 Spec No 2**: R271-277.

Korczak AL, Martynhak BJ, Pedrazzoli M, Brito AF, Louzada FM (2008). Influence of chronotype and social zeitgebers on sleep/wake patterns. *Braz J Med Biol Res* **41**(10): 914-919.

Kort WJ, Weijma JM (1982). Effect of chronic light-dark shift stress on the immune response of the rat. *Physiol Behav* **29**(6): 1083-1087.

Kosobud AE, Pecoraro NC, Rebec GV, Timberlake W (1998). Circadian activity precedes daily methamphetamine injections in the rat. *Neurosci Lett* **250**(2): 99-102.

Kreiss DS, Lucki I (1995). Effects of acute and repeated administration of antidepressant drugs on extracellular levels of 5-hydroxytryptamine measured in vivo. *J Pharmacol Exp Ther* **274**(2): 866-876.

Krishnan V, Nestler EJ (2008). The molecular neurobiology of depression. *Nature* **455**(7215): 894-902.

Kubera M, Kenis G, Bosmans E, Kajta M, Basta-Kaim A, Scharpe S*, et al* (2004). Stimulatory effect of antidepressants on the production of IL-6. *Int Immunopharmacol* **4**(2): 185-192.

Kubera M, Obuchowicz E, Goehler L, Brzeszcz J, Maes M (2011). In animal models, psychosocial stress-induced (neuro)inflammation, apoptosis and reduced neurogenesis are associated to the onset of depression. *Prog Neuropsychopharmacol Biol Psychiatry* **35**(3): 744-759.

Kudryavtseva NN, Bakshtanovskaya IV, Koryakina LA (1991). Social model of depression in mice of C57BL/6J strain. *Pharmacol Biochem Behav* **38**(2): 315-320.

Kuhn R (1958). The treatment of depressive states with G 22355 (imipramine hydrochloride). *Am J Psychiatry* **115**(5): 459-464.

Kurtuncu M, Arslan AD, Akhisaroglu M, Manev H, Uz T (2004). Involvement of the pineal gland in diurnal cocaine reward in mice. *Eur J Pharmacol* **489**(3): 203-205.

Lavoie J, Hebert M, Beaulieu JM (2013). Glycogen synthase kinase-3beta haploinsufficiency lengthens the circadian locomotor activity period in mice. *Behav Brain Res* **253**: 262-265.

LeGates TA, Altimus CM, Wang H, Lee HK, Yang S, Zhao H*, et al* (2012). Aberrant light directly impairs mood and learning through melanopsin-expressing neurons. *Nature* **491**(7425): 594-598.

LeGates TA, Fernandez DC, Hattar S (2014). Light as a central modulator of circadian rhythms, sleep and affect. *Nat Rev Neurosci* **15**(7): 443-454.

Lemmer B (2009). Discoveries of rhythms in human biological functions: a historical review. *Chronobiol Int* **26**(6): 1019-1068.

Levandovski R, Dantas G, Fernandes LC, Caumo W, Torres I, Roenneberg T*, et al* (2011). Depression scores associate with chronotype and social jetlag in a rural population. *Chronobiol Int* **28**(9): 771-778.

Lewy AJ, Lefler BJ, Emens JS, Bauer VK (2006). The circadian basis of winter depression. *Proc Natl Acad Sci U S A* **103**(19): 7414-7419.

Li J, Lu WQ, Beesley S, Loudon AS, Meng QJ (2012). Lithium impacts on the amplitude and period of the molecular circadian clockwork. *PLoS One* **7**(3): e33292.

Li JZ, Bunney BG, Meng F, Hagenauer MH, Walsh DM, Vawter MP*, et al* (2013). Circadian patterns of gene expression in the human brain and disruption in major depressive disorder. *Proc Natl Acad Sci U S A* **110**(24): 9950-9955.

Li W, Chen S, DeVries SH (2010). A fast rod photoreceptor signaling pathway in the mammalian retina. *Nat Neurosci* **13**(4): 414-416.

Liu YY, Liu TY, Qu WM, Hong ZY, Urade Y, Huang ZL (2012). Dopamine is involved in food-anticipatory activity in mice. *J Biol Rhythms* **27**(5): 398-409.

Loo H, Hale A, D'Haenen H (2002). Determination of the dose of agomelatine, a melatoninergic agonist and selective 5-HT(2C) antagonist, in the treatment of major depressive disorder: a placebo-controlled dose range study. *Int Clin Psychopharmacol* **17**(5): 239-247.

Lopez-Rodriguez F, Kim J, Poland RE (2004). Total sleep deprivation decreases immobility in the forced-swim test. *Neuropsychopharmacology* **29**(6): 1105-1111.

Luby MD, Hsu CT, Shuster SA, Gallardo CM, Mistlberger RE, King OD*, et al* (2012). Food anticipatory activity behavior of mice across a wide range of circadian and non-circadian intervals. *PLoS One* **7**(5): e37992.

Lucki I, O'Leary OF (2004). Distinguishing roles for norepinephrine and serotonin in the behavioral effects of antidepressant drugs. *J Clin Psychiatry* **65 Suppl 4**: 11-24.

Luo AH, Aston-Jones G (2009). Circuit projection from suprachiasmatic nucleus to ventral tegmental area: a novel circadian output pathway. *Eur J Neurosci* **29**(4): 748-760.

Lutter M, Krishnan V, Russo SJ, Jung S, McClung CA, Nestler EJ (2008). Orexin signaling mediates the antidepressant-like effect of calorie restriction. *J Neurosci* **28**(12): 3071-3075.

Maiden RJ (1987). Learned helplessness and depression: a test of the reformulated model. *J Gerontol* **42**(1): 60-64.

Martinek S, Inonog S, Manoukian AS, Young MW (2001). A role for the segment polarity gene shaggy/GSK-3 in the Drosophila circadian clock. *Cell* **105**(6): 769-779.

Martynhak BJ, Correia D, Morais LH, Araujo P, Andersen ML, Lima MM*, et al* (2011). Neonatal exposure to constant light prevents anhedonia-like behavior induced by constant light exposure in adulthood. *Behav Brain Res* **222**(1): 10-14.

Mayers AG, Baldwin DS (2005). Antidepressants and their effect on sleep. *Hum Psychopharmacol* **20**(8): 533-559.

McClung CA (2013). How might circadian rhythms control mood? Let me count the ways. *Biol Psychiatry* **74**(4): 242-249.

McEwen BS, Chattarji S, Diamond DM, Jay TM, Reagan LP, Svenningsson P*, et al* (2010). The neurobiological properties of tianeptine (Stablon): from monoamine hypothesis to glutamatergic modulation. *Mol Psychiatry* **15**(3): 237-249.

Meerlo P, Pragt BJ, Daan S (1997). Social stress induces high intensity sleep in rats. *Neurosci Lett* **225**(1): 41-44.

Meerlo P, Sgoifo A, Turek FW (2002). The effects of social defeat and other stressors on the expression of circadian rhythms. *Stress* **5**(1): 15-22.

Mendoza J, Angeles-Castellanos M, Escobar C (2005a). A daily palatable meal without food deprivation entrains the suprachiasmatic nucleus of rats. *Eur J Neurosci* **22**(11): 2855-2862.

Mendoza J, Angeles-Castellanos M, Escobar C (2005b). Entrainment by a palatable meal induces food-anticipatory activity and c-Fos expression in reward-related areas of the brain. *Neuroscience* **133**(1): 293-303.

Merikanto I, Lahti T, Kronholm E, Peltonen M, Laatikainen T, Vartiainen E*, et al* (2013). Evening types are prone to depression. *Chronobiol Int* **30**(5): 719-725.

Mersch PP, Middendorp HM, Bouhuys AL, Beersma DG, van den Hoofdakker RH (1999). Seasonal affective disorder and latitude: a review of the literature. *J Affect Disord* **53**(1): 35-48.

Millan MJ (2006). Multi-target strategies for the improved treatment of depressive states: Conceptual foundations and neuronal substrates, drug discovery and therapeutic application. *Pharmacol Ther* **110**(2): 135-370.

Millan MJ, Gobert A, Lejeune F, Dekeyne A, Newman-Tancredi A, Pasteau V*, et al* (2003). The novel melatonin agonist agomelatine (S20098) is an antagonist at 5-hydroxytryptamine2C receptors, blockade of which enhances the activity of frontocortical dopaminergic and adrenergic pathways. *J Pharmacol Exp Ther* **306**(3): 954-964.

Mirescu C, Peters JD, Noiman L, Gould E (2006). Sleep deprivation inhibits adult neurogenesis in the hippocampus by elevating glucocorticoids. *Proc Natl Acad Sci U S A* **103**(50): 19170-19175.

Mistlberger R, Rusak B (1987). Palatable daily meals entrain anticipatory activity rhythms in free-feeding rats: dependence on meal size and nutrient content. *Physiol Behav* **41**(3): 219-226.

Mistlberger RE (2009). Food-anticipatory circadian rhythms: concepts and methods. *Eur J Neurosci* **30**(9): 1718-1729.

Mitchell PB, Goodwin GM, Johnson GF, Hirschfeld RM (2008). Diagnostic guidelines for bipolar depression: a probabilistic approach. *Bipolar Disord* **10**(1 Pt 2): 144-152.

Miyauchi T, Kikuchi K, Satoh S (1981). Further studies on the potentiating effect of lithium chloride on methamphetamine-induced stereotypy in mice. *Jpn J Pharmacol* **31**(1): 61-68.

Mochcovitch MD, Nardi AE, Cardoso A (2012). Temperament and character dimensions and their relationship to major depression and panic disorder. *Rev Bras Psiquiatr* **34**(3): 342-351.

Mohawk JA, Miranda-Anaya M, Tataroglu O, Menaker M (2009). Lithium and genetic inhibition of GSK3beta enhance the effect of methamphetamine on circadian rhythms in the mouse. *Behav Pharmacol* **20**(2): 174-183.

Monje FJ, Cabatic M, Divisch I, Kim EJ, Herkner KR, Binder BR*, et al* (2011). Constant darkness induces IL-6-dependent depression-like behavior through the NF-kappaB signaling pathway. *J Neurosci* **31**(25): 9075-9083.

Monteleone P, Maj M (2008). The circadian basis of mood disorders: recent developments and treatment implications. *Eur Neuropsychopharmacol* **18**(10): 701-711.

Morris DW, Rush AJ, Jain S, Fava M, Wisniewski SR, Balasubramani GK*, et al* (2007). Diurnal mood variation in outpatients with major depressive disorder: implications for DSM-V from an analysis of the Sequenced Treatment Alternatives to Relieve Depression Study data. *J Clin Psychiatry* **68**(9): 1339-1347.

Morris DW, Trivedi MH, Fava M, Wisniewski SR, Balasubramani GK, Khan AY*, et al* (2009). Diurnal mood variation in outpatients with major depressive disorder. *Depress Anxiety* **26**(9): 851-863.

Mueller AD, Mear RJ, Mistlberger RE (2011). Inhibition of hippocampal neurogenesis by sleep deprivation is independent of circadian disruption and melatonin suppression. *Neuroscience* **193**: 170-181.

Mukherjee S, Coque L, Cao JL, Kumar J, Chakravarty S, Asaithamby A*, et al* (2010). Knockdown of Clock in the ventral tegmental area through RNA interference results in a mixed state of mania and depression-like behavior. *Biol Psychiatry* **68**(6): 503-511.

Murray G (2007). Diurnal mood variation in depression: a signal of disturbed circadian function? *J Affect Disord* **102**(1-3): 47-53.

Nagano M, Adachi A, Nakahama K, Nakamura T, Tamada M, Meyer-Bernstein E*, et al* (2003). An abrupt shift in the day/night cycle causes desynchrony in the mammalian circadian center. *J Neurosci* **23**(14): 6141-6151.

Nagayama H (1996). Chronic administration of imipramine and lithium changes the phase-angle relationship between the activity and core body temperature circadian rhythms in rats. *Chronobiol Int* **13**(4): 251-259.

Nemeroff CB, Weinberger D, Rutter M, MacMillan HL, Bryant RA, Wessely S*, et al* (2013). DSM-5: a collection of psychiatrist views on the changes, controversies, and future directions. *BMC Med* **11**: 202.

Nestler EJ, Carlezon WA, Jr. (2006). The mesolimbic dopamine reward circuit in depression. *Biol Psychiatry* **59**(12): 1151-1159.

Nomura K, Castanon-Cervantes O, Davidson A, Fukuhara C (2008). Selective serotonin reuptake inhibitors and raft inhibitors shorten the period of Period1-driven circadian bioluminescence rhythms in rat-1 fibroblasts. *Life Sci* **82**(23-24): 1169-1174.

Novati A, Roman V, Cetin T, Hagewoud R, den Boer JA, Luiten PG*, et al* (2008). Chronically restricted sleep leads to depression-like changes in neurotransmitter receptor sensitivity and neuroendocrine stress reactivity in rats. *Sleep* **31**(11): 1579-1585.

O'Brien WT, Harper AD, Jove F, Woodgett JR, Maretto S, Piccolo S*, et al* (2004). Glycogen synthase kinase-3beta haploinsufficiency mimics the behavioral and molecular effects of lithium. *J Neurosci* **24**(30): 6791-6798.

Oldham MA, Ciraulo DA (2014). Bright light therapy for depression: a review of its effects on chronobiology and the autonomic nervous system. *Chronobiol Int* **31**(3): 305-319.

Ozawa H, Miyauchi T (1977). Potentiating effect of lithium chloride on methamphetamine-induced stereotypy in mice. *Eur J Pharmacol* **41**(2): 213-216.

Panda S, Hogenesch JB (2004). It's all in the timing: many clocks, many outputs. *J Biol Rhythms* **19**(5): 374-387.

Pankevich DE, Teegarden SL, Hedin AD, Jensen CL, Bale TL (2010). Caloric restriction experience reprograms stress and orexigenic pathways and promotes binge eating. *J Neurosci* **30**(48): 16399-16407.

Papp M, Gruca P, Lason-Tyburkiewicz M, Adham N, Kiss B, Gyertyan I (2014). Attenuation of anhedonia by cariprazine in the chronic mild stress model of depression. *Behav Pharmacol* **25**(5-6): 567-574.

Paul JR, Johnson RL, Jope RS, Gamble KL (2012). Disruption of circadian rhythmicity and suprachiasmatic action potential frequency in a mouse model with constitutive activation of glycogen synthase kinase 3. *Neuroscience* **226**: 1-9.

Peixoto CA, da Silva AG, Carskadon MA, Louzada FM (2009). Adolescents living in homes without electric lighting have earlier sleep times. *Behav Sleep Med* **7**(2): 73-80.

Pereira DS, Tufik S, Louzada FM, Benedito-Silva AA, Lopez AR, Lemos NA*, et al* (2005). Association of the length polymorphism in the human Per3 gene with the delayed sleep-phase syndrome: does latitude have an influence upon it? *Sleep* **28**(1): 29-32.

Pereira DS, van der Veen DR, Goncalves BS, Tufik S, von Schantz M, Archer SN*, et al* (2014). The effect of different photoperiods in circadian rhythms of per3 knockout mice. *Biomed Res Int* **2014**: 170795.

Pereira M, Martynhak BJ, Baretta IP, Correia D, Siba IP, Andreatini R (2011). Antimanic-like effect of tamoxifen is not reproduced by acute or chronic administration of medroxyprogesterone or clomiphene. *Neurosci Lett* **500**(2): 95-98.

Pickard GE, Rea MA (1997). Serotonergic innervation of the hypothalamic suprachiasmatic nucleus and photic regulation of circadian rhythms. *Biol Cell* **89**(8): 513-523.

Pittenger C, Duman RS (2008). Stress, depression, and neuroplasticity: a convergence of mechanisms. *Neuropsychopharmacology* **33**(1): 88-109.

Porsolt RD, Bertin A, Jalfre M (1977). Behavioral despair in mice: a primary screening test for antidepressants. *Arch Int Pharmacodyn Ther* **229**(2): 327-336.

Possidente B, Lumia AR, McEldowney S, Rapp M (1992). Fluoxetine shortens circadian period for wheel running activity in mice. *Brain Res Bull* **28**(4): 629-631.

Possidente B, Lumia AR, McGinnis MY, Rapp M, McEldowney S (1996). Effects of fluoxetine and olfactory bulbectomy on mouse circadian activity rhythms. *Brain Res* **713**(1-2): 108-113.

Prendergast BJ, Kay LM (2008). Affective and adrenocorticotrophic responses to photoperiod in Wistar rats. *J Neuroendocrinol* **20**(2): 261-267.

Prendergast BJ, Nelson RJ (2005). Affective responses to changes in day length in Siberian hamsters (Phodopus sungorus). *Psychoneuroendocrinology* **30**(5): 438-452.

Prickaerts J, Moechars D, Cryns K, Lenaerts I, van Craenendonck H, Goris I*, et al* (2006). Transgenic mice overexpressing glycogen synthase kinase 3beta: a putative model of hyperactivity and mania. *J Neurosci* **26**(35): 9022-9029.

Raghavendra V, Kaur G, Kulkarni SK (2000). Anti-depressant action of melatonin in chronic forced swimming-induced behavioral despair in mice, role of peripheral benzodiazepine receptor modulation. *Eur Neuropsychopharmacol* **10**(6): 473-481.

Ramos-Brieva JA, Cordero-Villafafila A, Ayuso-Mateos JL, Rios B, Montejo ML, Rivera A*, et al* (1987). Distinct quality of depressed mood: an attempt to develop an objective measure. *J Affect Disord* **13**(3): 241-248.

Refinetti R, Menaker M (1993). Effects of imipramine on circadian rhythms in the golden hamster. *Pharmacol Biochem Behav* **45**(1): 27-33.

Reinink E, Bouhuys N, Wirz-Justice A, van den Hoofdakker R (1990). Prediction of the antidepressant response to total sleep deprivation by diurnal variation of mood. *Psychiatry Res* **32**(2): 113-124.

Riemann D, Konig A, Hohagen F, Kiemen A, Voderholzer U, Backhaus J*, et al* (1999). How to preserve the antidepressive effect of sleep deprivation: A comparison of sleep phase advance and sleep phase delay. *Eur Arch Psychiatry Clin Neurosci* **249**(5): 231-237.

Riemann D, Voderholzer U, Berger M (2002). Sleep and sleep-wake manipulations in bipolar depression. *Neuropsychobiology* **45 Suppl 1**: 7-12.

Rietveld WJ, van Schravendijk K (1987). Food intake and antiepileptic drugs: evidence for a role of GABA in circadian time keeping. *Prog Clin Biol Res* **227B**: 507-511.

Roberts DC, Brebner K, Vincler M, Lynch WJ (2002). Patterns of cocaine self-administration in rats produced by various access conditions under a discrete trials procedure. *Drug Alcohol Depend* **67**(3): 291-299.

Rosenwasser AM, Hayes MJ (1994). Neonatal desipramine treatment alters free-running circadian drinking rhythms in rats. *Psychopharmacology (Berl)* **115**(1-2): 237-244.

Roybal K, Theobold D, Graham A, DiNieri JA, Russo SJ, Krishnan V*, et al* (2007). Mania-like behavior induced by disruption of CLOCK. *Proc Natl Acad Sci U S A* **104**(15): 6406-6411.

Ruhe HG, Mason NS, Schene AH (2007). Mood is indirectly related to serotonin, norepinephrine and dopamine levels in humans: a meta-analysis of monoamine depletion studies. *Mol Psychiatry* **12**(4): 331-359.

Russo SJ, Murrough JW, Han MH, Charney DS, Nestler EJ (2012). Neurobiology of resilience. *Nat Neurosci* **15**(11): 1475-1484.

Rygula R, Papciak J, Popik P (2013). Trait pessimism predicts vulnerability to stress-induced anhedonia in rats. *Neuropsychopharmacology* **38**(11): 2188-2196.

Sabioni P, Baretta IP, Ninomiya EM, Gustafson L, Rodrigues AL, Andreatini R (2008). The antimanic-like effect of tamoxifen: Behavioural comparison with other PKC-inhibiting and antiestrogenic drugs. *Prog Neuropsychopharmacol Biol Psychiatry* **32**(8): 1927-1931.

Santiago RM, Barbiero J, Martynhak BJ, Boschen SL, da Silva LM, Werner MF*, et al* (2014). Antidepressant-like effect of celecoxib piroxicam in rat models of depression. *J Neural Transm* **121**(6): 671-682.

Seligman ME, Beagley G (1975). Learned helplessness in the rat. *J Comp Physiol Psychol* **88**(2): 534-541.

Selikoff IJ, Robitzek EH (1952). Tuberculosis chemotherapy with hydrazine derivatives of isonicotinic acid. *Diseases of the chest* **21**(4): 385-438.

Shearman LP, Jin X, Lee C, Reppert SM, Weaver DR (2000a). Targeted disruption of the mPer3 gene: subtle effects on circadian clock function. *Mol Cell Biol* **20**(17): 6269-6275.

Shearman LP, Sriram S, Weaver DR, Maywood ES, Chaves I, Zheng B*, et al* (2000b). Interacting molecular loops in the mammalian circadian clock. *Science* **288**(5468): 1013-1019.

Shimizu E, Hashimoto K, Okamura N, Koike K, Komatsu N, Kumakiri C*, et al* (2003). Alterations of serum levels of brain-derived neurotrophic factor (BDNF) in depressed patients with or without antidepressants. *Biol Psychiatry* **54**(1): 70-75.

Shiromani PJ, Overstreet D (1994). Free-running period of circadian rhythms is shorter in rats with a genetically upregulated central cholinergic system. *Biol Psychiatry* **36**(9): 622-626.

Silva MT, Galvao TF, Martins SS, Pereira MG (2014). Prevalence of depression morbidity among Brazilian adults: a systematic review and meta-analysis. *Rev Bras Psiquiatr* **36**(3): 262-270.

Sleipness EP, Jansen HT, Schenk JO, Sorg BA (2008). Time-of-day differences in dopamine clearance in the rat medial prefrontal cortex and nucleus accumbens. *Synapse* **62**(12): 877-885.

Sleipness EP, Sorg BA, Jansen HT (2007). Diurnal differences in dopamine transporter and tyrosine hydroxylase levels in rat brain: dependence on the suprachiasmatic nucleus. *Brain Res* **1129**(1): 34-42.

Song L, Che W, Min-Wei W, Murakami Y, Matsumoto K (2006). Impairment of the spatial learning and memory induced by learned helplessness and chronic mild stress. *Pharmacol Biochem Behav* **83**(2): 186-193.

Sprouse J, Braselton J, Reynolds L (2006). Fluoxetine modulates the circadian biological clock via phase advances of suprachiasmatic nucleus neuronal firing. *Biol Psychiatry* **60**(8): 896-899.

Stetler C, Dickerson SS, Miller GE (2004). Uncoupling of social zeitgebers and diurnal cortisol secretion in clinical depression. *Psychoneuroendocrinology* **29**(10): 1250-1259.

Stetson MH, Watson-Whitmyre M (1976). Nucleus suprachiasmaticus: the biological clock in the hamster? *Science* **191**(4223): 197-199.

Stewart KT, Rosenwasser AM, Hauser H, Volpicelli JR, Adler NT (1990). Circadian rhythmicity and behavioral depression: I. Effects of stress. *Physiol Behav* **48**(1): 149-155.

Suchecki D, Tiba PA, Machado RB (2012). REM Sleep Rebound as an Adaptive Response to Stressful Situations. *Front Neurol* **3**: 41.

Sullivan PF, Neale MC, Kendler KS (2000). Genetic epidemiology of major depression: review and meta-analysis. *Am J Psychiatry* **157**(10): 1552-1562.

Sun H, Kennedy PJ, Nestler EJ (2013). Epigenetics of the depressed brain: role of histone acetylation and methylation. *Neuropsychopharmacology* **38**(1): 124-137.

Suzuki K, Miyamoto T, Miyamoto M, Kaji Y, Takekawa H, Hirata K (2007). Circadian variation of core body temperature in Parkinson disease patients with depression: a potential biological marker for depression in Parkinson disease. *Neuropsychobiology* **56**(4): 172-179.

Tang X, Yang L, Sanford LD (2005). Rat strain differences in freezing and sleep alterations associated with contextual fear. *Sleep* **28**(10): 1235-1244.

Tapia-Osorio A, Salgado-Delgado R, Angeles-Castellanos M, Escobar C (2013). Disruption of circadian rhythms due to chronic constant light leads to depressive and anxiety-like behaviors in the rat. *Behav Brain Res* **252**: 1-9.

Tataroglu O, Aksoy A, Yilmaz A, Canbeyli R (2004). Effect of lesioning the suprachiasmatic nuclei on behavioral despair in rats. *Brain Res* **1001**(1-2): 118-124.

Tataroglu O, Davidson AJ, Benvenuto LJ, Menaker M (2006). The methamphetamine-sensitive circadian oscillator (MASCO) in mice. *J Biol Rhythms* **21**(3): 185-194.

Teng HK, Teng KK, Lee R, Wright S, Tevar S, Almeida RD*, et al* (2005). ProBDNF induces neuronal apoptosis via activation of a receptor complex of p75NTR and sortilin. *J Neurosci* **25**(22): 5455-5463.

Tonissaar M, Herm L, Rinken A, Harro J (2006). Individual differences in sucrose intake and preference in the rat: circadian variation and association with dopamine D2 receptor function in striatum and nucleus accumbens. *Neurosci Lett* **403**(1-2): 119-124.

Ushijima K, Morikawa T, To H, Higuchi S, Ohdo S (2006). Chronobiological disturbances with hyperthermia and hypercortisolism induced by chronic mild stress in rats. *Behav Brain Res* **173**(2): 326-330.

Valvassori SS, Bavaresco DV, Budni J, Bobsin TS, Goncalves CL, de Freitas KV*, et al* (2014). Effects of tamoxifen on tricarboxylic acid cycle enzymes in the brain of rats submitted to an animal model of mania induced by amphetamine. *Psychiatry Res* **215**(2): 483-487.

Valvassori SS, Budni J, Varela RB, Quevedo J (2013). Contributions of animal models to the study of mood disorders. *Rev Bras Psiquiatr* **35 Suppl 2**: S121-131.

van der Veen DR, Archer SN (2010). Light-dependent behavioral phenotypes in PER3-deficient mice. *J Biol Rhythms* **25**(1): 3-8.

van Londen L, Goekoop JG, Kerkhof GA, Zwinderman KH, Wiegant VM, De Wied D (2001). Weak 24-h periodicity of body temperature and increased plasma vasopressin in melancholic depression. *Eur Neuropsychopharmacol* **11**(1): 7-14.

van Praag HM (2004). Can stress cause depression? *Prog Neuropsychopharmacol Biol Psychiatry* **28**(5): 891-907.

Vandewalle G, Collignon O, Hull JT, Daneault V, Albouy G, Lepore F*, et al* (2013). Blue light stimulates cognitive brain activity in visually blind individuals. *J Cogn Neurosci* **25**(12): 2072-2085.

Vitaterna MH, King DP, Chang AM, Kornhauser JM, Lowrey PL, McDonald JD*, et al* (1994). Mutagenesis and mapping of a mouse gene, Clock, essential for circadian behavior. *Science* **264**(5159): 719-725.

Wallace DL, Han MH, Graham DL, Green TA, Vialou V, Iniguez SD*, et al* (2009). CREB regulation of nucleus accumbens excitability mediates social isolation-induced behavioral deficits. *Nat Neurosci* **12**(2): 200-209.

Warren WS, Hodges DB, Cassone VM (1993). Pinealectomized Rats Entrain and Phase-Shift to Melatonin Injections in a Dose-Dependent Manner. *J Biol Rhythm* **8**(3): 233-245.

Webb IC, Baltazar RM, Lehman MN, Coolen LM (2009a). Bidirectional interactions between the circadian and reward systems: is restricted food access a unique zeitgeber? *Eur J Neurosci* **30**(9): 1739-1748.

Webb IC, Baltazar RM, Wang X, Pitchers KK, Coolen LM, Lehman MN (2009b). Diurnal variations in natural and drug reward, mesolimbic tyrosine hydroxylase, and clock gene expression in the male rat. *J Biol Rhythms* **24**(6): 465-476.

Wefelmeyer T, Kuhs H (1996). Diurnal mood variation in melancholic patients and healthy controls. *Psychopathology* **29**(3): 184-192.

Wehr TA, Sack DA, Rosenthal NE (1987). Sleep reduction as a final common pathway in the genesis of mania. *Am J Psychiatry* **144**(2): 201-204.

Wehr TA, Wirz-Justice A, Goodwin FK, Duncan W, Gillin JC (1979). Phase advance of the circadian sleep-wake cycle as an antidepressant. *Science* **206**(4419): 710-713.

White W, Feldon J, Heidbreder CA, White IM (2000). Effects of administering cocaine at the same versus varying times of day on circadian activity patterns and sensitization in rats. *Behav Neurosci* **114**(5): 972-982.

Willner P, Towell A, Sampson D, Sophokleous S, Muscat R (1987). Reduction of sucrose preference by chronic unpredictable mild stress, and its restoration by a tricyclic antidepressant. *Psychopharmacology (Berl)* **93**(3): 358-364.

Wirz-Justice A (2003). Chronobiology and mood disorders. *Dialogues Clin Neurosci* **5**(4): 315-325.

Wirz-Justice A (2008). Diurnal variation of depressive symptoms. *Dialogues Clin Neurosci* **10**(3): 337-343.

Wittchen HU, Jacobi F, Rehm J, Gustavsson A, Svensson M, Jonsson B*, et al* (2011). The size and burden of mental disorders and other disorders of the brain in Europe 2010. *Eur Neuropsychopharmacol* **21**(9): 655-679.

Wollnik F (1992). Effects of chronic administration and withdrawal of antidepressant agents on circadian activity rhythms in rats. *Pharmacol Biochem Behav* **43**(2): 549-561.

Wood J, Birmaher B, Axelson D, Ehmann M, Kalas C, Monk K*, et al* (2009). Replicable differences in preferred circadian phase between bipolar disorder patients and control individuals. *Psychiatry Res* **166**(2-3): 201-209.

Wurtman RJ, Ozaki Y (1978). Physiological control of melatonin synthesis and secretion: mechanisms, generating rhythms in melatonin, methoxytryptophol, and arginine vasotocin levels and effects on the pineal of endogenous catecholamines, the estrous cycle, and environmental lighting. *J Neural Transm Suppl*(13): 59-70.

Yazici O (2014). Unipolar mania: a distinct entity? *J Affect Disord* **152-154**: 52-56.

Youdim MB, Arraf Z (2004). Prevention of MPTP (N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) dopaminergic neurotoxicity in mice by chronic lithium: involvements of Bcl-2 and Bax. *Neuropharmacology* **46**(8): 1130-1140.

Zarate C, Duman RS, Liu G, Sartori S, Quiroz J, Murck H (2013). New paradigms for treatment-resistant depression. *Annals of the New York Academy of Sciences* **1292**: 21-31.

Zarate CA, Jr., Singh JB, Carlson PJ, Brutsche NE, Ameli R, Luckenbaugh DA*, et al* (2006). A randomized trial of an N-methyl-D-aspartate antagonist in treatment-resistant major depression. *Arch Gen Psychiatry* **63**(8): 856-864.

Zeller EA, Barsky J (1952). In vivo inhibition of liver and brain monoamine oxidase by 1-Isonicotinyl-2-isopropyl hydrazine. *Proc Soc Exp Biol Med* **81**(2): 459-461.

Zheng B, Larkin DW, Albrecht U, Sun ZS, Sage M, Eichele G*, et al* (1999). The mPer2 gene encodes a functional component of the mammalian circadian clock. *Nature* **400**(6740): 169-173.