LEONARDO BRITO LOPES E SILVA

EFEITOS COMPORTAMENTAIS DA PRIVAÇÃO DE SONO EM CAMUNDONGOS SUBMETIDOS AO ISOLAMENTO SOCIAL: IMPLICAÇÕES NO COMPORTAMENTO TIPO ESQUIZOFRÊNICO

Dissertação apresentada à Universidade Federal de São Paulo – Escola Paulista de Medicina para obtenção do Título de Mestre em Ciências

São Paulo

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“O sentido da vida é aprender algo diferente hoje, algo que eu não sabia ontem...

Se eu não aprendo nada, o dia foi desperdiçado.”

Neil deGrasse Tyson

Dedicatória

DEDICATÓRIA

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Resumo & Abstract

RESUMO

O isolamento social em roedores é amplamente utilizado como modelo animal de transtornos psiquiátricos como depressão e esquizofrenia, por provocar nos animais o que se chama de síndrome do isolamento. Os efeitos nos animais incluem déficits cognitivos, comportamento antissocial, estereotipia, hiperlocomoção e aumento da reatividade à novidade, e são associadas à manifestações vistas nos pacientes. A esquizofrenia é um transtorno psiquiátrico grave e suas principais manifestações clínicas são sintomas positivos e negativos, déficits cognitivos e distúrbios do sono. O sono está intrinsecamente relacionado à esquizofrenia, com a insônia sendo um dos principais sintomas e podendo se apresentar como um prelúdio para o aparecimento dos outros sintomas.

Assim, a hipótese do presente trabalho é de que a privação do sono pode precipitar ou potencializar comportamentos do tipo esquizofrênico exibidos por camundongos submetidos ao modelo animal do isolamento social. Para testar essa hipótese buscou-se investigar os efeitos comportamentais da privação do sono em camundongos submetidos ao isolamento social.

Camundongos machos recém-desmamados foram submetidos ao isolamento social por 60 dias, e em seguida, submetidos a uma sessão de privação do sono por gentle handling (3 h) ou pelo método das plataformas múltiplas (24 h). Os camundongos passaram por testes de cognição, interação social, estereotipia induzida por apomorfina e hiperlocomoção induzida por anfetamina.

Os resultados mostraram que o isolamento social produz os efeitos esperados do modelo. Além disso, um curto período de privação de sono total pelo método do gentle handling pode potencializar os efeitos do isolamento social sobre a função cognitiva, comportamentos estereotipado e antissocial, bem como sobre o efeito estimulante agudo da anfetamina. Já o protocolo de privação de sono pelo método das plataformas múltiplas pode potencializar os efeitos do isolamento social sobre a função cognitiva, o comportamento antissocial e o efeito estimulante agudo da anfetamina.

Assim, foi demonstrado que a privação do sono pode constituir uma ferramenta útil para o estudo de modelos animais de esquizofrenia e que o tipo e a duração da privação de sono é um fator importante nesse contexto. O estudo da interação entre sono e esquizofrenia em modelos animais pode ser uma abordagem interessante para investigações posteriores sobre esta doença.

ABSTRACT

Social isolation in rodents is widely used as an animal model of psychiatric disorders such as depression and schizophrenia, because it causes the isolation syndrome in animals. The effects in animals include cognitive deficits, antisocial behavior, stereotyping, hyperlocomotion and increased reactivity to novelty, and are associated with the manifestations seen in patients. Schizophrenia is a serious psychiatric disorder and its main clinical manifestations are positive and negative symptoms, cognitive impairment and sleep disorders. Sleep is intrinsically linked to schizophrenia, and the insomnia can be one of the main symptoms and may present as a prelude to the appearance of other symptoms.

Thus, the hypothesis of this study is that sleep deprivation can precipitate or enhance the schizophrenic behaviors exhibited by mice subjected to the animal model of social isolation. To test this hypothesis we sought to investigate the behavioral effects of sleep deprivation in mice subjected to social isolation.

Newly weaned male mice were subjected to social isolation for 60 days and then subjected to sleep deprivation session by gentle handling (3 h) or by the method of multiple platforms (24 h). Mice underwent cognitive testing, social interaction, induced stereotypy Apomorphine and amphetamine-induced hyperlocomotion.

The results showed that social isolation produces the effects expected from the model. Furthermore, a short total sleep deprivation by the gentle handling method can potentiate the effects of social isolation on cognitive function, stereotyped, antisocial behavior, as well as acute amphetamine stimulating

effect. The protocol of sleep deprivation by the method of multiple platforms may potentiate the effects of social isolation on cognitive function, antisocial behavior and acute stimulating effect of amphetamine.

Thus, it was demonstrated that the sleep deprivation may be a useful tool for the study of animal models of schizophrenia and the type and duration of sleep deprivation is an important factor in this context. The study of the interaction between sleep and schizophrenia in animal models can be an interesting approach for future investigations into this disease.

ÍNDICE

DEDICATÓRIA vi
AGRADECIMENTOS viii
RESUMO xiii
ABSTRACT xv

1. INTRODUÇÃO 2
1.1 Esquizofrenia 2
1.2 Sono e Esquizofrenia 7
1.3 Isolamento social 12

2. JUSTIFICATIVA 16

3. OBJETIVOS 18
3.1 Objetivo Geral 18
3.2 Objetivos Específicos 18

4. POTENTIATING EFFECTS OF SLEEP DEPRIVATION ON SCHIZOPHRENIC-LIKE BEHAVIORS INDUCED BY SOCIAL ISOLATION IN MICE 20

5. BEHAVIORAL EVALUATION OF SOCIAL ISOLATION AND SLEEP DEPRIVATION INTERACTION IN MICE 59

6. DISCUSSÃO 91

7. CONCLUSÕES 96

8. REFERÊNCIAS 98

9. ANEXOS 108
9.1 Anexo 1: Comitê de Ética 108
9.2 Anexo 2: Comprovante de submissão - Manuscrito 1 109

Introdução

1. INTRODUÇÃO

1.1 Esquizofrenia

A esquizofrenia está entre as doenças mentais mais sérias e debilitantes, atingindo cerca de 1% da população mundial. É uma psicose crônica e idiopática, caracterizada por distúrbios nos processos mentais, aparentando ser um conjunto de diferentes doenças com sintomas que se assemelham e se sobrepõem (SILVA, 2006; KAPLAN, 2008; INSEL, 2010). Muitos autores costumam afirmar que na esquizofrenia existe uma ruptura entre o comportamento e a emoção. Fatores genéticos e ambientais estão envolvidos no seu desenvolvimento, mas ainda não se sabe claramente sua etiologia (SHORTER e MILLER, 2015; SCHMITT et al., 2011; SILVA, 2006).

Do ponto de vista comportamental, na sua forma mais comum, a esquizofrenia se apresenta com sintomas que caracterizam a psicose, como delírios e alucinações. Podem surgir transtornos na forma do pensamento (perda da lógica na associação de ideias com dificuldade de compreensão no discurso), comportamento desordenado, comportamento catatônico (fenômenos como estupor, catalepsia, automatismo, maneirismo, estereotipia e ecopraxia) e agitação psicomotora. Todos esses elementos juntos caracterizam os sintomas positivos da esquizofrenia (SCHULTZ e ANDREASEN, 1999; SILVA, 2006; KAPLAN, 2008; INSEL, 2010). Já os sintomas negativos são caracterizados por comportamento antissocial, falta de motivação, apatia, anedonia, discurso e pensamento empobrecido, embotamento afetivo e prejuízos ocupacionais (AUSTIN, 2005, ROSS et al., 2006; VAN OS E KAPUR, 2009; EHRLICH et al., 2012). Todo esse conjunto de sinais e sintomas aparece

geralmente no final da adolescência e início da vida adulta. No entanto, a doença já se faz presente muito antes na vida do indivíduo por meio de manifestações mais sutis. Déficits cognitivos e distúrbios de sono, por exemplo, são sinais que podem estar presentes muito antes da manifestação dos sintomas positivos e negativos e parecem não se inserir nessa classificação (INSEL, 2010; SILVA, 2006).

Pacientes com esquizofrenia apresentam um déficit cognitivo generalizado (SCHULTZ e ANDREASEN, 1999; AUSTIN, 2005, KAPLAN, 2008; INSEL, 2010). As alterações cognitivas na esquizofrenia não são classificadas em sintomas positivos ou negativos. De fato, os déficits cognitivos são alterações primárias da doença, evidentes durante todo o seu curso evolutivo até mesmo antes do primeiro surto (KEEFE e FENTON, 2007; FERREIRA JUNIOR et al., 2010). Mais especificamente, pacientes com esquizofrenia podem apresentar déficits de velocidade de processamento cognitivo e velocidade psicomotora, de memória de trabalho, de aprendizado verbal e memória imediata e de longo prazo, memória visual, nas habilidades motoras, sensoriais e perceptuais. As alterações incluem ainda déficits em atenção e vigilância, bem como em raciocínio e resolução de problemas (NUECHTERLEIN et al., 2004; SILVA, 2006; KAPLAN, 2008; INSEL, 2010).

Além das anormalidades comportamentais, são encontradas diversas alterações estruturais no cérebro de pacientes com esquizofrenia. Dentre elas se destacam redução no córtex frontal e temporal, hipocampo e tálamo, e aumento dos ventrículos laterais e do volume de sulcos, além de uma ligeira diminuição do cérebro como um todo (CANNON et al.,2003; RAPOPORT et al., 2005; PIONTKEWITZ et al., 2012).

Apesar dos avanços nos estudos de neuroimagem, neuroquímica, neuroendocrinologia e genética, as causas da esquizofrenia ainda não estão completamente esclarecidas. Seu quadro clínico complexo dificulta o melhor entendimento de seus mecanismos fisiopatológicos. Nenhum tratamento disponível atualmente garante remissão de todos os sintomas, sejam os positivos, negativos, cognitivos ou relacionados ao ciclo vigília-sono (BUMB et al., 2015). O diagnóstico muitas vezes é tardio e, frequentemente, confundido com outros transtornos psiquiátricos, como depressão, mania e transtorno bipolar. Com relação à fisiopatologia, algumas hipóteses tentam explicar a seus mecanismos. Há evidências de que a esquizofrenia é uma doença multifatorial, ou seja, determinada pela interação de fatores genéticos e ambientais (CANNON e ROSSO, 2002; SILVA, 2006; FATEMI e FOLSOM, 2009).

Dentre as várias hipóteses desenvolvidas para explicar a esquizofrenia, a hipótese dopaminérgica tem sido nos últimos anos uma das mais investigadas e aceitas. Segundo esta hipótese, os sintomas negativos da esquizofrenia seriam resultantes da hipofunção dopaminérgica no córtex pré-frontal. Essa diminuição de atividade dopaminérgica no córtex levaria a uma hiperatividade funcional de neurônios dopaminérgicos que se projetam para estruturas subcorticais como o núcleo *accumbens* e o estriado dorsal, o que por sua vez promoveria os sintomas positivos da esquizofrenia (AUSTIN, 2005; ROSS et al., 2006; SILVA, 2006; VAN OS e KAPUR, 2009; ISEL, 2010). Esta hipótese foi formulada baseada em evidências farmacológicas. Diversos estudos com drogas que atuam no sistema dopaminérgico, aumentando ou diminuindo sua atividade, corroboram essa teoria. A anfetamina, por exemplo, estimula a neurotransmissão dopaminérgica e provoca quadros psicóticos em indivíduos

sem a doença, bem como antecipa crises em pacientes portadores da esquizofrenia (ROSS, 2006). Por outro lado, o antagonismo dopaminérgico é o mecanismo de ação dos principais fármacos utilizados no tratamento da doença (FATEMI e FOLSOM, 2009; BUMB et al., 2015).

Hoje se sabe que além do sistema dopaminérgico, outros sistemas de neurotransmissão desempenham algum papel na etiologia da esquizofrenia, sendo provável que vários sistemas estejam envolvidos simultaneamente na fisiopatologia dessa complexa doença (LIEBERMAN et al., 1998; SILVA, 2006; EGERTON e STONE, 2012). Nesse contexto, o glutamato figura nos mais recentes estudos como um sistema de neurotransmissão-chave na fisiopatologia da esquizofrenia. Segundo essa nova linha, os sistemas dopaminérgico e glutamatérgico seriam co-moduladores e a desregulação dopaminérgica poderia surgir como um resultado direto de anormalidades na transmissão glutamatérgica (EGERTON e STONE, 2012). Mais especificamente, uma hipofunção de receptores NMDA de glutamato localizados no córtex pré-frontal em interneuronios GABAérgicos levaria à hipoatividade destes. Estes interneurônios GABAérgicos fazem sinápses com vias glutamatérgicas no córtex, que permaneceriam hiperativadas. Tais neurônios gutamatérgicos retornam às regiões mesencefálicas onde localizam-se os corpos celulares dos neurônios dopaminérgicos da via mesolímbica e mesocortical. Desta forma, tais alterações contribuiriam tanto para uma hipoatividade da via mesocortical, durante os sintomas negativos, quanto para a hiperatividade da via mesolímbica durante sintomas positivos (EGERTON e STONE, 2012; NAKAZAWA et al., 2012; SHORTER e MILLER, 2015).

Outra hipótese bastante aceita é a do neurodesenvolvimento. Baseada em estudos clínicos, de neuroimagem, genéticos e ambientais, ela parece se completar às outras hipóteses (ROSS et al., 2006). Segundo essa teoria, a esquizofrenia estaria relacionada a prejuízos nos processos de desenvolvimento neuronal, resultando em alterações físicas e comportamentais que estão presentes muito mais cedo na vida do indivíduo do que o aparecimento dos sintomas clínicos clássicos (LEWIS e LEVITT, 2002; RAPOPORT et al., 2005; PIPER et al., 2012). Inúmeros eventos adversos são capazes de interferir no desenvolvimento de estruturas cerebrais específicas, podendo promover prejuízos que aumentam a vulnerabilidade do indivíduo ao surgimento dos sintomas da doença. Tais eventos podem ser interferências precoces (períodos pré e perinatal) ou tardias (infância e adolescência) no processo de neurodesenvolvimento, tendo forte relação com a etiologia e fisiopatologia da esquizofrenia e estão presentes no histórico de um grande número de pacientes (CANNON et al., 2003; RAPOPORT et al., 2005; SILVA, 2006; PIPER et al., 2012).

Dados epidemiológicos evidenciam que indivíduos que desenvolveram esquizofrenia possuem maior probabilidade de ter sofrido eventos adversos nos períodos pré ou perinatal ou de terem sido expostos a eventos estressores quando comparados a indivíduos saudáveis. Esses eventos incluem complicações na gravidez, como falta de nutrientes, pré-eclâmpsia, diabetes, incompatibilidade sanguínea referente ao fator Rh e infecções, além de malformações congênitas e complicações no parto, como hipóxia, hemorragia pré-parto, atonia uterina e cesariana de emergência (LEWIS e LEVITT, 2002; RAPOPORT et al., 2005; PIPER et al., 2012). A separação materna, o

isolamento social e outros eventos psicossociais estressores podem, também, desencadear alterações no desenvolvimento cerebral e provocar alterações comportamentais associadas à esquizofrenia na fase adulta (CANNON et al., 2003; FONE e PORKESS, 2008).

De acordo com a hipótese do desenvolvimento neurológico, a etiologia da esquizofrenia pode envolver processos patológicos causados por fatores genéticos e ambientais que começam antes de o cérebro atingir a maturidade. Essas anomalias de desenvolvimento neurológico têm sido sugeridas como responsáveis por ativar circuitos neurais patológicos durante a adolescência ou início da idade adulta (por vezes devido ao estresse grave), o que contribuiria para o surgimento de sintomas positivos e/ou negativos (RAPOPORT et al., 2005; SILVA, 2006; PIPER et al., 2012).

1.2 Sono e Esquizofrenia

O sono é um processo complexo, regulado por uma grande interação entre múltiplos sistemas de neurotransmissores e regiões do cérebro. Assim, é intuitivo pensar que qualquer anormalidade no funcionamento cerebral possa interferir no sono. Não é surpresa que a esquizofrenia comprometa o sono e o ritmo circadiano dos pacientes por ser uma doença que afeta diversas regiões do cérebro e sistemas de neurotransmissão, muitas delas envolvidas com a regulação do ciclo vigília-sono. Por isso os distúrbios de sono e no ciclo circadiano são sintomas frequentes na esquizofrenia, reportados em 30 a 80% dos pacientes (COHRS, 2008; WULFF et al. 2009; PRITCHETT et al., 2012).

Desde o início do século XX já existem relatos de alterações no sono em pacientes com esquizofrenia e que o conteúdo onírico desses indivíduos (AFONSO, 2011). Na prática clínica verifica-se que, na esquizofrenia, a insônia é uma queixa frequentemente relatada pelos doentes e familiares. A insônia parece estar relacionada com a gravidade da doença e tem sido referenciada como um sintoma prodrômico, muitas vezes se manifestando como um prelúdio do surto psicótico (CHEMERINSKI et al., 2002; PALMESE et al, 2011; AFONSO et al., 2011). Especificamente, o paciente pode apresentar uma dificuldade tanto em iniciar quanto em manter um sono reparador, fragmentação de sono, menor tempo total de sono, menor eficiência de sono, sono agitado, pesadelos frequentes, quantidade de sono não-REM reduzida, especialmente sono de ondas lentas (TANDON et al., 1992; MONTI & MONTI, 2004; BENSON, 2006; AFONSO et al., 2011). A avaliação objetiva do sono, alcançada pelo advento da técnica de polissonografia, mostrou que a quantidade, a qualidade, e a distribuição da fase de sono REM não apresenta diferenças significativas entre doentes com esquizofrenia não medicados e indivíduos saudáveis (DEMENT, 1955; BENSON, 2006; AFONSO, 2011). Por outro lado, a latência para o sono REM, definida como o período que compreende o início do sono e o aparecimento do primeiro episódio de sono REM, encontra-se frequentemente diminuída na esquizofrenia (TAYLOR et al, 1991; POULIN et al, 2003; AFONSO et al, 2011). Estudos sugerem que uma exacerbação da transmissão colinérgica seria o mecanismo neuroquímico responsável por essa redução (RIEMANN et al, 1994; AFONSO et al, 2011), que tem sido associada ao aumento da atividade delirante e alucinatória, à desorganização do pensamento e do comportamento, e também se

correlaciona a um pior prognóstico da doença (POULIN et al, 2003; AFONSO et al, 2011).

Se observa também com frequência em pacientes com esquizofrenia uma dificuldade em manter um ritmo circadiano regular. Esse fator compromete o processo de reabilitação, uma vez que dificulta o cumprimento dos horários laborais e o ajustamento às atividades sociais (AFONSO, 2011; PRITCHETT et al., 2012). Assim como os déficits cognitivos, as alterações do sono na esquizofrenia podem estar presente durante todo o curso da doença, podem surgir na fase aguda e persistir, em muitos casos, na fase crônica. Apesar da arquitetura do sono melhorar com o tratamento com antipsicóticos, o sono permanece fragmentado e não regressa ao padrão normal, em muitos casos, mesmo em pacientes que se encontram estabilizados clinicamente. Esses achados sugerem a existência de mecanismos fisiopatológicos da própria doença que comprometem diretamente o curso normal do processo vigília-sono (BOIVIN, 2000; AFONSO, 2011; PRITCHETT et al, 2012).

Mas se por um lado os próprios mecanismos da doença provocam distúrbios de sono e do ritmo circadiano, por outro, interferências paralelas no sono podem amplificar os sintomas psiquiátricos e afetar o curso da doença (CHEMERINSKI et al., 2002; AFONSO et al., 2011; PRITCHETT et al., 2012). Um exemplo disso é o fato de episódios de insônia precederem surtos psicóticos em pacientes. Na esquizofrenia, a má qualidade do sono está associada com uma diminuição da qualidade de vida, maiores sintomas positivos e prejuízo de funções cognitivas, incluindo prejuízo na consolidação da memória dependente do sono (KLINGAMAN et al., 2015).

A privação de sono em indivíduos saudáveis compartilha muitas semelhanças com manifestações clínicas observadas na esquizofrenia. Ainda, alguns sinais típicos da doença, a exemplo de sintomas psicóticos como alterações perceptuais e paranóia, déficits cognitivos, redução da conectividade funcional e do metabolismo córtico-frontal e talâmico, e alguns sintomas negativos podem ser induzidos pela privação de sono em indivíduos saudáveis e potencializados em pacientes com esquizofrenia (TANDON, 1992; AFONSO et al., 2011; PRITCHETT et al., 2012; KLINGAMAN et al., 2015). A perda de sono pode atuar como um fator estressor desencadeante do surto, uma vez que a privação de sono provoca uma hiperatividade dopaminérgica decorrente da supersensibilidade dos receptores dopaminérgicos pós-sinápticos (NUNES et al., 1994; TRONCONE et al., 1988). Assim, a hiperfunção de vias dopaminérgicas pode atuar como um gatilho para o surto psicótico. De fato, diversos comportamentos relacionados à ação da dopamina, e que podem ser induzidos experimentalmente pela administração de agonistas diretos ou indiretos, também são observados após a privação de sono (FERGUSON e DEMENT, 1969; TUFIK et al., 1978; TUFIK, 1981a, b; TRONCONE et al., 1988; ANDERSEN et al., 2003; 2005; BERRO et al, 2014). Por exemplo, em roedores a privação de sono promove um aumento da atividade locomotora espontânea e também é capaz de potencializar a hiperlocomoção induzida por anfetamina, comportamento que é considerado um endofenótipo associado à esquizofrenia (FRUSSA-FILHO et al., 2004). Também em roedores, a inibição por pré-pulso, um importante marcador para a esquizofrenia, também é reduzida após a privação de sono e este efeito pode ser revertido por antipsicóticos, mas não por ansiolíticos ou antidepressivos (FRAU et al., 2008). Na clínica, a inibição

por pré-pulso também pode ser prejudicada pela privação de sono (PETROVSKY et al., 2014).

Os sintomas negativos também podem ser sensíveis à fragmentação do sono e do ritmo circadiano, uma vez que a dessincronizarão circadiana provoca piora do humor, irritabilidade e volatilidade afetiva em voluntários saudáveis. É visto na clínica que a melhora dos sintomas negativos é frequentemente associada à da qualidade do sono (YAMASHITA et al. 2004; MURRAY e HARVEY 2010). Também tem sido relatada uma associação entre o desempenho cognitivo e o sono na esquizofrenia. É amplamente sabido que prejuízos no sono ou no ritmo circadiano afetam a cognição em indivíduos saudáveis (BANKS e DINGES, 2007). Em pacientes com esquizofrenia, em tratamento ou não, os déficits cognitivos característicos da doença também são exacerbados pela privação de sono (YANG e WINKELMAN, 2006; WULFF e JOYCE, 2011).

Corroborando essa abordagem, além do tratamento com antipsicóticos melhorar alguns parâmetros relacionados ao sono, o uso de terapias farmacológicas e intervenções cognitivas e comportamentais para tratar distúrbios do sono, em especial a insônia, tem o potencial de melhorar a qualidade de vida, função cognitiva, e reduzir a gravidade dos sintomas psiquiátricos (AFONSO et al., 2011; PRITCHETT et al., 2012; KLINGAMAN et al., 2015).

1.3 Isolamento social

Os modelos animais são fundamentais para a investigação de uma doença humana, seja sobre a etiologia, a fisiopatologia, sinais e sintomas, ou tratamento. Porém, quando se trata de transtornos psiquiátricos, o desenvolvimento de modelos animais torna-se um desafio devido à complexidade do sistema nervoso humano, dificultando a avaliação de parâmetros subjetivos em animais. Assim, nenhum modelo animal de um transtorno psiquiátrico é capaz de ter uma condição experimental que represente fielmente o contexto humano, nem tampouco sujeitos experimentais que reproduzam perfeitamente os achados clínicos de um paciente (LIPSKA e WEINBERGER, 2000; NESTLER e HYMAN, 2010).

Devido à dificuldade de se atingir um modelo animal ideal, Paul Willner propôs em 1984 critérios crescentes de validade de um modelo: a validade preditiva, a validade fenomenológica e a validade de constructo. A validade preditiva indica que o modelo corresponde ao isomorfismo farmacológico no qual o tratamento com drogas produz alterações semelhantes na doença e no modelo. Já a validade fenomenológica busca similaridades bioquímicas, neuropatológicas ou comportamentais. A validade de constructo refere-se à relação entre o modelo e a fisiopatologia da doença (SILVA, 2006; NESTLER e HYMAN 2010).

Assim, quando se propõe um modelo animal é preciso utilizar ferramentas e manipulações que produzam efeitos relacionáveis à condição que se quer estudar. O objetivo dos modelos animais de esquizofrenia é avaliar parâmetros específicos isolados a respeito da doença. Esses parâmetros podem ser

comportamentais, anatômicos ou neuroquímicos e servem como marcadores aproximados para a esquizofrenia. Os parâmetros comportamentais são amplamente utilizados e tem fornecido achados que vem conduzindo as principais descobertas acerca da doença. Dentre eles, estão a inibição por pré-pulso do reflexo do sobressalto, a inibição latente, e, de relevância para o presente estudo, a hiperlocomoção e a estereotipia induzidas por agonistas dopaminérgicos, a interação social e a avaliação neuropsicológica. Podem ser usados psicoestimulantes como agonistas dopaminérgicos, capazes de produzir hiperlocomoção e estereotipia associadas à esquizofrenia, que podem ser revertidas pela ação de antipsicóticos antagonistas dopaminérgicos (GEYER e ELLENBROEK, 2003; CHINEN et al., 2006; JONES et al., 2011; PIONTKEWITZ et al., 2012). Por sua vez, os testes de interação social quantificam o número de interações entre indivíduos, e podem indicar sintomas negativos da esquizofrenia (SILVA, 2006; JONES et al., 2011). Com relação à cognição, prejuízos nessa função são alterações primárias da esquizofrenia, e são evidentes durante todo o curso evolutivo da doença, inclusive no seu período prodrômico. Dessa forma, avaliações cognitivas em animais submetidos a modelos de esquizofrenia evidenciam comprometimentos dos tipos de memória espacial e executiva (GEYER e ELLENBROEK, 2003; DERE et al., 2007; JONES et al., 2011).

Nesse contexto, um dos principais modelos animais de esquizofrenia é o isolamento social em roedores. Esse modelo é capaz de promover alterações duradouras nos animais, as quais podem ser associadas à doença na fase adulta. Do ponto de vista neuroquímico, o isolamento social provoca uma diminuição da transmissão dopaminérgica na via mesocortical e em regiões do

córtex pré-frontal, e um aumento da transmissão dopaminérgica na via mesolímbica e em regiões do estriado em roedores (FONE e PORKESS, 2008). Do ponto de vista neuroanatômico, animais submetidos a esses protocolos experimentais apresentam redução na estrutura do complexo hipocampal e do córtex pré-frontal e também redução de interneurônios GABAérgicos (HEIDBREDER et al., 2000; HALL, 1998). No que diz respeito ao comportamento, o isolamento social promove hiperatividade, aumento da reatividade à novidade (neofobia), déficits da inibição por pré-pulso, déficits cognitivos, aumento da ansiedade e da agressividade e comportamento antissocial. Muitos desses comportamentos parecem ser revertidos pela administração de neurolépticos típicos e atípicos (VALZELLI et al., 1973; HEIDBREDER et al., 2000; WEISS et al., 2004; FONE e PORKESS, 2008; MARSDEN et al., 2011).

Em conjunto, essas alterações são chamadas de “síndrome de isolamento” e muitas delas são semelhantes aos sintomas presentes na esquizofrenia. Esses efeitos se devem ao fato de que os roedores possuem uma estrutura social definida e desenvolvem uma hierarquia que participa criticamente de seu desenvolvimento (JONES et al., 2011). Dessa forma, a privação do contato desde o desmame prejudica o desenvolvimento cerebral e promove tais alterações no animal adulto e que não são revertidas pela reintegração social (LAPIZ et al., 2003; FONE e PORKESS, 2008).

Justificativa

2. JUSTIFICATIVA

Perturbações do sono e do ciclo circadiano são bastante prevalentes em pacientes com esquizofrenia e impactam na saúde física e mental, bem como a qualidade de vida desses indivíduos. Ainda, a privação de sono, como eventos de insônia, pode desencadear o surto psicótico. Assim, diante da forte associação bidirecional existente entre o sono e a esquizofrenia, torna-se de grande importância a avaliação dessa interação. Essa abordagem pode fornecer um melhor entendimento dos mecanismos fisiopatológicos da doença, bem como dos distúrbio de sono e do ciclo circadiano envolvidos. Apesar de estudos avaliarem essa interface na clínica, poucos estudos pré-clinicos têm avaliado essa interação, que poderia constituir uma excelente ferramenta na elucidação dos mecanismos fisiopatológicos da esquizofrenia e no seu tratamento.

Diante desse contexto, a privação de sono, dependendo da sua natureza, poderia intensificar alguns espectros comportamentais induzidos pelo isolamento social em camundongos, um modelo animal de esquizofrenia.

Objetivos

3. OBJETIVOS

3.1 Objetivo Geral

O objetivo da presente dissertação foi investigar os efeitos comportamentais de dois métodos de privação de sono (*gentle handling* e plataformas múltiplas) em camundongos submetidos ao isolamento social.

3.2 Objetivos Específicos

Verificar os efeitos da privação de sono sobre:

- O desempenho na tarefa de esquiva discriminativa em labirinto em cruz elevado em camundongos submetidos ao isolamento social;
- O desempenho na tarefa de reconhecimento de objetos em camundongos submetidos ao isolamento social;
- A interação social em camundongos submetidos ao isolamento social;
- O comportamento estereotipado induzido pela administração de apomorfina em camundongos submetidos ao isolamento social;
- A hiperlocomoção e sensibilização comportamental induzida pela administração aguda de anfetamina em camundongos submetidos ao isolamento social.

Manuscrito 1

POTENTIATING EFFECTS OF SLEEP DEPRIVATION ON SCHIZOPHRENIC-LIKE BEHAVIORS INDUCED BY SOCIAL ISOLATION IN MICE

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ABSTRACT

Schizophrenia is a serious psychiatric disorder, with its main clinical manifestations being positive and negative symptoms and cognitive. Social isolation in rodents has been proposed as an animal model of schizophrenic-like behaviors. Concurrently, sleep disturbance is intrinsically related to schizophrenia, as insomnia is a prelude to the appearance of clinical manifestations. We hypothesized that sleep deprivation could potentiate schizophrenic-like behaviors displayed by social isolated mice. We sought to investigate the behavioral effects of sleep deprivation in mice subjected to social isolation. Newly weaned male mice were subjected to social isolation for 60 days, and then submitted to a 3-h session of sleep deprivation by gentle handling. The mice were tested for cognition, apomorphine-induced stereotypy, social interaction and amphetamine-induced hyperlocomotion. The results showed that a short period of total sleep deprivation could potentiate the effects of social isolation on stereotyped and aggressive behaviors as well as on the acute stimulant effect of amphetamine. Thus, we demonstrated that sleep deprivation can constitute a useful tool for studying animal models of schizophrenia.

Keywords: Schizophrenia, social isolation, sleep, total sleep deprivation, mice.

INTRODUCTION

Schizophrenia is a devastating psychiatric disorder in which clinical manifestations can be present in the form of positive (e.g. delusions and hallucinations), negative symptoms (e.g. blunting of affect and depressive behaviors), cognitive deficits and sleep disturbances (ROSS et al, 2006; SILVA, 2006; INSEL, 2010; AFONSO, 2011). The sleep disturbances include increased sleep latency and reduction in total sleep time, sleep efficiency, REM sleep latency, REM sleep density and slow-wave sleep duration (COHRS, 2008; MANOACH and STICKGOLD, 2009). Moreover, it is also associated with significant circadian disruption, such as abnormal phasing or instability and fragmentation of rest-activity rhythms (MARTIN et al., 2005; WULFF et al., 2009). In agreement, patients under treatment with typical antipsychotics show an increase in sleep efficiency and total sleep time (COHRS, 2008). Importantly, sleep disturbances such as insomnia episodes often appear as prodromal symptoms of schizophrenia (AFONSO, 2011). Thus, sleep deprivation acts as a stressor that can precipitate a psychotic episode in schizophrenic patients. One possible explanation is the supersensitivity of dopamine receptors induced by sleep deprivation (TRONCONE et al., 1988; NUNES JUNIOR et al., 1994).

Several hypotheses have been developed to explain the schizophrenia etiology, one of the most widely accepted and studied being the dopaminergic hypothesis. According that, there is a dopaminergic hypofunction in the prefrontal cortex and a subsequent dopaminergic hyperactivity in the dorsal striatum and nucleus accumbens (ROSS et al, 2006; SILVA, 2006). Another proposal is the neurodevelopmental hypothesis, which postulates that

schizophrenia is related to impairments in neuronal development. Such damage would be caused by adverse events during pregnancy (e.g. maternal infection), at birth (e.g. fetal hypoxia) or during childhood (e.g. psychosocial stress) (LEWIS and LEVITT, 2002; CANNON, et al., 2003; RAPOPORT et al., 2005; PIPER et al., 2012).

Regarding animal models, social isolation in rodents has been proposed for the investigation of schizophrenic-like behaviors considering that rodents have a defined social structure and hierarchy, which critically influence their development (JONES et al., 2011). Contact deprivation from weaning may lead to alterations in brain development, resulting in behavioral, neuroanatomical and neurochemical changes which may be associated with schizophrenia (LAPIZ et al., 2003; FONE and PORKESS, 2008). Specifically, neuronal alterations can be a reduction in the structure of the hippocampal complex and prefrontal cortex, as well as changes in mesocortical dopaminergic transmission (HALL, 1998; HAIDBREDER at al., 2000). Increased reactivity to novelty, deficits in prepulse inhibition, cognitive impairment and increased anxiety, aggression and locomotor activity are among the behavioral effects promoted by social isolation (VALZELLI, 1973; HALL, 1998; HAIDBREDER at al., 2000; LAPIZ et al., 2003; FONE and PORKESS, 2008). It is interesting to note that some of these behavioral changes can be reversed by typical and atypical neuroleptics (HALL, 1998; HAIDBREDER at al., 2000).

Considering that insomnia can be a prelude to the appearance of clinical manifestations of schizophrenia, we hypothesized that sleep deprivation could precipitate the schizophrenic-like behaviors observed in social isolated mice. Despite the fact that the interaction between schizophrenia and sleep has been

extensively examined in clinical scenary, the evaluation of the effects of sleep deprivation on schizophrenic-like behaviors in an animal model may constitute a valuable tool. In this study we sought to investigate the behavioral effects of sleep deprivation in mice subjected to social isolation.

METHODS

Subjects

Twenty-one-day-old Swiss male mice were maintained at 22º C in 12:12 h light-dark cycle (lights on at 07:00h). Food and water were available *ad libitum*. Animals were maintained in accordance with the Brazilian Law for Procedures for Animal Scientific Use (#11794/2008) and experimental procedures were approved by the Institutional Animal Care and Use Committee under the protocol #330300.

Social isolation (ISO)

For the social isolation, animals were maintained alone for 60 days or housed in groups of 6 per cage (control group). The isolation was carried out in cages that prevented any visual or social interactions, allowing only auditory and olfactory contacts with mice from neighboring cages (BORÇOI et al., 2015).

Sleep deprivation (SD)

Animals were subjected to either control condition (not sleep deprived) or to sleep deprivation through the gentle handling method (PATTI et al., 2010; FERNANDES-SANTOS et al., 2012). It consists of keeping the animal awake

by tapping on the cage and, if necessary, by gently touching them with a soft brush. Sleep deprivation lasted 3 h, starting at 10 am.

Drugs

D-amphetamine sulfate (2.5 mg/kg body weight) (Sigma® Chemical Co.) was diluted in saline 0.9 %, which was also the control solution, administered intraperitoneally. Apomorphine hydrochloride (1.5 mg/kg body weight) (Sigma® Chemical Co.) was diluted in 0.2% ascorbic acid, which was also the vehicle solution, administered subcutaneously. All solutions were administered in the volume of 10 ml/kg body weight.

Plus-maze discriminative avoidance task (PM-DAT)

The PM-DAT was used to concomitantly evaluate learning, memory, anxiety-like behavior, and motor activity, as described previously (SILVA and FRUSSA-FILHO, 2000; FERNANDES et al., 2015). The aversive stimuli consisted of a 100-watt light and an air blow produced by a 110-V hairdryer positioned over the aversive enclosed arm. These stimuli were present during the 10-min training session. Test session lasted 3 min, performed in the absence of the aversive stimuli. In all sessions, the apparatus was cleaned with a 5% alcohol solution before each animal observation. Total number of entries, percent time spent in the aversive enclosed arm (time spent in aversive enclosed arm/ time spent in both enclosed arms), and percent time spent in open arms (time spent in open arms/time spent in both open and enclosed arms) were calculated. Learning and memory were evaluated by the percent time spent in the aversive enclosed arm during training and testing,

respectively. Anxiety-like behavior and motor activity were assessed by the percent time spent in the open arms and the total number of entries in all the arms of the apparatus, respectively.

Object recognition task (ORT)

Object recognition task was conducted in the open field. Firstly, there was a habituation session in the open field without objects. In the next day, animals were subjected to session I. In this session, mice were placed in the arena containing two similar objects (object A and object A') and left to explore them freely for 10 min. The session II occurred 180 minutes later, in order to evaluate short-term memory. In this session, one of the objects was substituted for a new object (object B) and the mouse was introduced in the arena for more 10 min. The positions of the objects (familiar or novel) were randomly permuted for each experimental animal. Exploration was defined as sniffing or touching the object with the nose and/or forepaws. Sitting on or turning around the object was not considered exploratory behavior. Time spent exploring each object and the numbers of crossings through quadrants were recorded by an observer blind to the treatment. The objects and the apparatus were cleaned with a 5% alcohol solution after each behavioral session. Memory was measured by the relative time spent exploring the familiar object compared to the time spent exploring a new object. If an animal remembers a previously familiar object, this object should be explored less than the new object. Conversely, a previously familiar object that was not remembered would be explored just as much as a new objet (ENNACEUR, 2010).

Social interaction

The assessment of social behavior was adapted from Linck and colleagues (2008). The social interaction was evaluated in an opaque polypropylene cage (25 x 20 x 20 cm) with no top. The day before testing, mice were habituated, when each mouse remained alone in the test box for 10 min. On the test day, mice were allocated in pairs composed of an unknown animal matched for body weight with the experimental animal and placed in the test box. The time spent interacting (sniffing, grooming, following, mounting and attacking the partner and nonspecific friendly contacts) were quantified. Passive contact (sitting or lying with bodies in contact) was not quantified. Only contacts that came from the experimental animal were considered.

Stereotyped behavior

Animals were observed for stereotyped behavior in wire mesh cages (16 x 30 x 18 cm) free of water and food. Stereotyped behavior was quantified every 5 min for 15 s per animal in a 100-min session. The scoring system applied was proposed by Setler and colleagues (1976), with modifications validated in our laboratory for mice (CARVALHO et al., 2006). Scores from 0 to 4 were attributed to each animal as follows: 0 asleep or stationary; 1, active; 2, active with predominantly stereotyped sniffing and rearing; 3, stereotyped sniffing with bursts of licking and/or gnawing and biting; 4, continual licking and/or gnawing of cage grids.

Open field test

The amphetamine-induced hyperlocomotion was evaluated in the open field arena. It consists of an opaque cylindrical white polyethylene arena (50cm height) and wooden base (40cm diameter) divided into 19 squares. In each session, the animal was observed for 10 min and the locomotion (number of entries in quadrants with all 4 paws) and the number of rearing (amount of lift on 2 paws supported or not on the device wall) were quantified (CHINEN et al., 2006).

Experimental design

Experiment I – Effects of SD on the PM-DAT in social-isolated mice

After 60 days of social isolation, mice were submitted to SD. Immediately after the SD procedure, mice were trained in PM-DAT forming the following groups: CTRL – home cage controls (N=11-12), SD –only sleep deprived (N=12), ISO – only isolated (N=12) and ISO-SD – isolated and sleep-deprived (N=12). Test session was performed 7 days after training.

Experiment II – Effects of SD on ORT in social-isolated mice

On the 59th day of social isolation, animals were habituated in the open field. On the next day, animals were submitted to SD forming the following groups: CTRL (N=10), SD (N=10), ISO (N=10) and ISO-SD (N=10). Immediately after the SD period, each animal was submitted to the session I in the open field with two similar objects. Three hours later, they were submitted to the session II, in which they were exposed to one of the familiar objects and one new object in the open field.

Experiment III – Effects of SD on social behavior in social-isolated mice

On the 59th day of social isolation, animals were habituated. On the next day, animals were submitted to SD, forming the following groups: CTRL (N=12), SD (N=11), ISO (N=12) and ISO-SD (N=12). Immediately after the SD period, each animal was again placed in the test box with an unfamiliar mouse for 10 min for the social interaction evaluation.

Experiment IV – Effects of SD on stereotyped behavior in social-isolated mice

After 60 days of social isolation, mice were submitted to SD, forming the following groups: CTRL (N=11), SD (N=11), ISO (N=11) and ISO-SD (N=11). Immediately after the SD period, all animals received an apomorphine injection and 5 min later, their stereotyped behavior was evaluated.

Experiment V – Effects of SD on amphetamine-induced hyperlocomotion in social-isolated mice.

From the 57th to 59th day of social isolation, animals were habituated to the open field arena after a saline injection. On the 60th day, animals were submitted to SD. Immediately after it, animals received either a saline (SAL) or 2.5mg/Kg amphetamine (AMP) injection, forming the groups: SAL (N=9) –only treated with SAL; AMP (N=11) – only treated with AMP: SD-SAL (N=12) – sleep deprived treated with SAL: SD-AMP (N=10) – sleep deprived treated with AMP: ISO-SAL (N=11) – isolated treated with SAL : ISO-AMP (N=12) – isolated treated with AMP: ISO-SD-SAL (N=12) – isolated and sleep deprived treated with SAL; and ISO-SD-AMP (N=10-11) – isolated and sleep deprived treated with AMP. Fifteen min later, mice were exposed to the open field arena. Seven

days later, mice were submitted to a challenge session, in which all animals received the amphetamine injection before the behavior evaluation.

Statistical analysis

Data were compared by MANOVA and Duncan's test when necessary. When applicable, independent or paired samples T-test were used. A probability of $p<0.05$ was considered to show significant differences.

RESULTS

Experiment I – Effects of SD on the PM-DAT in social-isolated mice

In the training, MANOVA for the time percent spent in the aversive arm showed significant effects of social condition (not isolated *vs*. isolated) [$F(1,44)=10.18$; $p=0.003$] and of social condition x sleep condition (not sleep deprived *vs*. sleep deprived) interaction [$F(1,44)=6.48$; $p=0.015$]. Duncan's test revealed that the ISO group spent less time in the aversive arm compared to CTRL and SD groups (Figure 1A). Concerning anxiety, when the percent time in the open arms was analyzed, there was no significant difference (Figure 1B). Regarding locomotion, MANOVA followed by Duncan's test demonstrated significant effects of sleep condition [$F(1,44)=17.40$; $p<0.001$] and social condition x sleep condition interaction [$F(1,44)=4.80$; $p=0.034$]. Animals in the ISO group had a decrease in the number of entries compared to CTRL and SD groups. Oppositely, animals in the ISO-SD group presented an increase in this parameter compared to CTRL and ISO groups (Figure 1C).

During test, there were no differences among groups in the percent of time spent in the aversive arm, in the open arms or in the number of entries (Figures 1D, 1E and 1F).

Experiment II – Effects of SD on ORT in social-isolated mice

MANOVA followed by Duncan's test showed significant effect of social condition on the locomotor activity in both sessions (Session I - [$F(1,36)=22.96$; $p<0,001$]; Session II - [$F(1,36)=16.71$; $p<0,001$]). In both sessions, social-isolated animals had an increased locomotion compared to respective non-social-isolated groups (Figure 2A and 2C). Regarding the interaction with objects, there was no difference among groups in the 1st session (Figure 2B). In the 2nd session, paired samples t test showed that CTRL, SD and ISO groups explored more the object B than the object A (CTRL – [$t(1,9)=4.12$; $p=0,003$]; SD – [$t(1,9)=2.60$; $p=0.029$]; ISO – [$t(1,9)=2.74$; $p=0.023$]) (Figure 2D).

Experiment III – Effects of SD on social behavior in social-isolated mice

MANOVA and Duncan's test showed effect of social condition on the number of friendly contacts [$F(1,43)=45.44$; $p<0.001$]. Groups ISO and ISO-SD had lower amount of contacts compared to their respective controls for social condition (Figure 3A). In addition, isolated animals, regardless of sleep condition, showed a significant increase in the number of attacks compared to the SD and CTRL groups, respectively [$F(1,43)=42.54$; $p<0.001$] (Figure 3B).

Experiment IV – Effects of SD on stereotyped behavior in social-isolated mice

In the stereotyped behavior, MANOVA followed by Duncan's test showed significant effects of social [$F(1,40)=11.35$; $p=0.002$] and sleep [$F(1,40)=11.79$; $p=0.001$] conditions. The SD and ISO groups had an increase in stereotypy score when compared to the CTRL group. When combined, these manipulations promoted an even greater increase in stereotypy. The SD-ISO group had higher stereotypy score than SD or ISO groups (Figure 4).

Experiment V – Effects of SD on amphetamine-induced hyperlocomotion in social-isolated mice

In the habituation sessions, the independent samples T-test showed that isolated animals had an increase in locomotion frequency compared to control animals in all of the behavioral sessions (1^{st} habituation day, [$t(1,86)=2.08$; $p=0.041$] and 3^{rd} habituation day, [$t(1,86)=3.67$; $p<0.001$]). However, even though isolated mice presented an increased motor activity in the 3^{rd} habituation session compared to control, the paired samples T-test showed that both groups had a decrease in locomotion in the 3^{rd} exposure to the apparatus compared to the to the 1^{st} one ([$t(1,41)=8.58$; $p<0.001$] and [$t(1,45)=5.72$; $p<0.001$] for not isolated and isolated animals, respectively) (Figure 5).

In the priming session, MANOVA followed by Duncan's test showed significant effects of treatment (SAL *vs.* AMP) [$F(1,79)=80.19$; $p<0.001$], social condition [$F(1,79)=37.26$; $p<0.001$], social condition x sleep condition [$F(1,79)=5.45$; $p=0.022$] and social condition x treatment interactions [$F(1,79)=9.77$; $p=0.002$] on motor activity. All animals that received AMP showed an increase in locomotion compared to their respective control. In

addition, ISO-SAL, ISO-AMP and ISO-SD-AMP groups had an increase in locomotion compared to SAL, CTRL-AMP and SD-AMP groups, respectively. The effect of social condition x sleep condition interaction was shown by increased locomotion presented by ISO-AMP, ISO-SD-SAL and ISO-SD-AMP groups compared to SD-AMP, SAL and AMP, respectively. The effect of social condition x treatment interaction was showed by increased locomotion presented by ISO-AMP and ISO-SD-AMP groups compared to SAL and SD-SAL, respectively (Figure 6A).

Paired samples t-test showed that all groups had an increase in locomotion in the challenge session compared to the priming session [$t(1,8)=3.76$; $p=0.006$] – SAL; [$t(1,10)=3.73$; $p=0.004$] – AMP; [$t(1,11)=2.63$; $p=0.023$] – SD-SAL; [$t(1,9)=4.59$; $p=0.001$] – SD-AMP; [$t(1,10)=4.85$; $p=0.001$] – ISO-SAL; [$t(1,11)=4.64$; $p=0.001$] – ISO-AMP; [$t(1,11)=5.96$; $p<0.001$] – ISO-SD-SAL; [$t(1,9)=5.20$; $p=0.001$] – ISO-SD-AMP (Figure 6A).

Considering the locomotion frequency in the challenge session, three-way ANOVA followed by Duncan's test showed that there were significant effects of treatment [$F(1,79)=10.17$; $p=0.002$] and social condition factors [$F(1,79)=16.16$; $p<0.001$], treatment x sleep condition [$F(1,79)=4.0$; $p=0.049$] and treatment x sleep condition x social condition [$F(1,79)=5.45$; $p=0.02$] interactions. The AMP and ISO-AMP groups showed higher locomotion compared to their respective controls (SAL and ISO-SAL). The effect of social isolation was evidenced by the increased motor activity presented by the groups ISO-SAL, ISO-AMP, and ISO-SD-SAL compared to their respective controls (SAL, AMP, and SD-SAL). The effect of treatment x sleep condition interaction was shown by difference among isolated animals, which displayed an increase in locomotion in the ISO-AMP

and ISO-SD-SAL groups (but not the group IS-SD-AMP), in relation to the ISO-SAL group. Additionally, the treatment x social condition x sleep condition interaction effect was shown by increased locomotion presented by the groups ISO-AMP, ISO-SD-SAL and ISO-SD-AMP compared to the groups SAL and SD-SAL (Figure 6A).

Regarding rearing behavior, MANOVA followed by Duncan's test showed that there was significant effect of sleep condition [$F(1,79)=4.36$; $p=0.04$] and treatment factors [$F(1,79)=4.97$; $p=0.03$] in the priming session. The groups ISO-SD-AMP and SD-SAL had an increased rearing frequency compared to the ISO-AMP and SAL, respectively. Also, the ISO-SD-AMP and AMP groups had an increased rearing frequency compared to the ISO-SD-SAL and SAL, respectively. Concerning the challenge session, there were significant effects of sleep [$F(1,79)=6.54$; $p=0.012$] and social [$F(1,79)=5.17$; $p=0.026$] condition factor. The groups ISO-SD-AMP, ISO-SD-SAL and SD-SAL presented an increase in rearing frequency compared to the ISO-AMP, ISO-SAL and SAL groups, respectively. Still, ISO-SD-AMP, ISO-SD-SAL and ISO-SAL groups presented an increase in this parameter compared to SD-AMP, SD-SAL and SAL, respectively (Figure 6B).

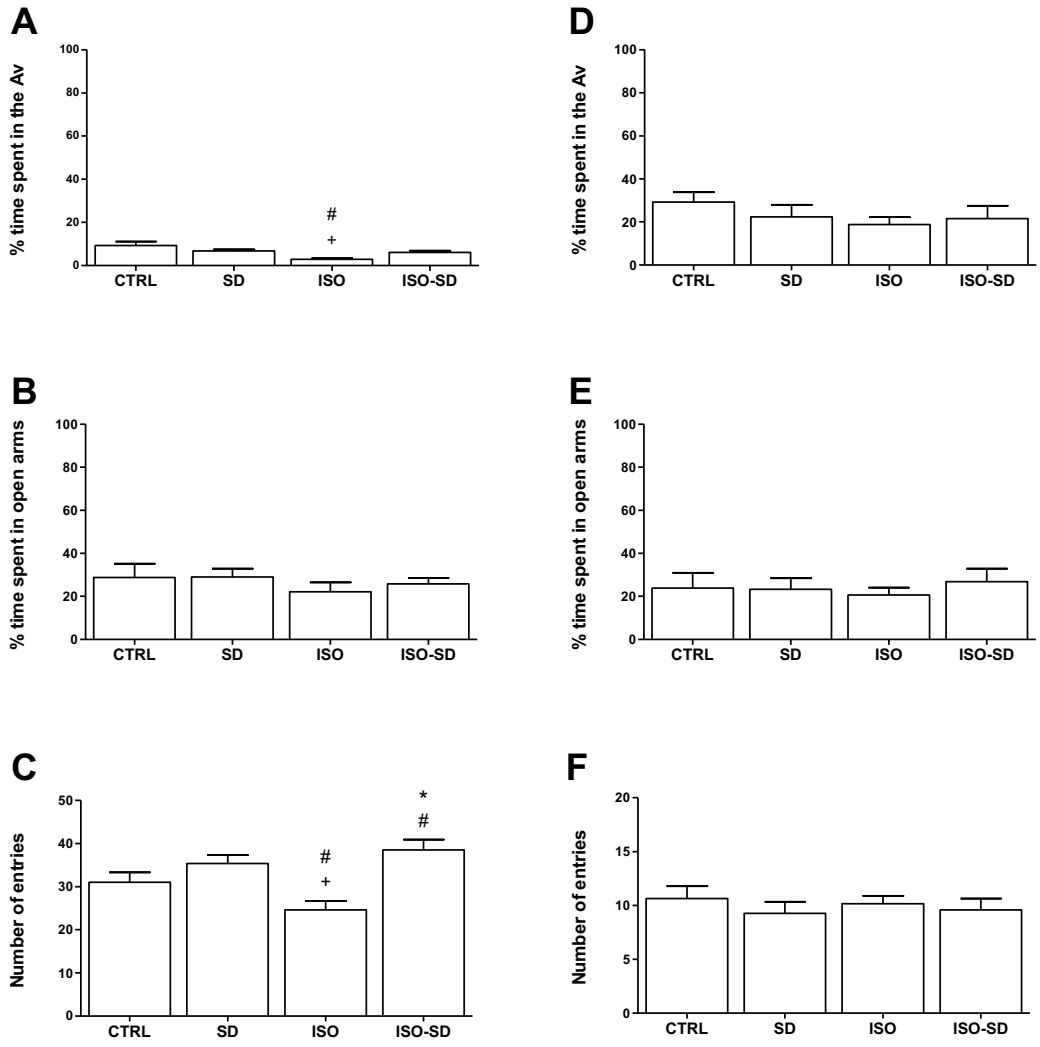


Figure 1: Effects of sleep deprivation on PM-DAT in social-isolated mice. CTRL: control condition; SD: only sleep-deprived group; ISO: only social-isolated group; ISO-SD: social-isolated and sleep-deprived group. Results are presented as mean ± S.E. (MANOVA and Duncan's test) of percent time spent in the aversive enclosed arm (A – Training session; D – Test session), percent time spent in the open arms (B – Training session; E – Test session) and total number of entries (C – Training session; F – Test session). $^{+}p<0.05$ compared to group of different social condition; $^{*}p<0.05$ compared to group of different sleep condition; $^{\#}p<0.05$ compared to group of different social and sleep conditions.

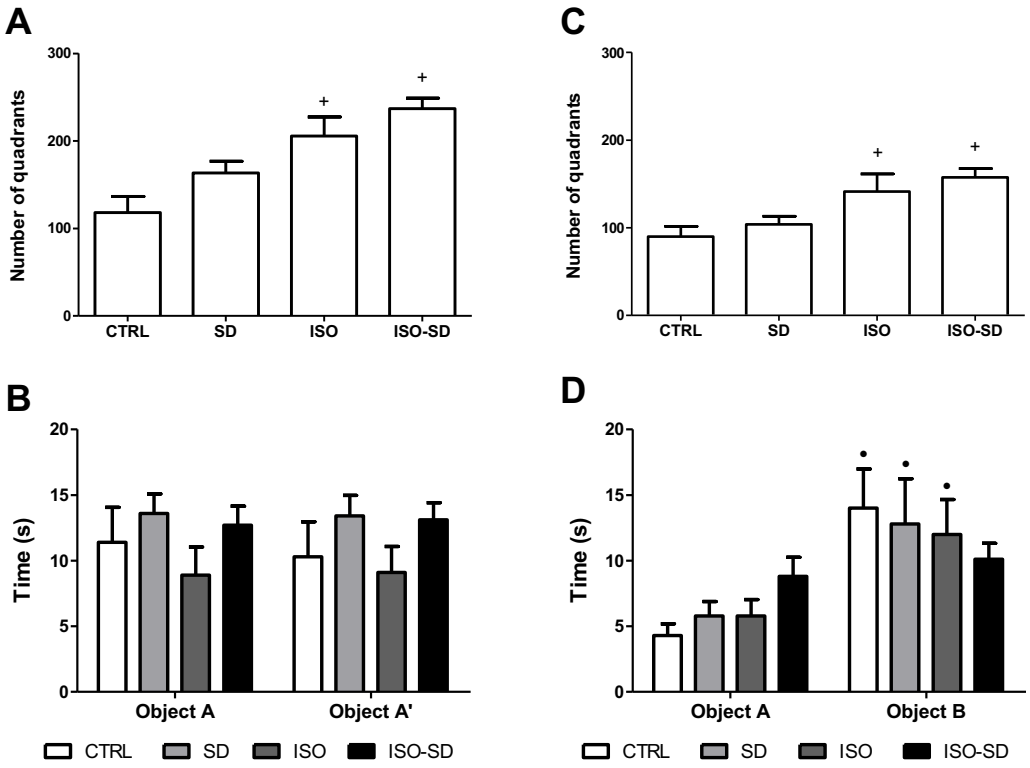


Figure 2: Effects of sleep deprivation on ORT in social-isolated mice. CTRL: control condition; SD: only sleep-deprived group; ISO: only social-isolated group; ISO-SD: social-isolated and sleep-deprived group. Results are presented as mean ± S.E. (MANOVA, Duncan's test and paired samples t-test) of number of crossing through quadrants (A – Session I; C – Session II) and time spent exploring objects (B – Session I; D – Session II). $^{+}p<0.05$ compared to groups with different social condition; $^{\bullet}p<0.05$ compared to the exploration time with the other object by the same group.

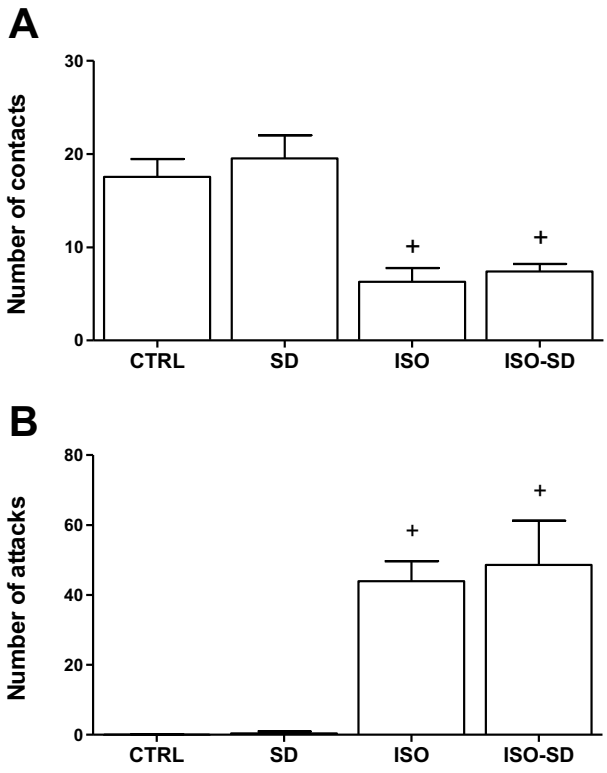


Figure 3: Effects of sleep deprivation on social behavior in social-isolated mice. CTRL: control condition; SD: only sleep-deprived group; ISO: only social-isolated group; ISO-SD: social-isolated and sleep-deprived group. Results are presented as mean ± S.E. (MANOVA and Duncan's test) of number of friendly contacts (**A**) and number of attacks (**B**). $^{+}p<0.05$ compared to groups with different social condition.

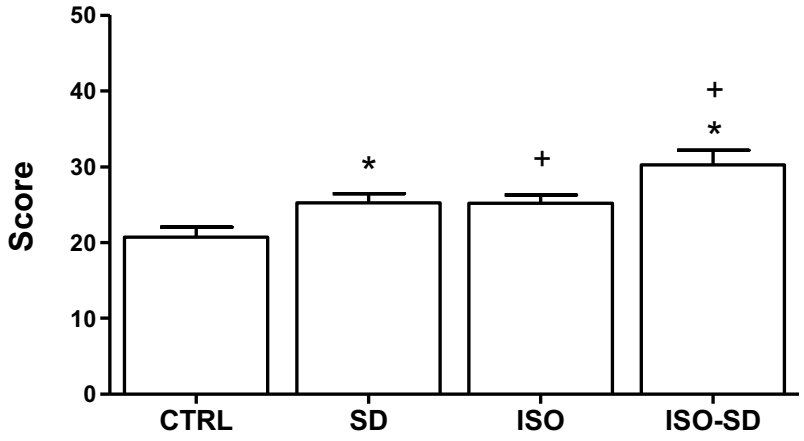


Figure 4: Effects of sleep deprivation on stereotyped behavior in social-isolated mice. CTRL: control condition; SD: only sleep-deprived group; ISO: only social-isolated group; ISO-SD: social-isolated and sleep-deprived group. Results are presented as the mean ± S.E. (MANOVA and Duncan's test) of stereotypy score during a 100-min session. $^{+}p<0.05$ compared to group of different social condition; *$p<0.05$ compared to group of different sleep condition.

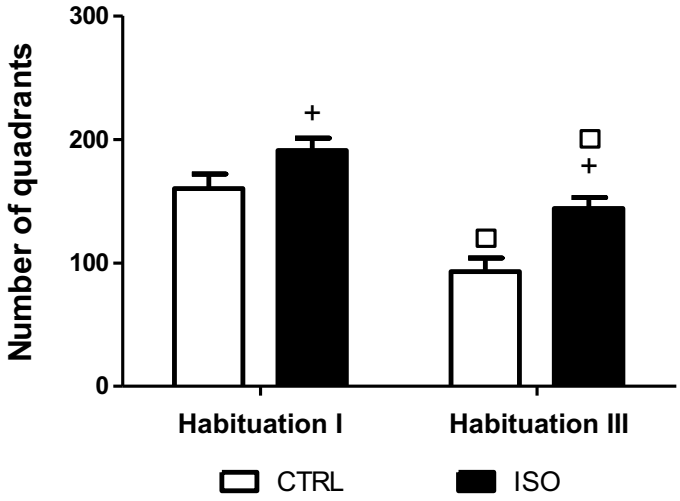
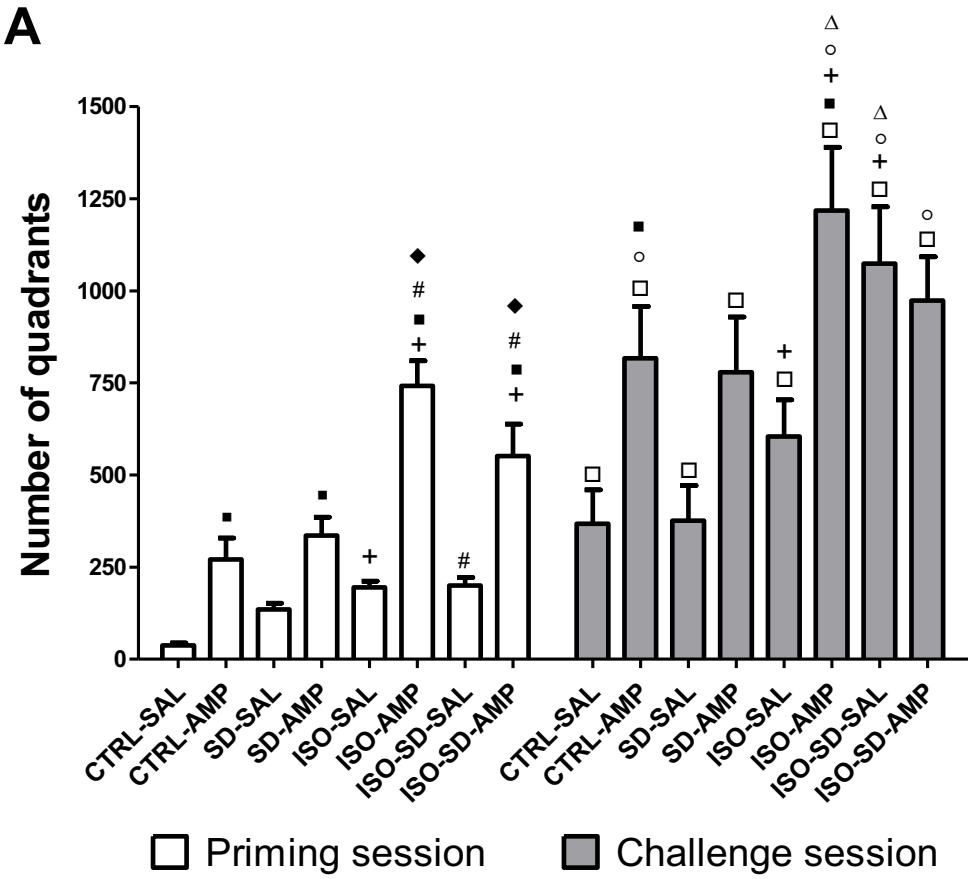


Figure 5: Habituation sessions of experiment V (before the sleep deprivation protocol). CTRL: control condition; ISO: only social-isolated group. Results are presented as mean ± S.E. (independent samples t-test and paired samples t-test) of number of crossings through quadrants in the 1st and the 3rd habituation sessions. $^{+}p<0.05$ compared to group of different social condition. $^{\square}p<0.05$ compared to the same group in the first habituation session.


A
Number of quadrants
1500
1250
1000
750
500
250
0
CTRL-SAL
CTRL-AMP
SD-SAL
SD-AMP
ISO-SAL
ISO-AMP
ISO-SD-SAL
ISO-SD-AMP
Priming session
Challenge session

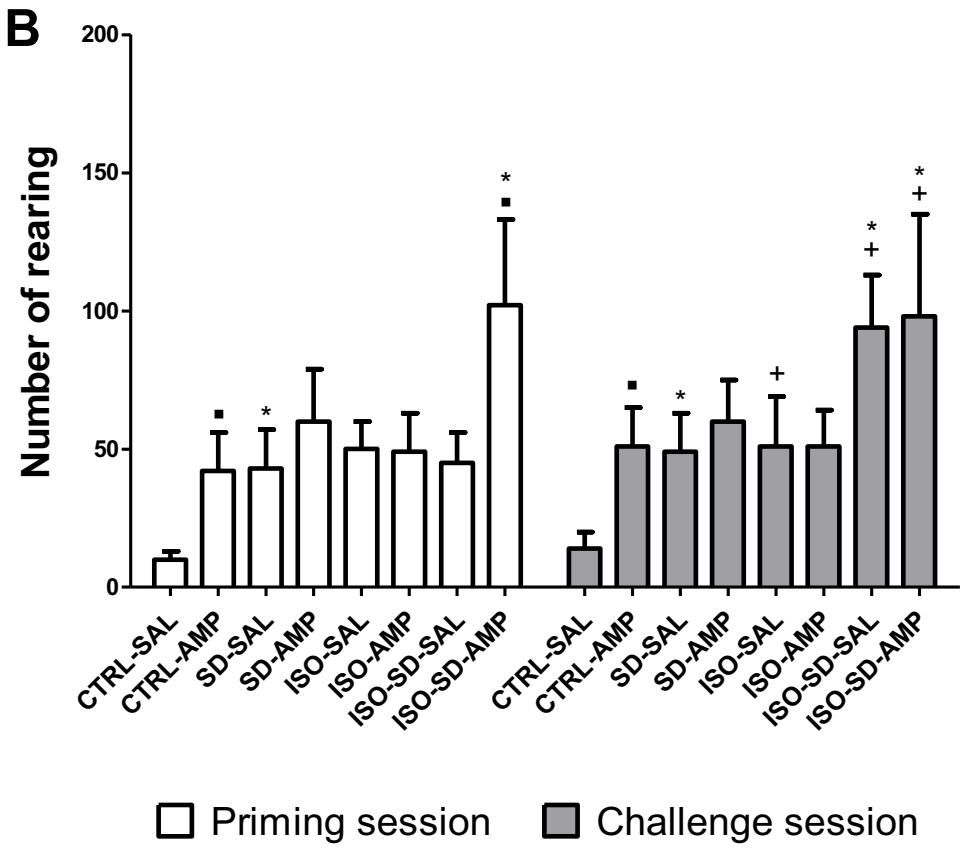

B
Number of rearing
200
150
100
50
0
CTRL-SAL
CTRL-AMP
SD-SAL
SD-AMP
ISO-SAL
ISO-AMP
ISO-SD-SAL
ISO-SD-AMP
Priming session
Challenge session

Figure 6: Effects of sleep deprivation on amphetamine-induced hyperlocomotion in social-isolated mice. CTRL- : control condition; SD- : only sleep deprived group; ISO- : only social-isolated group; ISO-SD- : social-isolated and sleep deprived group; -SAL: groups that received saline in the priming session; -AMP: groups that received amphetamine in the priming session. Results are presented as the mean ± S.E. (MANOVA, Duncan's test and paired samples t-test) of number of crossings through quadrants (**A**) and rearing frequency (**B**) in the priming and challenged sessions. $^{+}p<0.05$ compared to groups with the same sleep and treatment conditions, but different social condition; $^{*}p<0.05$ compared to groups of the same social and treatment conditions, but different sleep condition; $^{\#}p<0.05$ compared to groups with the same treatment but different social and sleep condition; $^{\blacksquare}$compared to groups with the same social and sleep conditions, but different treatment; $^{\blacklozenge}p<0.05$ compared to groups with the same sleep condition but different treatment and social condition; $^{\circ}p<0.05$ compared to SAL and SD-SAL groups; $^{\Delta}p<0.05$ compared to ISO-SAL group; $^{\square}p<0.05$ compared with the same group in the priming session.

DISCUSSION

In the present study, we evaluated the behavioral effects of sleep deprivation on the schizophrenic-like behavior induced by social isolation in mice. Together, sleep deprivation and social isolation promoted impaired memory of a non-aversive task but not of an aversive one. In addition, we have found that a short period of total sleep deprivation (3 h) potentiated the effects of social isolation on stereotyped and aggressive behaviors. Moreover, this sleep condition also potentiated the stimulant effect of amphetamine in mice subjected to social isolation since weaning.

In experiment I, the PM-DAT was employed to evaluate cognition. Notably, this behavioral model has also been shown to be effective in concomitantly evaluating cognition, anxiety-related behaviors and exploratory activity (SILVA and FRUSSA-FILHO, 2000; CARVALHO et al., 2006; SILVA et al., 1997; PATTI et al., 2006; ALVARENGA et al., 2008). Thus, the social isolation protocol employed here seemed to improve learning of the task, which is assessed by the magnitude of the avoidance of the aversive enclosed arm in the training session (PATTI et al., 2010). However, this does not seem to be the case in this study, since in the test session social-isolated animals displayed similar exploration of the arm that had been aversive during training compared to the control group. Within this context, the decreased time spent in the aversive enclosed arm during training could be a higher reactivity to the aversive stimuli of social-isolated mice.

When memory was tested in a non-aversive context (experiment II), the amnestic effects of sleep deprivation and social isolation were verified. In this

way, only the group of animals isolated and sleep-deprived did not discriminate the familiar and the new object. This task is based on the natural tendency of rodents to prefer new stimuli over familiar ones. Thus, if memory is preserved, the animal will spend more time exploring the novel object, thereby signaling recognition memory (WINTERS et al., 2008; ENNACEUR, 2010; BLASER and HEYSER, 2015). Of note, the ORT assesses declarative memory in rodents (DERE et al., 2007). Impairment in declarative memory is among the largest and most robust cognitive deficits in patients with schizophrenia (STONE and HSI, 2011; GODER et al., 2015).

Regarding the test session in experiment I, our group has already demonstrated that total sleep deprivation by gentle handling for 6 h before training impaired performance in the testing and that this impairment is not state-dependent (PATTI et al., 2010). Here, all of the groups retrieved the task in the test session in experiment I. There was no significant difference in the percent of time in the aversive enclosed arm, suggesting the same magnitude of retention. These results suggest that sleep deprivation for 3 h (unlike 6 h) was not sufficient to induce memory deficits. Social isolation was not able to impair the retention of the aversive discriminative task. Similarly, no effects were found in the exploration of the open arm in both behavioral sessions of the PM-DAT.

There are qualitative environmental differences between the PM-DAT and the ORT in the open field arena. While the open-field arena can be a neutral environment, the PM-DAT contains open arms naturally avoided by rodents and one aversive enclosed arm, which can be actively avoided, making this apparatus more anxiogenic than the open-field. Thus, the aversive context in

the PM-DAT may have boosted the higher reactivity of isolated animals, leading to less exploration in aversive enclosed arm. In this regard, studies have shown that social isolation promotes increased novelty reactivity in rodents (LAPIZ et al., 2003; FONE and PORKESS, 2008; FABRICIUS et al., 2010; LOMANOWSKA et al., 2010).

Curiously, social-isolated mice had a hypolocomotion during the training session in the PM-DAT. The discrepancy between the PM-DAT and the open field findings could be due to the experimental context again. Along these lines, highly illuminated (i.e., more aversive) open field has been shown to decrease spontaneous (BOUWKNECHT et al., 2007) or drug-induced (FUKUSHIRO et al., 2010) locomotor activity. Notably, the total number of entries in the PM-DAT has been shown to be as sensitive as the open field model in evaluating hypolocomotion induced by different drugs (CARVALHO et al., 2003; ARAUJO et al., 2009; GULICK and GOULD, 2009). In addition, we have previously demonstrated that hyperlocomotion induced by non-pharmacological methods such as continuous exposure to light (ABILIO et al., 1999) or paradoxical sleep deprivation for 72h (FRUSSA-FILHO et al., 2004) can be accurately detected by the total number of entries in the PM-DAT.

Although total sleep deprivation for 3h alone did not modify locomotion either in the PM-DAT training session or in the open field, it increased this parameter in social isolated mice in experiment I and V. It is recognized that both social isolation (LAPIZ et al., 2003; FONE and PORKESS, 2008; HALL, 1998) and sleep deprivation (NUNES JUNIOR et al., 1994; TUFIK et al., 1978; TUFIK, 1981a,b) can modulate dopaminergic transmission, leading to dopaminergic supersensitivity. Indeed, hyperlocomotion in rodents has been

extensively related to increased dopaminergic neurotransmission in the mesoaccumbens system (DELFS et al., 1990; PIJNENBURG et al., 1975). It could be proposed that social isolation and total sleep deprivation induced an increase in dopaminergic transmission that each condition alone was not sufficient to promote hyperlocomotion. However, when these conditions occurred simultaneously, a synergic effect was observed.

In agreement, both social isolation and sleep deprivation potentiated apomorphine-induced stereotyped behavior (experiment V). Furthermore, this potentiation had a greater magnitude when these conditions were combined. Within this context, stereotypy is an important feature of schizophrenia and other psychiatric disorders and motor stereotypies can be experimentally induced by dopaminergic stimulation. It has already been shown that social isolation is able to potentiate the apomorphine-induced stereotyped behavior in mice (BORÇOI et al., 2015) and rats (SAHAKIAN et al., 1975). Still, amphetamine can also promote higher stereotypy index in sleep-deprived rats compared to control animals (TRONCONE et al., 1988).

The present study sought to evaluate the socialization pattern of social isolated mice subjected to sleep deprivation. Due to the ability of social isolation to induce social withdrawn and aggressive behavior, social interaction measures in rodents subjected to isolation have been used to study depression and negative symptoms of schizophrenia (HALL, 1998; LINCK et al., 2008; POWELL and MIYAKAWA, 2006). In our study, both social isolation and sleep deprivation independently induced aggressive behaviors as demonstrated by a decrease in friendly contacts and an increase in the number of attacks (in social-isolated mice) and persecutions (in sleep-deprived mice). Studies have

reported an increase in social interaction, regardless of the type of interaction, in social-isolated animals. Importantly, this effect is due to an increase in aggressive behavior instead of an increase in friendly socialization (WONGWITDECHA and MARSDEN, 1996; HALL, 1998; FONE and PORKESS, 2008).

In experiment V, we analyzed hyperlocomotion induced by amphetamine administration in the open field arena. This evaluation is one of the most frequently reported endophenotypes of schizophrenia. This behavior is the result of an increased activity of the mesolimbic dopaminergic pathway and is associated with the positive symptoms of the disease (HALL, 1998; SILVA, 2006; JONES, 2011). Herein, the habituation sessions showed that social-isolated animals have a high spontaneous locomotion. This increased locomotion could be the result of a greater reactivity to novelty induced by prolonged social isolation (HALL, 1998; HEIDBREDER et al., 2000; LAPIZ et al., 2003; FONE and PORKESS, 2008; FABRICIUS et al., 2010; LOMANOWSKA et al., 2010). During the 1st habituation session, the exposure to an unfamiliar environment was the stimulus for this hyperlocomotion, which seems to be related to an increased dopaminergic transmission in the mesolimbic system and could be reversed by dopaminergic antagonists (FABRICIUS et al., 2010; JONES, 2011). This spontaneous hyperlocomotion did not prevent habituation to the open-field apparatus, since isolated mice displayed decreased locomotion from the 1st to 3rd day.

In the priming session, social isolation potentiated the acute stimulant effect of amphetamine. Curiously, this effect was not observed in the sleep-deprived mice previously subjected to social isolation. A possible explanation

for this finding could be the increased rearing frequency. In line with experiment IV, it could be argued that sleep deprivation before the priming session potentiated the stereotyped behavior increasing rearing frequency. The simultaneous decrease in locomotor frequency may be interpreted as a consequence of the behavioral competition phenomenon, masking the potentiating effect of sleep deprivation on motor activity.

In the challenge session, amphetamine increased locomotion in relation to the 1st treatment day in mice that were not submitted to sleep deprivation, irrespective of social condition. When repeatedly administered, psychostimulants induce behavioral sensitization. This phenomenon is defined by a progressive increase in drug-induced behavioral responses following repeated administration of the same dose of the drug in rodents (FUKUSHIRO and FRUSSA-FILHO, 2011; SAITO et al., 2014). In the same way, a single injection of amphetamine has been reported to enhance locomotor stimulation produced by a subsequent injection of the drug given hours, days or weeks later (CHINEN et al., 2006; FRUSSA-FILHO et al., 2004; ALVAREZ et al., 2006; CALZAVARA et al., 2008).

Although our group has already demonstrated that paradoxical sleep deprivation potentiates amphetamine-induced behavioral sensitization (FRUSSA-FILHO et al., 2004), we did not observe this effect in the present study. This discrepancy could be due to methodological issues, i.e., different regimes of sleep deprivation (48 h of paradoxical sleep deprivation x 3 h of total sleep deprivation) and mice strains (C57BL/6 x Swiss). Conversely, sleep deprivation potentiated the acute stimulant effect of amphetamine in mice subjected to social isolation, suggesting an enduring effect. In agreement,

Kameda and colleagues (2014) have shown that the acute stimulant effect of amphetamine was potentiated by sleep deprivation when it occurred 7 days before the administration of the drug in young mice. Collectively, these findings suggest that sleep deprivation, social isolation and amphetamine-induced behavioral sensitization may share similar mesolimbic dopaminergic alterations.

Taken together, we showed that sleep deprivation interacted with the schizophrenic-like behaviors in mice submitted to social isolation, potentiating some behaviors associated with schizophrenia. Specifically, a short period of total sleep deprivation potentiated the effects of social isolation on stereotyped and aggressive behaviors, as well as in the response to the acute stimulant effect of amphetamine. Still, sleep deprivation and social isolation together lead to a memory impairment in a non-aversive task. Thus, sleep deprivation can constitute a relevant tool in the investigation of animal models of schizophrenia, since sleep disturbances are intrinsically found in this disorder.

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Manuscrito 2

BEHAVIORAL EVALUATION OF SOCIAL ISOLATION AND SLEEP DEPRIVATION INTERACTION IN MICE

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ABSTRACT

Social isolation in rodents has been used as a potential animal model for study of schizophrenia. This model promotes neurochemical, neuroanatomical and behavioral changes in animals that can be associated with the disease. In parallel, sleep and circadian disturbances have high prevalence and can be a prelude to the appearance of the others clinical manifestations in schizophrenic patients. Despite the widespread knowledge about sleep and circadian disorders in schizophrenia, the mechanisms underlying these symptoms remain overlooked. Therefore, we hypothesized that sleep deprivation could potentiate schizophrenic-like behaviors displayed by social-isolated mice. Newly weaned male mice were subjected to social isolation for 60 days and then submitted to a 24-h session of sleep deprivation by multiple platform method. Mice were tested for cognition, social interaction, apomorphine-induced stereotypy, and amphetamine-induced hyperlocomotion. The results showed that this protocol of sleep deprivation could potentiate the effects of social isolation on social behavior and the acute stimulant effect of amphetamine. The study of interaction between sleep and schizophrenia in animal models can be an interesting approach for further investigations on this disease.

Keywords: Schizophrenia, social isolation, sleep, sleep deprivation, mice.

INTRODUCTION

Social isolation in rodents has been used as a potential animal model for study of schizophrenia. Contact deprivation from weaning may impair the brain development in rodents leading to critical alterations (LAPIZ et al., 2003; WEISS et al., 2004; JONES et al., 2011). This model promotes neurochemical, neuroanatomical and behavioral changes that can be associated with changes in schizophrenia. Such neurochemical modifications include, for example, decreased dopaminergic transmission in the mesocortical pathway and in regions of pre-frontal cortex, and increased dopaminergic transmission in the mesolimbic pathways (HALL, 1998; FONE and PORKESS, 2008). From a neuroanatomical perspective, social isolation induces a reduction in hippocampal complex and prefrontal cortex structures (HEIDBREDER et al., 2000; HALL, 1998). Behavioral studies have demonstrated deficit in sensorimotor gating, antisocial behavior, stereotypy and cognitive deficits. Still, some behavioral changes appear to be reversed by the administration of neuroleptics, but not by the socialization (VALZELLI, 1973; FONE and PORKESS, 2008; MARSDEN et al., 2011).

All those behavioral manifestations in animals are associated to some schizophrenia symptoms in humans. The disease is characterized by positive (e.g. delusions and hallucinations) and negative symptoms (e.g. blunting of affect and depressive behaviors), cognitive deficits and sleep and circadian rhythm disruptions (KAPLAN, 2008; INSEL, 2010; AFONSO, 2011; EHRLICH et al., 2012). However, few studies have considered the role of sleep in schizophrenia in comparison with others symptoms. Sleep-onset and

maintenance insomnia is a common symptom in schizophrenic patients regardless of either their medication status (drug-naive or previously treated) or the phase of the clinical course (acute or chronic) (AFONSO, 2011). Regarding sleep architecture, the majority of studies indicate that non-rapid eye movement (NREM), N3 sleep and REM sleep onset latency are reduced in schizophrenia. Still, patients can present generalized circadian dysfunctions (MONTI and MONTI, 2004; BENSON, 2006; AFONSO et al., 2011).

Such disturbances could be partly related to a presumed hyperactivity of the dopaminergic system and dysfunction of the GABAergic system, both associated with core features of schizophrenia (MONTI et al., 2013). Some of those symptoms, like insomnia, can appear as prodromal symptoms. Furthermore, several studies suggest that the sleep deprivation can precipitate a psychotic episodic in schizophrenic patients (PRITCHETT et al., 2012). A possible explanation for this sleep deprivation property is its capacity to promote an exacerbation of dopaminergic transmission, by supersensitivity of dopamine receptors (TUFIK et al., 1978; TUFIK, 1981; LIMA et al., 2008). Indeed, the hyperactivity of dopaminergic neurons is strongly related to the physiopathology of schizophrenia (ROSS et al., 2006; SILVA et al., 2006; INSEL et al., 2010). In this context, considering the role of insomnia as a prelude to the appearance of clinical manifestations, the evaluation of the interactions between sleep and schizophrenia became of great importance. Thus, the present study sought to investigate whether sleep deprivation could precipitate or potentiate the known behaviors induced by the social isolation, an animal model of schizophrenia.

MATERIAL AND METHODS

Subjects

Twenty-one-day-old Swiss male mice were maintained at 22º C in 12:12 h light-dark cycle (lights on at 07:00am). Food and water were available *ad libitum*. Animals were maintained in accordance with the Brazilian Law for Procedures for Animal Scientific Use (#11794/2008) and experimental procedures were approved by the Institutional Animal Care and Use Committee under the protocol #330300.

Social isolation (ISO)

For the social isolation procedure, animals were maintained alone for 60 days from the 21st day of life or housed in groups of 6 per cage (control group). The isolation was carried out in cages that prevented any visual or social interactions, allowing only auditory and olfactory contacts with mice from neighboring cages (BORÇOI et al., 2015).

Sleep deprivation (SD)

Animals were placed in water tanks (41 cm x 34 cm x 16.5 cm) containing platforms (3 cm in diameter) in a proportion of 2 platforms per animal, surrounded by water up to 1 cm beneath the surface. In this method, the animals are capable of moving inside the tank, jumping from one platform to the other. Home cage control animals were maintained in their cages in the same room. The animals were sleep deprived for 24h starting at 1:00pm (SUCHECKI and TUFIK, 2000).

Drugs

D-amphetamine sulfate (2.5 mg/kg body weight) (Sigma® Chemical Co.) was diluted in saline 0.9%, which was also the control solution, administered intraperitoneally. Apomorphine hydrochloride (1.5 mg/kg body weight) (Sigma® Chemical Co.) was diluted in 0.2% ascorbic acid, which was also the vehicle solution, administered subcutaneously. All solutions were administered in the volume of 10 ml/kg body weight.

Plus-maze discriminative avoidance task (PM-DAT)

The PM-DAT was used to concomitantly evaluate learning, memory, anxiety-like behavior, and motor activity, as described previously (SILVA and FRUSSA-FILHO, 2000; FERNANDES et al., 2015). The aversive stimuli consisted of a 100-watt light and an air blow produced by a 110-V hairdryer positioned over the aversive enclosed arm. These stimuli were present during the 10-min training session. Test session lasted 3 min, performed in the absence of the aversive stimuli. In all sessions, the apparatus was cleaned with a 5% alcohol solution before each animal observation. Total number of entries, percent time spent in the aversive enclosed arm (time spent in aversive enclosed arm/ time spent in both enclosed arms), and percent time spent in open arms (time spent in open arms/time spent in both open and enclosed arms) were calculated. Learning and memory were evaluated by the percent time spent in the aversive enclosed arm during training and testing, respectively. Anxiety-like behavior and motor activity were assessed by the

percent time spent in the open arms and the total number of entries in all the arms of the apparatus, respectively.

Object recognition task (ORT)

Object recognition task was conducted in the open field. Firstly, there was a habituation session in the open field without objects. In the next day, animals were subjected to the session I. In this session, mice were placed in the arena containing two similar objects (object A and object A') and left to explore them freely for 10 min. The session II occurred 180 minutes later, in order to evaluate short-term memory. In the session II, one of the objects was substituted for a new object (B) and the mouse was introduced in the arena for more 10 min. The positions of the objects (familiar or novel) were randomly permuted for each experimental animal. Exploration was defined as sniffing or touching the object with the nose and/or forepaws. Sitting on or turning around the object was not considered exploratory behavior. Time spent exploring each object and the numbers of crossings through quadrants were recorded by an observer blind to the treatment. The objects and the apparatus were cleaned with a 5% alcohol solution after each behavioral session.

In this task, the natural tendency of rodents to explore new objects in their environment is used to determine whether an animal remembers a previously familiar object. Memory for a familiar object is measured by the relative time spent exploring this object compared to the time spent exploring a new object. If an animal remembers a previously familiar object, this object should be explored less than the new object. Conversely, a previously familiar object that is not remembered will be explored just as much as a new objet (ENNACEUR, 2010).

Social interaction

The assessment of social behavior was adapted from Linck and colleagues (2008). The social interaction was evaluated in an opaque polypropylene cage (25 x 20 x 20 cm) with no top. The day before testing, mice were habituated, when each mouse remained alone in the test box for 10 min. In the test day, mice were allocated in pairs composed of an unknown animal matched for body weight with the experimental animal and placed in the test box. The time spent interacting (sniffing, grooming, following, mounting and attacking the partner and nonspecific friendly contacts) were quantified. Passive contact (sitting or lying with bodies in contact) was not quantified. Only contacts that came from the experimental animal were considered.

Stereotyped behavior

The animals were observed for stereotyped behavior in wire mesh cages (16 x 30 x 18 cm) free of water and food. Stereotyped behavior was quantified every 5 min for 15 s per animal in a 100-min session. The scoring system proposed by Setler and colleagues (1976) with modifications validated in our laboratory for mice (CARVALHO et al, 2006) was applied. Scores from 0 to 4 were attributed to each animal as follows: 0 asleep or stationary; 1, active; 2, active with predominantly stereotyped sniffing and rearing; 3, stereotyped sniffing with bursts of licking and/or gnawing and biting; 4, continual licking and/or gnawing of cage grids.

Open field test

The amphetamine-induced hyperlocomotion was evaluated in the open-field arena. It consists of an opaque cylindrical white polyethylene arena (50 cm height) and wooden base (40 cm diameter) divided into 19 squares. In each session, the animal was observed for 10 min and the locomotion (number of entries in quadrants with all 4 paws) and the number of rearing (amount of lift on 2 paws supported or not on the device wall) were quantified (CHINEN et al., 2006).

Experimental design

Experiment I – Effects of SD on the PM-DAT in social-isolated mice

Mice were deprived from sleep at 1:00 pm in the 59th day of social isolation. Twenty-four hour later, mice were trained in PM-DAT forming the following groups: CTRL – home cage controls (N=12), SD – only sleep-deprived (N=12), ISO – only isolated (N=11) and ISO-SD – isolated and sleep-deprived (N=12). Test session was performed 7 days after training.

Experiment II – Effects of SD on ORT in social-isolated mice

In the 59th day of social isolation, animals were habituated to open field apparatus and immediately after, the sleep deprivation procedure started likewise in experiment I. The same groups were formed: CTRL (N=11), SD (N=11), ISO (N=12) and ISO-SD (N=12). In the next day, immediately after SD, animals were submitted to the session I, in which they ware exposure to two

similar objects in the open field. Three hours later, they were submitted to the session II, in which they were exposed to one of the familiar objects and one new object in the open field.

Experiment III – Effects of SD on social behavior in social-isolated mice

On the 59th day of social isolation, animals were habituated and immediately after, the sleep deprivation procedure started. The same groups were formed: CTRL (N=12), SD (N=12), ISO (N=12) and ISO-SD (N=11). In the next day, each animal was placed in the test box with an unfamiliar mouse for 10 min for the social interaction evaluation.

Experiment IV – Effects of SD on stereotyped behavior in social-isolated mice

In the 59th day of social isolation, the sleep deprivation procedure started. In the next day, immediately after the end of SD period, all animals received an apomorphine injection, forming the same groups: CTRL (N=12), SD (N=12), ISO (N=12) and ISO-SD (N=12). Five min later, their stereotyped behavior was evaluated.

Experiment V – Effects of SD on amphetamine-induced hyperlocomotion in social-isolated mice.

From the 57th to 59th day of social isolation, animals were habituated to the open-field arena after a saline injection. Immediately after the 3rd habituation session, the sleep deprivation procedure started. In the next day, immediately after the end of sleep deprivation period, animals received a saline (SAL) or 2.5 mg/Kg amphetamine (AMP) injection, forming the groups: SAL (N=12) – only

treated with SAL; AMP (N=12) – only treated with AMP; SD-SAL (N=12) – sleep-deprived treated with SAL; SD-AMP (N=12) – sleep-deprived treated with AMP; ISO-SAL (N=12) – isolated treated with SAL; ISO-AMP (N=12) – isolated treated with AMP; ISO-SD-SAL (N=12) – isolated and sleep-deprived treated with SAL; and ISO-SD-AMP (N=12) – isolated and sleep-deprived treated with AMP. Fifteen min later, mice were submitted to the priming session in the open field arena. Seven days later, mice were submitted to a challenge session, in which all animals received an amphetamine injection 15 min before the behavior evaluation.

Statistical analysis

Data were compared by MANOVA and Duncan's test when necessary. When applicable, independent or paired samples T-test were used. A probability of $p<0.05$ was considered to show significant differences.

RESULTS

Experiment I – Effects of SD on the PM-DAT in social-isolated mice

Both in the training and in the test sessions, MANOVA revealed that there were no differences among groups in the percent of time spent in the aversive arm, in the open arms and the total number of entries (Table 1).

Experiment II – Effects of SD on the ORT in social-isolated mice

In the session I, MANOVA followed by Duncan's test revealed significant effect of social condition (not isolated vs. isolated) [$F(1,42)=14.46$; $p<0.001$] on locomotor activity. Both ISO and ISO-SD groups showed an increased locomotion compered to CTRL and SD groups, respectively (Figure 1A). Regarding interaction with objects in the same session, paired samples t-test showed that there was no significant difference in the time spent exploring the objects among groups (Figure 1B). In the test session, MANOVA followed by Duncan's test revealed significant effect of social isolation [$F(1,42)=13.02$; $p=0.001$] on locomotor activity. Thus, ISO and ISO-SD groups showed an increase in locomotion compered to CTRL and SD groups, respectively (Figure 1C). Concerning the interaction with the objects in the test session, paired samples t-test showed that only CTRL [$t(1,10)=2.24$; $p=0.049$] and ISO [$t(1,11)=3.09$; $p=0.010$] groups explored significantly more the object B than the object A (Figure 1D).

Experiment III – Effects of SD on social behavior in social-isolated mice

Analyzing the amount of friendly contacts, MANOVA followed by Duncan's test revealed significant effects of sleep condition (not sleep-deprived vs. sleep-deprived) [$F(1,43)=5.53$; $p<0.039$] and social condition [$F(1,43)=4.82$; $p<0.034$]. Thus, SD and ISO groups presented a decrease in the number of friendly contacts compared to CTRL group (Figure 2A). In relation to the number of attacks, MANOVA followed by Duncan's test revealed significant effects of sleep [$F(1,43)=5.10$; $p<0.029$] and social [$F(1,43)=31.81$; $p<0.001$] conditions and sleep condition x social condition interaction [$F(1,43)=5.85$; $p<0.020$]. The effect of sleep deprivation was shown by an increase in the number of attacks by ISO-SD group compared to ISO group. The effect of social isolation was shown by an increase in the number of attacks by ISO and ISO-SD groups compared to CTRL and SD groups, respectively. Finally, the effect of sleep condition x social condition interaction was shown by the increase in the same parameter by ISO and ISO-SD groups compared to SD and CTRL groups, respectively (Figure 2B).

Experiment IV – Effects of SD on stereotyped behavior in social-isolated mice

In the stereotyped behavior, MANOVA followed by Duncan's test showed significant effect of social condition [$F(1,44)=6.44$; $p=0.015$]. Thus, the ISO and ISO-SD groups showed an increase in stereotypy score when compared to CTRL and SD groups, respectively (Figure 3).

Experiment V – Effects of SD on amphetamine-induced hyperlocomotion in social-isolated mice

In the habituation sessions, the independent samples t-test showed that isolated animals had an increase in locomotion frequency compared to control animals in both sessions (1st habituation session, [t(1,94)=5.80; p<0.001] and 3rd habituation session, [t(1,94)=3.68; p<0.001]). Moreover, the paired samples t-test showed that both groups had a decrease in locomotion in the 3rd exposure to the apparatus compared to the to the 1st one (CTRL group, [t(1,47)=8.66; p<0.001] and ISO group, [t(1,47)=8.26; p<0.001]) (Figure 4A). In the priming session, MANOVA followed by Duncan's test showed significant effects of treatment (SAL *vs.* AMP) [F(1,88)=118.92; p<0.001] and social condition [F(1,88)=14.96; p<0.001]. All animals that received amphetamine showed an increase in locomotion compared to their respective control treated with saline. In addition, all social isolated groups presented an increased locomotor activity compared to their respective non-isolated groups (Figure 4B). In the challenge session, MANOVA followed by Duncan's test showed significant effects of treatment [F(1,88)=75.86; p<0.001] and social condition [F(1,88)=8.22; p=0.005]. Again, all amphetamine-treated groups showed an increase in locomotion compared to their respective saline control groups (Figure 4B). Finally, paired samples t-test showed that all groups had an increase in locomotion in the challenge session compared to the priming session ([t(1,11)=4.65; p=0.001] – SAL; [t(1,11)=3.42; p=0.006] – AMP; [t(1,11)=2.85; p=0.016] – SD-SAL; [t(1,11)=2.30; p=0.042] – SD-AMP; [t(1,11)=3.11; p=0.010] – ISO-SAL; [t(1,11)=3.10; p=0.010] – ISO-SD-SAL (Figure 4B).

Performance of mice in the PM-DAT in experiment I.

	Training			
	CTRL	SD	ISO	ISO-SD
PTAv	4.6 ± 0.8	3.6 ± 0.9	4.3 ± 0.9	4.9 ± 1.0
PTO	12.9 ± 3.6	7.8 ± 2.9	16.9 ± 3.9	15.8 ± 5.0
NE	26.2 ± 2.6	20.9 ± 2.7	28.0 ± 4.3	27.8 ± 4.1
	Test			
	CTRL	SD	ISO	ISO-SD
PTAv	16.1 ± 6.1	19.1 ± 8.2	10.3 ± 3.0	28.1 ± 10.1
PTOA	6.2 ± 2.8	3.4 ± 1.6	15.6 ± 8.6	3.9 ± 1.5
NE	4.4 ± 0.9	4.5 ± 0.9	9.9 ± 1.4	4.7 ± 0.6

Table 1: Effects of sleep deprivation on PM-DAT in social-isolated mice. CTRL: control condition; SD: only sleep-deprived group; ISO: only social-isolated group; ISO-SD: social-isolated and sleep-deprived group. Results are presented as the mean ± SE of percent time spent in the aversive enclosed arm (PTAv), percent time spent in the open arms (PTO) and total number of entries (NE) during the training and test sessions. There was no difference among groups in any parameter analyzed in both sessions (MANOVA and Duncan's test).

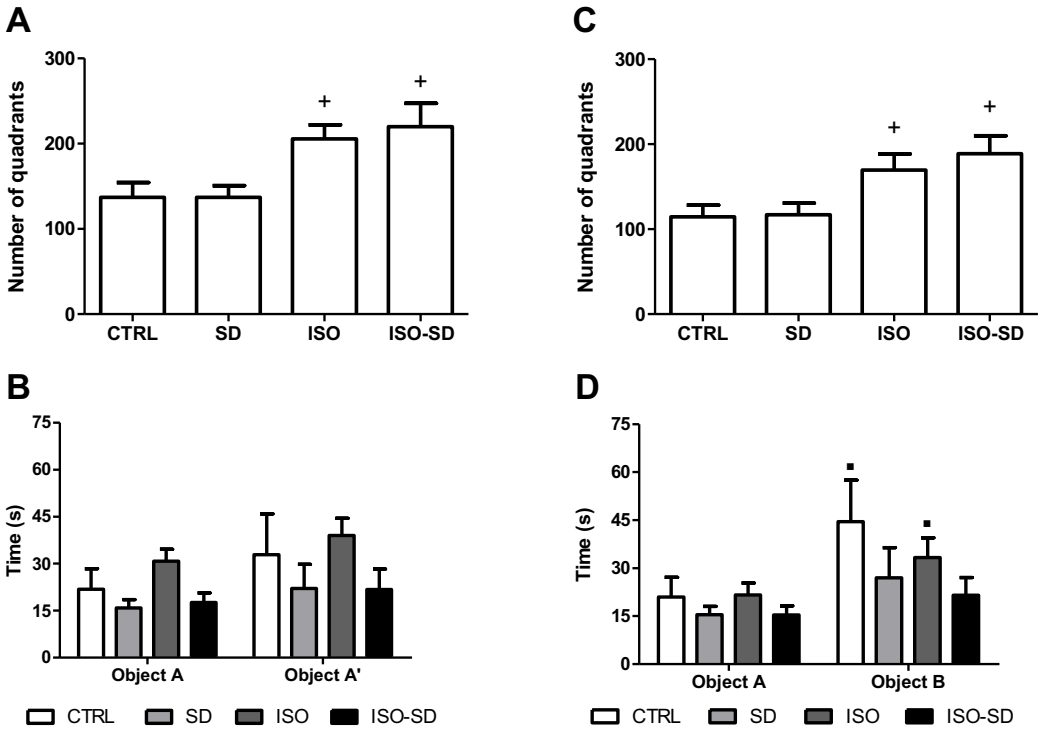

Figure 1: Effects of sleep deprivation on ORT in social-isolated mice. CTRL: control condition; SD: only sleep-deprived group; ISO: only social-isolated group; ISO-SD: social-isolated and sleep-deprived group. Results are presented as the mean ± SE (MANOVA and Duncan's test). Locomotor activity and time spent interacting with the objects in the training (A and B, respectively) and testing (C and D, respectively) sessions; $^{+}p<0.005$ compared to group of different social condition; $^{\bullet}p<0.005$ compared to time exploring the familiar object.

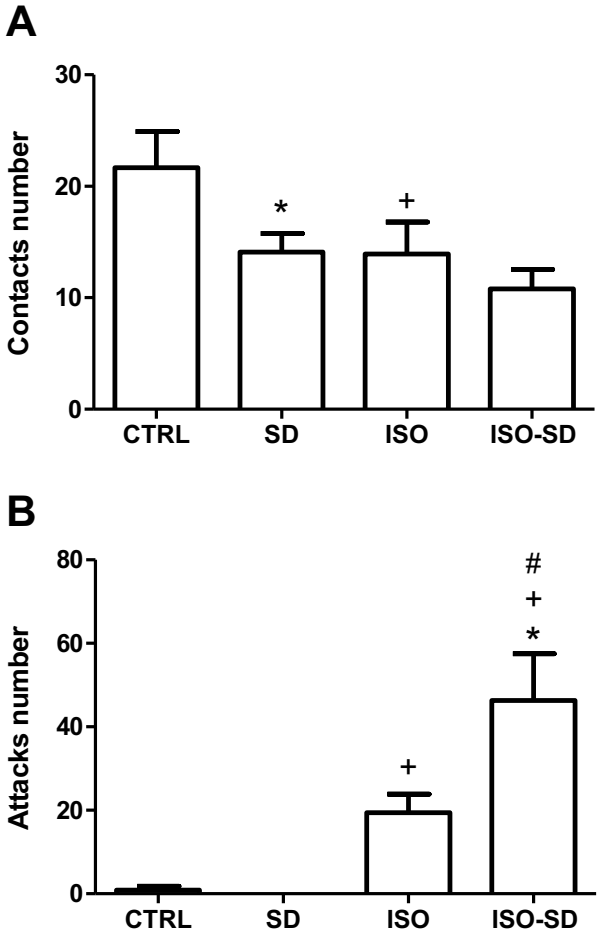

Figure 2: Effects of sleep deprivation on social behavior in social-isolated mice. CTRL: control condition; SD: only sleep deprived group; ISO: only social-isolated group; ISO-SD: social-isolated and sleep deprived group. Results are presented as the mean ± SE (MANOVA and Duncan's test). Friendly contacts (**A**) and attacks (**B**). *$p<0.005$ compared to group of different sleep condition; $^{+}p<0.005$ compared to group of different social condition; $^{\#}p<0.005$ compared to group of different social and sleep conditions.

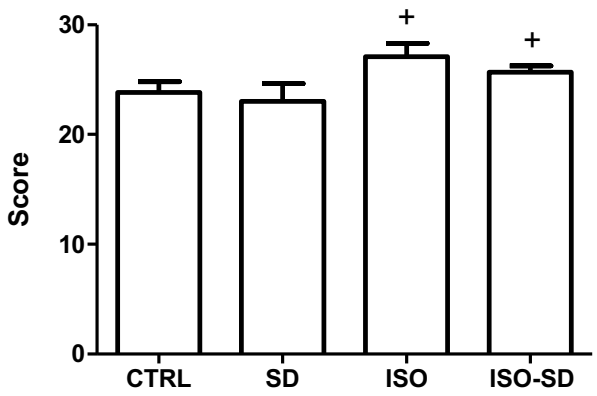

Figure 3: Effects of sleep deprivation on stereotyped behavior in social-isolated mice. CTRL: control condition; SD: only sleep deprived group; ISO: only social-isolated group; ISO-SD: social-isolated and sleep deprived group. Results are presented as the mean ± SE (MANOVA and Duncan's test). $^{+}p<0.005$ compared to group of different social condition.

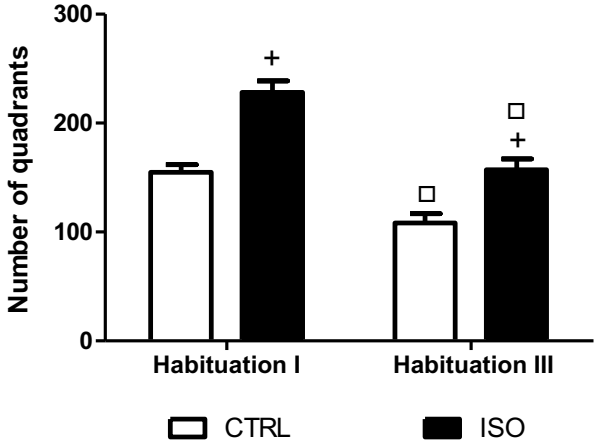

Figure 4: Habituation sessions of experiment IV (before the sleep deprivation protocol). CTRL: control condition; ISO: only social-isolated group. Results are presented as the mean ± SE (MANOVA and Duncan's test). $^{+}p<0.005$ compared to group of different social condition. $^{\square}p<0.005$ compared to the same group in the first habituation session.

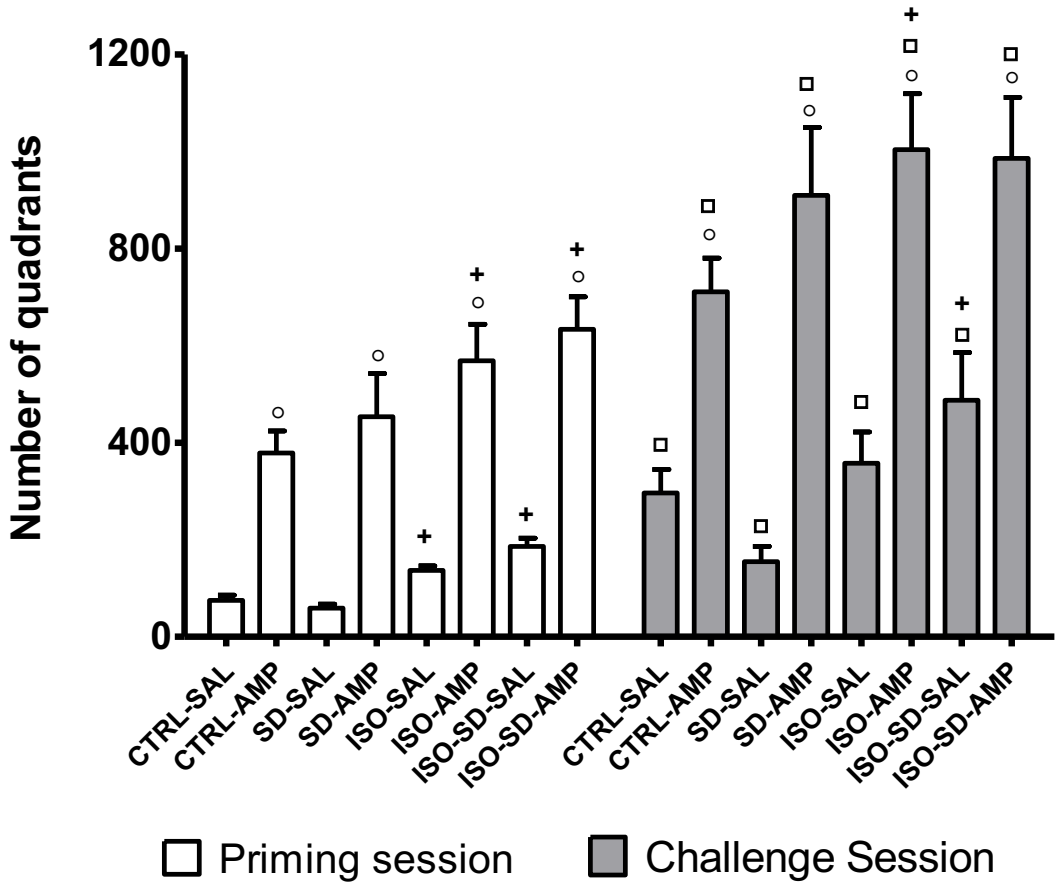

Figure 5: Effects of sleep deprivation on amphetamine-induced hyperlocomotion in social-isolated mice. CTRL-: control condition; SD-: only sleep deprived group; ISO-: only social-isolated group; ISO-SD-: social-isolated and sleep deprived group; -SAL: groups that received saline in the priming session; -AMP: groups that received amphetamine in the priming session. Results are presented as the mean ± SE (MANOVA and Duncan's test). ºp<0.005 compared to groups of different treatment. $^{+}$p<0.005 compared to group of different social condition. $^{\square}$p<0.005 compared to the same group in the priming session.

DISCUSSION

Previous evidence demonstrated that social isolation can lead to alterations in the dopaminergic neurotransmission, making this manipulation a useful animal model of schizophrenia. In this sense, the present study was tailored to evaluate the behavioral effects of sleep deprivation on the schizophrenic-like behavior induced by social isolation in mice.

In experiment I, we used the PM-DAT to evaluate cognition in mice. This model is able to measure learning, memory, anxiety and locomotor activity (SILVA and FRUSSA-FILHO, 2000; CARVALHO et al., 2006). In the present study, we failed to detect any behavioral alteration displayed by mice submitted to social isolation and/or sleep deprivation. In previous studies we have verified amnestic effects induced by 72 h of sleep deprivation achieved by multiple platforms. Such memory impairments were associated with state-dependent learning (PATTI et al., 2010). In addition, unpublished data of our group showed that total sleep deprivation by gentle handling for 3 h enhanced locomotor activity in isolated animals evaluated in the PM-DAT. Herein, paradoxical sleep deprivation for 24 h did not modify memory in an aversive task.

In experiment II, which evaluated memory in a non-aversive task (ORT), the same protocol of sleep deprivation impaired performance. Thus, in the 1st session, as expected, there was no difference in exploration of the objects. In the 2nd session (3 h later) sleep-deprived animals irrespective of social condition, showed impairment in short-term memory. Previous studies of our group have shown that sleep deprivation by gentle handling for 3 h alone was not able to impair object recognition short-term memory. On the hand, when this

protocol was associated with social isolation, it induced amnestic effects (LOPES-SILVA et al., unpublished data). This discrepancy could be due to different sleep deprivation methods.

Regarding the social behavior, social isolation induced a decrease in friendly contacts and increased aggressive behavior. Indeed, the antisocial behavior is one of the most common findings of all sort of social isolation protocols and it is largely used to study negative symptoms of schizophrenia and depression in animal models (HALL, 1998; FONE and PORKESS, 2008; LINCK et al., 2008). Notwithstanding, the paradoxical sleep deprivation method employed in the present study decreased friendly contacts as well as increased attacks. Thus, both social isolation and sleep deprivation were able to increase aggressive behavior and when those conditions were combined, the effect was potentiated. These results can be explained by the aforementioned property of both social isolation and sleep deprivation to induce an increase in dopaminergic transmission. In fact, aggressive behavior is associated with dopaminergic transmission. In this sense, Yu and colleagues (2014) demonstrate that activation of dopaminergic neurons in ventral tegmental area increases aggression. Still, this effect may be dependent on the method and the duration of the sleep deprivation procedure.

In the 4th experiment, we evaluated the stereotyped behavior induced by apomorphine. Our results have shown that the social isolation lead to an increase in the stereotypy, corroborating previously data from our group (BORÇOI et al., 2015). Yet, the sleep deprivation protocol used here was not sufficient to produce modifications. Oppositely, Troncone and coworkers (1988) have shown that 96 h of paradoxical sleep deprivation in rats increased

stereotyped behavior induced by apomorphine. This discrepancy may be due to the duration of sleep deprivation (24 h vs. 96 h) or the species employed (mice vs. rats).

In Experiment V, it was assessed the amphetamine-induced hyperlocomotion in an open field. Accordingly, Frussa-Filho and colleagues (2004) showed that either the repeated administration or even a single injection of amphetamine enhanced the behavioral effects of a subsequent injection of the drug in sleep-deprived animals. This phenomenon, called behavioral sensitization, is usually measured in terms of locomotion or stereotypy and has been widely recognized as an animal model of lasting susceptibility to exacerbation to psychostimulant-induced psychosis. In this way, it is an important model to study the positives symptoms of schizophrenia. In the habituation phase, social-isolated animals presented a greater spontaneous locomotion though they displayed habituation to the open-field arena (decrease in the locomotor activity from the 1st session to the 3rd exposure). This increased basal locomotion in social-isolated mice may be the result of a greater reactivity to novelty induced by prolonged social isolation. This phenomenon is called isolation syndrome (HALL, 1998; FONE and PORKESS, 2008; LAPIZ et al, 2003). In the priming session, the acute hyperlocomotor effect of amphetamine was observed in all of the groups. Importantly, the social isolation potentiated this effect. In this sense, isolated groups had an increased motor activity compared to their respective non-isolated group. This finding supports the ideia that social isolation may cause an increase of the mesolimbic dopaminergic transmission (FABRICIUS et al., 2010) which potentiates amphetamine-induced hyperlocomotor effect.

It is well established that sleep deprivation can increase impulsiveness (BERRO et al., 2014), as well as spontaneous (FRUSSA-FILHO at al., 2004) and drug induced (ARRIAGA et al., 1988; FERGUSON and DEMENT, 1969; FRUSSA-FILHO et al., 2004) motor activity in the open-field test in laboratory animals. In fact, those behaviors are closely related to dopaminergic neurotransmission, which in turn, is widely known to be altered by sleep deprivation (TUFIK et al., 1978; TUFIK, 1981; TRONCONE et al., 1988; FRUSSA-FILHO et al., 2004). We have shown that sleep deprivation by multiple platforms for 24 h (present data) or gentle handling for 3 h (LOPES-SILVA et al., unpublished data), can increase the spontaneous motor activity in the open field arena. On the other hand, paradoxical sleep deprivation for 24 h employed herein did not potentiate the amphetamine-induced hyperlocomotor effect. However, the same sleep deprivation protocol when performed during 48 h potentiates the acute effect of amphetamine (KAMEDA et al., 2014), corroborating our hypothesis that the duration of sleep deprivation is a key-factor.

In the challenge session, all of the groups that received the drug for the 2nd time had an increase in the locomotor activity compared to groups that were receiving the 1st injection. Moreover, all groups in this session presented an increased locomotor activity compared to the same group in the priming session, revealing the behavioral sensitization phenomenon. Of note, the ISO-AMP and ISO-SD-SAL groups showed an increased motor activity compared to their respective non-isolated controls, suggesting that social isolation potentiated the behavioral sensitization induced by amphetamine.

Taken together, our results show that social isolation promotes behavioral effects that can be associated to positive and negative symptoms of schizophrenia, constituting an interesting animal model in the study of this disease. Moreover, the paradoxical sleep deprivation for 24 h can potentiate some of these behaviors (aggression and amphetamine-induced hyperlocomotion). Comparing to previous studies of our group, the method and the duration of sleep deprivation seem to be important issues that may modulate social-isolation-induced behavioral modifications. Thus, further studies are necessary to understand the relationship between sleep and schizophrenia and the approaches employed herein could be extremely useful for such evaluation.

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Discussão

6. DISCUSSÃO

Os transtornos neuropsiquiátricos são altamente prevalentes e debilitantes. Contudo, apesar do impacto negativo desses distúrbios na saúde pública, os progressos na compreensão de suas fisiopatologias e a implementação de novos mecanismos terapêuticos têm ocorrido lentamente. O grande desafio ao estudar essas doenças é a complexidade de seus mecanismos fisiopatológicos e manifestações clínicas. Embora nas últimas décadas houvesse um rápido progresso no desenvolvimento de tecnologias não invasivas para estudar a estrutura e função do cérebro humano, continuam a existir limitações substanciais na capacidade de investigar os detalhes da fisiologia e biologia molecular desse órgão. Dadas essas limitações, o uso de bons modelos animais poderia contribuir para um progresso significativo no estudo da fisiopatologia e terapêutica de diversas doenças psiquiátricas. Nesse contexto, reconhecida a importância do uso dos modelos animais para a investigação dos transtornos psiquiátricos, é imprescindível o desenvolvimento de novos modelos, além do aprimoramento de modelos existentes.

Assim, o presente trabalho buscou implementar um modelo animal de comportamento esquizofrênico já existente, trazendo uma abordagem pouco comum no contexto dos estudos com modelos animais. A observação de que o paciente portador de esquizofrenia apresenta distúrbios de sono e do ritmo circadiano é amplamente conhecida. No entanto, a avaliação das interações entre o sono e a esquizofrenia em modelos animais perde espaço para outros tipos de abordagem.

Já está estabelecido que o isolamento social em roedores pode alterar a neurotransmissão dopaminérgica. Essa modificação seria análoga às alterações observadas na esquizofrenia, tornando essa manipulação um importante modelo animal para a doença. Somado a isso, levamos em conta a existência de alterações no sono e no ritmo circadiano nessa doença e a capacidade da privação do sono em precipitar os sintomas. De fato, muito se relata sobre a ocorrência de episódios de insônia seguidos de surto psicóticos em pacientes. Nesse sentido, o presente estudo procurou avaliar os efeitos comportamentais da privação de sono sobre o comportamento do tipo esquizofrênico induzido pelo isolamento social em camundongos.

Demonstramos que o tipo e a duração da privação de sono são pontos importantes nesse tipo de abordagem. Assim, um curto período de privação de sono total (3 h) potencializou o efeito do isolamento social no que diz respeito aos comportamentos estereotipados e antissociais. Além disso, esse mesmo protocolo de privação de sono potencializou o efeito estimulante de anfetamina nos camundongos submetidos ao isolamento social. Já a privação de sono paradoxal por 24 h também foi capaz de potencializar alguns comportamentos. Dessa forma, esse método privação de sono potencializou os prejuízos cognitivos em uma tarefa não-aversiva (mas não em uma tarefa aversiva), o comportamento antissocial, a atividade locomotora e efeito estimulante da anfetamina. Esses achados sugerem que os protocolos de privação de sono empregados no presente estudo apresentam propriedades diferentes, e consequentemente seus efeitos comportamentais em um mesmo modelo animal de esquizofrenia também podem ser distintos. Enquanto na privação de sono total o animal é impedido de ter qualquer quantidade de sono, na privação

pelo método das plataformas múltiplas eles eram privados especificamente de uma fase do sono (sono paradoxal). Porém, é importante ressaltar que os protocolos de privação apresentam vieses. Isto é, na privação de sono total existe a intrusão de curtos períodos de sono e na privação pelo método das plataformas múltiplas existe também a privação de pequena parcela de sono NREM (COLAVITO et al., 2013). No entanto, os protocolos de privação de sono empregados apresentam características distintas. Por exemplo, do ponto de vista clínico, o sono REM está mais fortemente ligado à memória não-declarativa enquanto que o sono não-REM está associado à memória declarativa. Assim, a privação de sono REM afetaria principalmente as memórias não-declarativas e a privação de sono total teria potencial para afetar ambas (SAXVIG et al, 2008; GAIS e BORN, 2004; HARRISON e HORNE, 2000). Ainda, a privação de sono total, mas não de sono REM, diminui o limiar de dor (ONEN et al., 2001). Com relação à parâmetros imunológicos, a privação de sono total por uma noite produz aumento de células T-CD4 e por duas noites produz aumento de Leucócitos pelo aumento especificamente de Neutrófilos. Já a privação de sono paradoxal por uma noite produz uma diminuição dos níveis de IgA (RUIZ et al., 2012).

Assim, a(s) fase(s) e a duração da privação de sono são determinantes para os efeitos produzidos. Dessa forma, tem-se mais possibilidades de abordagens no estudo da interação entre o sono e a esquizofrenia, ou qualquer outra doença neuropsiquiátrica. Essa riqueza de possibilidades pode ser ainda maior quando se considera a existência de diferentes modelos animais de esquizofrenia. Apesar de todos os modelos buscarem mimetizar uma mesma condição patológica, existem diferenças entre eles no que diz respeito às

manifestações comportamentais, resposta à fármacos e alterações neuroquímicas ou neuroanatômicas. Dessa forma, os modelos animais associados à uma abordagem que leve em consideração o fator sono podem ter muito a contribuir para um melhor entendimento da esquizofrenia e do papel do sono na mesma. Nesse contexto, Pritchett e colaboradores (2012) estabeleceram quatro abordagens chave para um melhor entendimento da interação da doença com o sono em modelos animais, a saber:

1) Distúrbios de sono e do ritmo circadiano podem ser observados em modelos animais de esquizofrenia?

2) Interferências no sono ou no ritmo circadiano podem provocar ou potencializar comportamento tipo esquizofrênico em modelos animais?

3) Tratamentos que visem melhorar os distúrbios de sono e do ritmo circadiano são capazes de promover melhorias também nos comportamentos tipo esquizofrênicos nos modelos animais?

4) Tratamentos que visem melhorar os comportamentos tipo esquizofrênicos nos modelos animais também são capazes de aliviar os distúrbios de sono e do ritmo circadiano?

Cada uma dessas perguntas pode ser explorada do pondo de vista comportamental e neuroquímico, buscando atribuir a cada estudo validação fenomenológica, farmacológica e de constructo. As perguntas são muitas e as possibilidade de estudos também. Muito ainda há para se esclarecer sobre a esquizofrenia e sobre o papel do sono nessa doença.

Conclusões

7. CONCLUSÕES

O protocolo de isolamento social empregado no presente trabalho foi capaz de promover alterações comportamentais nos camundongos subjacentes à esquizofrenia, replicando dados da literatura que apontam o isolamento social como um modelo animal para esta doença.

Foi mostrado no presente trabalho que um curto período de privação de sono total (3 h) potencializou o efeito do isolamento social no que diz respeito aos comportamentos estereotipados e antissociais. Além disso, esse protocolo de privação de sono potencializou o efeito estimulante de anfetamina nos camundongos submetidos ao isolamento social. Ainda, a privação de sono paradoxal por 24 h também foi capaz de potencializar os prejuízos cognitivos em uma tarefa não-aversiva (mas não em uma tarefa aversiva), o comportamento antissocial, a atividade locomotora e o efeito estimulante da anfetamina nos camundongos submetidos ao modelo. Os protocolos de privação de sono empregados no estudo apresentam propriedades diferentes, e consequentemente seus efeitos comportamentais em um mesmo modelo animal de esquizofrenia também podem ser distintos. A duração da privação de sono também é um ponto importante nesse tipo de abordagem.

Diante da importante interação entre o sono e a esquizofrenia, os resultados do presente trabalho permitem concluir que utilização de modelos animais em associação com o fator sono pode ser uma ferramenta muito útil nos estudos dos mecanismos neuroquímicos da esquizofrenia e do papel do sono nessa doença, bem como no desenvolvimento de novas estratégias terapêuticas.

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Anexos

9. ANEXOS

9.1 Anexo 1: Comitê de Ética

São Paulo, 27 de junho de 2013
CEUA N 330300

Ilmo(a). Sr(a).
Pesquisador(a): Leonardo Brito Lopes E Silva
Depto/Disc: Psicobiologia
Roberto Frussa Filho (orientador)

Título do projeto: "INTERAÇÕES ENTRE ESQUIZOFRENIA E PRIVAÇÃO DE SONO: UM ESTUDO PRÉ CLÍNICO".

Parecer Consubstanciado da Comissão de Ética no Uso de Animais UNIFESP/HSP

A esquizofrenia está entre as mais sérias e debilitantes doenças mentais existentes, atingindo geralmente adultos jovens no início de suas vidas independentes e produtivas. Esse transtorno pode apresentar dois tipos de manifestações, chamados de sintomas positivos, no qual aparecem as alucinações e delírios, e sintomas negativos, em que os principais achados são embotamento afetivo e pobreza do discurso, além dos déficits cognitivos. Ainda, o sono é geralmente perturbado em pacientes com esquizofrenia. De fato, a insônia é uma queixa frequentemente relatada pelos pacientes e seus familiares. Dentre as várias hipóteses desenvolvidas para explicar a gênese da esquizofrenia, a da hiperfunção dopaminérgica central é atualmente uma das mais bem investigadas e aceitas. De importância, diversas evidências experimentais demonstram um papel crítico da dopamina na regulação do ciclo vigília-sono. Ainda, a privação de sono é capaz de potencializar diversos comportamentos relacionados à neurotransmissão dopaminérgica, induzidos pela administração de agonistas diretos ou indiretos. Nesse cenário, uma vez que eventos de insônia têm sido frequentemente relatados como um prelúdio do aparecimento dos sinais e sintomas desse transtorno, buscaremos investigar os efeitos da privação de sono sobre as alterações comportamentais induzidas pela exposição pré-natal ao POLI:IC ou ao isolamento social em camundongos, dois modelos animais de esquizofrenia.

A Comissão de Ética no Uso de Animais da Universidade Federal de São Paulo/Hospital São Paulo, na reunião de 27/06/2013, **ANALISOU** e **APROVOU** todos os procedimentos apresentados neste protocolo.
1. Comunicar toda e qualquer alteração do protocolo.
2. Comunicar imediatamente ao Comitê qualquer evento adverso ocorrido durante o desenvolvimento do protocolo.
3. Os dados individuais de todas as etapas da pesquisa devem ser mantidos em local seguro por 5 anos para possível auditoria dos órgãos competentes.
4. **Relatórios parciais** de andamento deverão ser enviados **anualmente** à CEUA até a conclusão do protocolo.

Atenciosamente,

Prof. Dr. José Osmar Medina Pestana
Coordenador da Comissão de Ética no Uso de Animais
Universidade Federal de São Paulo/Hospital São Paulo

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9.2 Anexo 2: Comprovante de submissão - Manuscrito 1

Journal of Psychiatry and Neuroscience

Submission Confirmation

Thank you for submitting your manuscript to *Journal of Psychiatry and Neuroscience*.

Manuscript ID:	JPN-15-0174
Title:	BEHAVIORAL EFFECTS OF SLEEP DEPRIVATION ON MALE MICE SUBMITTED TO SOCIAL ISOLATION: IMPLICATIONS FOR SCHIZOPHRENIA
Authors:	Lopes-Silva, Leonardo Patti, Camilla Zanin, Karina Bizerra, Carolina Wuo-Silva, Raphael Kawamoto, Elisa Santos-Baldaia, Renan Hollais, André Andersen, Monica Frussa-Filho, Roberto Tufik, Sergio
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