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EFEITOS DA TAURINA EM MODELOS PRÉ-CLINICOS DE ESQUIZOFRENIA

Porto Alegre
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Dissertação apresentada ao Programa de Pós-Graduação em Ciências Biológicas: Farmacologia e Terapêutica do Instituto de Ciências Básicas da Saúde da Universidade Federal do Rio Grande do Sul como requisito parcial para a obtenção do título de mestra em Farmacologia e Terapêutica.

Orientadora: Prof.ª Dr.ª Ana Paula Herrmann

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Aprovado em 05 de maio de 2022.

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“What gets us into trouble is not what we don't know. It's what we know for sure that just ain't so.”

Mark Twain

RESUMO

Os avanços significativos conquistados nas últimas décadas no entendimento da neurobiologia de transtornos mentais, como a esquizofrenia, infelizmente não resultaram em tratamentos mais eficazes para os pacientes acometidos por tais condições. Antagonistas de receptores glutamatérgicos do tipo NMDA induzem um estado psicótico em indivíduos saudáveis semelhante à psicose observada na esquizofrenia, além de serem amplamente utilizados para induzir sintomas psicóticos em modelos pré-clínicos da doença. A taurina é um aminoácido com ação neuromoduladora inibitória do sistema nervoso central, neuroprotetora e antioxidante. Nesse trabalho investigamos o potencial da taurina em prevenir os déficits induzidos por administração aguda de MK-801 (dizocilpina), um antagonista NMDA, em ensaios comportamentais relacionados à esquizofrenia em camundongos C57BL/6 e peixes-zebra. Nos experimentos em roedores, os animais foram injetados intraperitonealmente (i.p.) com solução salina ou taurina (50, 100 e 200 mg/kg), e 30 minutos depois receberam outra injeção i.p. de solução salina ou MK-801 (0,15 mg/kg). Os testes comportamentais de inibição por pré-pulso da resposta de sobressalto e interação social foram realizados 30 minutos após a última injeção, enquanto a atividade locomotora foi avaliada continuamente desde a primeira injeção, finalizando 60 minutos após a última. Já no caso de peixes-zebra, os animais foram colocados em um béquer contendo 200 mL de água ou taurina (42, 150 ou 400 mg/L) durante 20 minutos, sendo subsequentemente expostos a água ou MK-801 (5 μM) durante mais 20 minutos. O teste de interação social foi realizado imediatamente após o término da última exposição. Como esperado, a administração de MK-801 causou hiperlocomoção e déficit de inibição por pré-pulso em camundongos, enquanto em peixes-zebra induziu hiperlocomoção e déficit de interação social. Curiosamente, camundongos tratados com MK-801 passaram mais tempo interagindo com os animais estímulo; taurina na dose de 50 mg/kg teve o mesmo efeito, o que pode estar relacionado aos seus efeitos ansiolíticos já documentados. Em nenhuma das espécies foram observados efeitos preventivos da taurina sobre as alterações comportamentais induzidas agudamente por MK-801, contrariando evidências prévias da literatura. Evidências recentes têm fomentado a ideia de que o curso da esquizofrenia pode ser modificado por estratégias de intervenção iniciadas precocemente, antes do primeiro surto psicótico e do estabelecimento do transtorno em sua forma plena. Deste modo, é uma perspectiva desse trabalho avaliar o tratamento precoce e contínuo com taurina em um modelo desenvolvimental de esquizofrenia para melhor elucidar o potencial preventivo dessa molécula.

Palavras-chave: esquizofrenia, taurina, MK-801, C57BL/6, peixes-zebra, comportamento

ABSTRACT

The significant advances made in recent decades in understanding the neurobiology of mental disorders such as schizophrenia have not, unfortunately, resulted in more effective treatments for patients afflicted with such conditions. Glutamate NMDA receptor antagonists induce a psychotic state in healthy individuals similar to the psychosis observed in schizophrenia and are also widely used to induce psychotic symptoms in preclinical models of the disease. Taurine is an amino acid that acts as an inhibitory neuromodulator of the central nervous system with neuroprotective and antioxidant properties. In this work we investigated the potential of taurine to prevent deficits induced by acute administration of MK-801 (dizocilpine), an NMDA antagonist, in behavioral assays with relevance to schizophrenia in C57BL/6 mice and zebrafish. In rodent experiments, animals were injected intraperitoneally (i.p.) with either saline or taurine (50, 100 e 200 mg/kg), and 30 minutes later they received another i.p. injection of saline or MK-801 (0.15 mg/kg). The behavioral tests of prepulse inhibition of the startle response and social interaction were performed 30 minutes after the last injection, while locomotor activity was assessed continuously from the first injection, ending 60 minutes after the last one. For zebrafish experiments, the animals were placed in a beaker containing 200 mL of tank water or taurine (42, 150 or 400 mg/L) for 20 minutes, being subsequently exposed to tank water or MK-801 (5 µM) for another 20 minutes. The social interaction test was performed immediately after the end of the last exposure. As expected, MK-801 administration induced hyperlocomotion and prepulse inhibition deficits in rodents, whereas in zebrafish it induced hyperlocomotion and social interaction deficit. Interestingly, mice treated with MK-801 spent more time interacting with the stimulus animals; taurine at a dose of 50 mg/kg had the same effect, which may be related to its already documented anxiolytic effects. Preventive effects of taurine on behavioral changes acutely induced by MK-801 were not observed in any of the species, contradicting previous evidence in the literature. Recent evidence has fostered the idea that the course of schizophrenia can be modified by intervention strategies initiated early, before the first psychotic break and the establishment of the full-blown disorder. Thus, it is a perspective of this work to evaluate early and continuous treatment with taurine in a developmental model of schizophrenia to better elucidate the preventive potential of this molecule.

Keywords: schizophrenia, taurine, MK-801, C57BL/6, zebrafish, behavior

LISTA DE FIGURAS

LISTA DE ABREVIATURAS

GABA	Ácido gama-aminobutírico
GSH	Glutationa
i.p.	Intraperitoneal
MK-801	Dizocilpina
NAC	N-acetil-cisteína
NMDA	N-Metil-D-aspartato
PCP	Fenciclidina
PPI	Inibição por pré-pulso
SNC	Sistema nervoso central

SUMÁRIO

1 INTRODUÇÃO

1.1 Esquizofrenia

A discussão do que é loucura é um tópico remoto, e gera dúvidas desde a Grécia antiga. Para muitos filósofos, como Descartes, a loucura era símbolo da fragilidade da mente humana, enquanto outros, como Platão e Nietzsche, a viam como uma maneira de escapar as limitações da realidade (Ahonen, 2019). A verdade é que o tema até hoje é alvo de incertezas e curiosidade. Os primeiros relatos relacionados à esquizofrenia foram feitos pelo psiquiatra alemão Emil Kraepelin, ao diagnosticar *"dementia praecox"* em 1897 (Kraepelin, 1897), e, como dito um século depois por Jablensky (1997), *"seria difícil encontrar outras doenças que foram investigadas com o mesmo vigor e persistência ao longo de um século e se provaram ser tão intratáveis e pouco compreendidas quanto a esquizofrenia".*

Afetando 24 milhões de pessoas no mundo (World Health Organization, 2022), a esquizofrenia é um transtorno mental crônico e incapacitante caracterizado por uma ampla gama de sintomas, os quais normalmente aparecem durante a adolescência ou começo da vida adulta (Boison et al., 2012; Marsman et al., 2013). Atualmente, o diagnóstico da doença, de acordo com o Manual Diagnóstico e Estatístico de Transtornos Mentais, inclui delírios, alucinações, discurso desorganizado, déficits cognitivos incapacitantes e prejuízo do funcionamento psicossocial (American Psychiatric Association, 2014).

Os sintomas mais comumente relacionados aos indivíduos com esquizofrenia são os chamados sintomas positivos, também conhecidos como sintomas psicóticos ou psicose, onde o indivíduo pode apresentar alucinações (visuais e/ou auditivas), delírios, discurso desorganizado e agitação. Além dos sintomas positivos, sintomas negativos e cognitivos também estão presentes, sendo fatores importantes para o comprometimento do funcionamento psicossocial do indivíduo (Keefe et al., 2007). Os sintomas negativos abrangem comportamentos relevantes para a inserção social, tais como isolamento social, embotamento emocional, timidez excessiva, anedonia, alogia, e avolição (Tandon et al., 2009; Larson et al., 2010). Os sintomas cognitivos foram os últimos a de fato serem incluídos na tripartite de sintomas da esquizofrenia (Shafer & Dazzi, 2019), embora já fossem observados em estudos desde 1930

(Heaton et al., 1978). Em 1998, uma metanálise demonstrou de maneira inegável a presença de déficits cognitivos, de memória e atenção em pacientes com esquizofrenia, os quais podem variar em grande magnitude (Heinrichs & Zakzanis, 1998; Kremen et al., 2000; MacCabe et al., 2012).

Enquanto em homens os sintomas da esquizofrenia aparecem principalmente no começo da segunda década de vida, a ocorrência em mulheres tem uma variação maior, superando os casos em homens entre os quarenta e cinquenta anos de idade (**Figura 1**; Kirkbride et al., 2012). Uma revisão sistemática recente revelou que, ao contrário do que se acreditava, a suscetibilidade não é igual entre homens e mulheres – a incidência da doença é levemente maior no sexo masculino. Já a prevalência na população se mantém a mesma, em um caso a cada cem indivíduos (McGrath et al., 2008; Jongsma et al., 2019).

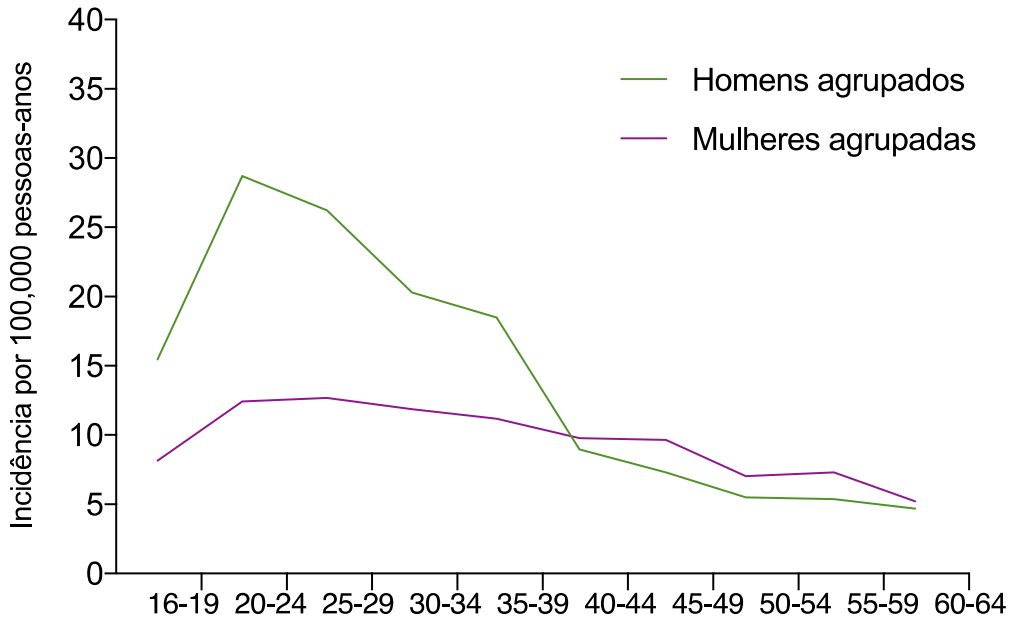

Figura 1. Incidência da esquizofrenia em homens e mulheres na Inglaterra durante o período de 1950-2009. A incidência em homens e mulheres encontra-se agrupada. Adaptado de Kirkbride e colaboradores (2012).

Além de todo estigma enfrentado por indivíduos com esquizofrenia, soma-se uma série de dados preocupantes – alta taxa de desemprego, abuso de substâncias, sedentarismo, menores chances da encontrar um parceiro e tabagismo são apenas alguns dos obstáculos enfrentados por quem é diagnosticado com a condição (Lasser et al., 2000; Marwaha et al., 2007; Dipasquale et al., 2013; Hjorthøj et al., 2015).

Devido a estes e outros fatores, a expectativa de vida de indivíduos com esquizofrenia é, em geral, reduzida entre 13 a 15 anos, tendo como agravante um índice de suicídio em torno de 5% (Hor & Taylor, 2010; Hjorthøj et al., 2017).

Ainda não há protocolos de prevenção bem estabelecidos, fazendo com que a principal estratégia de tratamento para indivíduos com esquizofrenia seja uma intervenção farmacológica precoce e contínua, principalmente com fármacos antipsicóticos, além de tratamentos psicoterápicos (Bruijnzeel et al., 2014). Apesar disso, alguns indivíduos não apresentam melhora significativa ou não se adaptam devido aos diversos efeitos adversos dos fármacos atualmente disponíveis, como efeitos extrapiramidais, alterações metabólicas e endócrinas (Bruijnzeel et al., 2014; Ellenbroek, 2012; Tandon & Halbreich, 2003). Além disso, esses fármacos têm impacto mínimo ou nulo sobre os sintomas negativos e cognitivos da doença, considerados fatores centrais para o déficit funcional da esquizofrenia (Keefe et al., 2007).

Enquanto nos últimos anos é possível perceber uma redução significativa em relação à mortalidade e morbidade de doenças cardiovasculares e câncer, poucos avanços foram feitos em relação aos transtornos mentais (Insel, 2010). Desta maneira, é imprescindível a descoberta de novas abordagens terapêuticas que consigam retardar ou moderar os sintomas de pacientes com esquizofrenia, ou até mesmo evitar o primeiro surto psicótico, especialmente em jovens em risco de transição para esquizofrenia.

1.2 Patofisiologia da esquizofrenia

Aproximadamente 73% dos indivíduos que são diagnosticados com esquizofrenia apresentam sintomas prodrômicos, os quais têm duração, em média, de doze meses (Häfner et al., 1998; Møller & Husby, 2000). Os sintomas prodrômicos apresentam grande heterogeneidade, podendo variar de ansiedade, depressão, irritabilidade, raiva, retração social e até mesmo sintomas de psicose breves, intermitentes e limitados (também conhecidos como BLIPS, do inglês Brief Limited Intermittent Psychotic Symptoms) (Yung & McGorry, 1996; Fusar-Poli et al., 2013, 2017). Embora esses sintomas possam antecipar o aparecimento da esquizofrenia em sua forma plena, acredita-se que alterações ainda durante a gestação e a infância

estejam relacionadas com o desenvolvimento da doença (Brown & Derkits, 2010; Brown, 2011).

Um dos maiores desafios da esquizofrenia é que, apesar da descoberta de novas evidências, o mecanismo da patofisiologia central da doença permanece um mistério, bem como seu diagnóstico neuropatológico e a ausência de biomarcadores (Liu et al., 2021). Devido à falta de sinais neurodegenerativos, acredita-se que processos neurodesenvolvimentais anormais aconteçam muitos anos antes do início dos sintomas, como resultado da susceptibilidade genética do indivíduo e da interação do mesmo com fatores externos. Abuso de substâncias, migração, infecções virais durante a gestação e complicações perinatais são fatores de risco comumente associados a indivíduos que desenvolvem a forma plena da esquizofrenia (Brown, 2011). Acredita-se que essa complexa interação entre genética e ambiente é a responsável pelas supostas disfunções nos sistemas dopaminérgico, glutamatérgico e GABAérgico que emergem no início da vida adulta (Howes & Kapur, 2009; Rapoport et al., 2012; Grace & Gomes, 2019).

O sistema dopaminérgico é o último sistema monoaminérgico a ser formado no cérebro durante o processo de ontogenia, o que sugere que o mesmo apresente funções importantes na estabilização e integração dos circuitos cerebrais (Lauder & Bloom, 1974). Embora o aumento de atividade dopaminérgica esteja intimamente relacionado à esquizofrenia, ainda não há evidências substanciais de que esse transtorno se origine devido a uma disfunção patológica dentro do próprio sistema dopaminérgico (Grace, 2016). Na última década, a hipótese de que o sistema dopaminérgico é afetado devido a disfunções de estruturas aferentes que regulam seu funcionamento tem ganhado força, principalmente devido à introdução do modelo pré-clínico de lesão neonatal no hipocampo ventral (Murray, 2002). O modelo de lesão hipocampal surgiu durante a observação de um menor volume hipocampal em um gêmeo monozigótico com esquizofrenia (Weinberger et al., 1992). Posteriormente, o modelo pré-clínico confirmou o aparecimento de um estado hiperdopaminérgico em animais adultos que sofreram lesão no hipocampo ventral (equivalente ao hipocampo anterior em humanos) durante o período pós-natal (Lipska & Weinberger, 2000). Atualmente, estudos *post mortem* e estudos em modelos animais elucidaram a diminuição dos interneurônios GABAérgicos *fast-spiking* positivos para parvalbumina no hipocampo, corroborando a hipótese de que uma disfunção hipocampal está envolvida com o aparecimento da esquizofrenia (Lewis et al., 2005; Benes et al., 2007;

Lodge et al., 2009; Gill & Grace, 2014). A perda dos interneurônicos GABAérgicos de parvalbumina no subículo ventral do hipocampo levaria a uma hiperativação e disritmicidade dos neurônios piramidais, resultando também na hiperativação da área tegmentar ventral e estriado associativo, hiperativação da amígdala basolateral e perturbação da atividade e ritmicidade do córtex pré-frontal, associados ao aparecimento dos sintomas positivos, negativos e cognitivos, respectivamente (**Figura 2**; Grace, 2016).

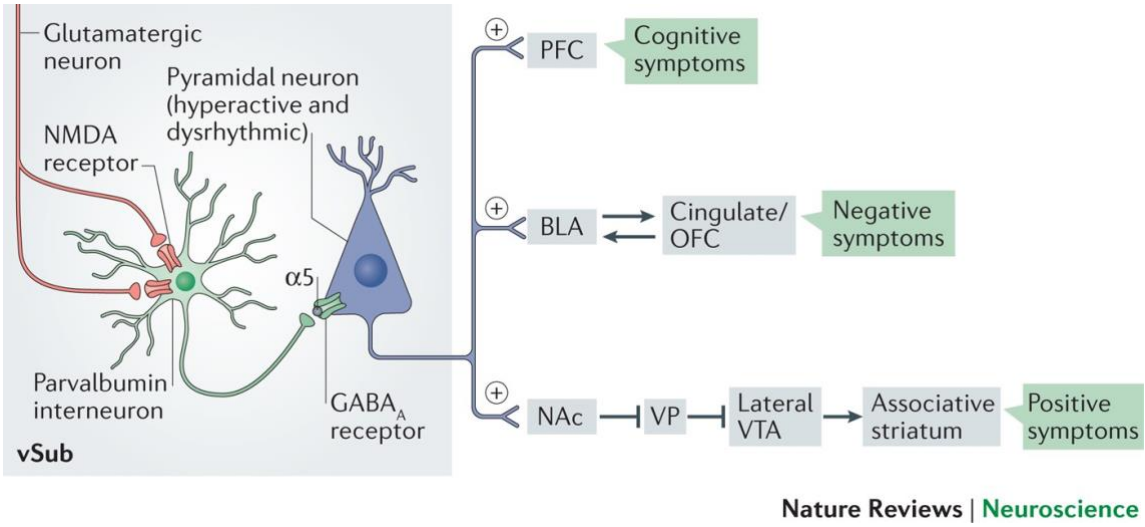

Figura 2. Disfunção do subículo ventral e sintomatologia da esquizofrenia. Os interneurônicos GABAérgicos positivos para parvalbumina presentes no subículo ventral do hipocampo (vSub) são ativados via receptores NMDA, e inibem os neurônios piramidais via receptor $GABA_A$ contendo a subunidade α5. Na esquizofrenia, acredita-se que a perda desses interneurônios inibitórios leve a uma desinibição do neurônio piramidal, o qual tem projeções para o cortéx pré-frontal (PFC), amígdala basolateral (BLA) e núcleo accumbens (NAc). Uma maior ativação do núcleo accumbens leva a uma inibição do pálido ventral (VP) e aumenta a responsividade de neurônios dopaminérgicos na área tegmentar ventral (VTA), a qual se projeta para o estriado associativo. Este é o mecanismo proposto para o aparecimento dos sintomas positivos. Além disso, a perda de neurônios piramidais pode levar a disfunções e perda de ritmo no córtex pré-frontal, o que explica o aparecimento dos sintomas cognitivos. Já os sintomas negativos seriam derivados da interferência gerada entre a conexão da amígdala basolateral com a área cortical límbica, responsável pelo controle e responsividade emocional. Fonte: Grace (2016) (reprodução autorizada – Anexo B).

1.3 Taurina

A taurina, ácido 2-aminoetanossulfônico, é o segundo aminoácido endógeno mais abundante no sistema nervoso central (Huxtable, 1992). Pode ser sintetizada a partir do aminoácido cisteína no fígado (Kimura et al., 2009). É o aminoácido livre mais abundante em humanos, encontrado principalmente no coração e fígado, bem como

no sistema nervoso central, incluindo tronco encefálico e hipocampo (Vohra & Hui, 2000; Ito et al., 2009), onde atua na osmorregulação, estabilização de membrana, neuromodulação e regulação dos níveis intracelulares de cálcio (Junyent et al., 2009, 2011; Marcinkiewicz & Kontny, 2014). Ainda, como neurotransmissor e neuromodulador inibitório do sistema nervoso central (Oja & Saransaari, 1996a), a taurina também pode atenuar a apoptose e funcionar como agente neuroprotetor, antioxidante e imunomodulador (Almarghini et al., 1991a; Redmond et al., 1998a), possuindo também potente capacidade neuroprotetora em casos de neurotoxicidade induzida por glutamato (Wu et al., 2009).

O papel da taurina tem sido testado em diversas patologias, incluindo depressão, falência cardíaca, degeneração de retina e problemas de crescimento (Lourenço & Camilo, 2002). No sistema nervoso central, sabe-se que a taurina atua como agonista de receptores GABAérgicos e glicinérgicos, entretanto ainda não há um consenso se seus efeitos fisiológicos são mediados exclusivamente por esses receptores ou se receptores taurina-específicos estão envolvidos (Oja & Saransaari, 2015). Foi relatado por Ikeda (1977), ainda, o potencial da taurina na melhora de estados psicóticos no contexto da abstinência ao álcool, como delírios, alucinações, prejuízo cognitivo ou crises epilépticas.

O fármaco análogo da taurina, acamprosato, vem sendo proposto como uma alternativa para indivíduos com esquizofrenia por possuir um perfil farmacológico mais favorável em estágios precoces de doença quando comparado a risperidona e olanzapina (Paz et al., 2008). Além disso, a N-acetilcisteína (NAC), um precursor de glutationa (GSH), é tido como alternativa para redução do estresse oxidativo em pacientes com esquizofrenia (Bošković et al., 2011; Reddy & Reddy, 2011). Em um ensaio clínico randomizado duplo-cego, a administração de NAC foi capaz de melhorar moderadamente quadros de esquizofrenia crônica (Berk et al., 2008). Como a taurina também depende da cisteína para sua biossíntese, é possível que seus níveis fisiológicos também possam ter sido modificados pela NAC (Schuller-Levis & Park, 2003).

No espectro clínico, aumento dos níveis de taurina foram observados no cortéx pré-frontal de indivíduos com esquizofrenia, além de uma correlação ter sido vista entre o seu aumento e a duração da doença (Shirayama et al., 2010). Em contraste, a concentração da taurina foi observada diminuída no fluido cerebroespinal de pacientes com esquizofrenia e doença de Alzheimer que nunca haviam sido

medicados (Do et al., 1995; Engelborghs et al., 2003). Ainda, em modelos animais de esquizofrenia por infecção pré-natal também foi observada uma diminuição dos níveis de taurina no hipocampo fetal, corroborando para a relação causal entre infecção/inflamação durante a gestação e aumento do risco de transtornos psicóticos em adultos (Winter et al., 2009a; Yang et al., 2019). Por fim, um recente ensaio clínico duplo-cego, realizado com 86 pacientes com esquizofrenia em primeiro episódio de psicose, mostrou uma melhora significativa nos sintomas de psicopatologia naqueles que receberam taurina como tratamento adjuvante em comparação ao grupo placebo (O'Donnell et al., 2016). A taurina se mostra como um composto promissor e com grande potencial para o tratamento da esquizofrenia, tanto como agente preventivo como tratamento adjuvante; entretanto, ainda são necessários estudos clínicos e estudos em modelos animais para que seu potencial terapêutico possa ser elucidado com mais clareza no espectro da esquizofrenia.

1.4 Modelos animais de esquizofrenia

Modelos animais são ferramentas de grande valia para a investigação das bases patológicas e possíveis abordagens terapêuticas de doenças humanas. A criação de modelos animais adequados para doenças neuropsiquiátricas complexas, como a esquizofrenia, é especialmente desafiadora. No caso da esquizofrenia, a etiologia da doença e os mecanismos patológicos por trás dos sintomas ainda não são bem compreendidos (Liu et al., 2021). Além disso, muitos dos sintomas da doença são de difícil reprodução em roedores por se tratar de experiências de percepção, até então (Schmack et al., 2021), unicamente humanas.

Em roedores, modelos farmacológicos, genéticos ou neurodesenvolvimentais são utilizados para estudar diferentes aspectos da esquizofrenia, justamente por apresentam diferentes vantagens quanto a validade de face e/ou construto (Winship et al., 2019). Os modelos farmacológicos são os mais tradicionalmente empregados, normalmente utilizando substâncias que aumentam a liberação de dopamina, como a anfetamina, ou utilizando antagonistas não-competitivos do receptor NMDA, como a fenciclidina (PCP) e o MK-801 (Winship et al., 2019). Nestes modelos, é possível observar comportamentos relevantes para a esquizofrenia, tal como a inibição por pré-pulso da resposta de sobressalto (PPI), onde o déficit sensório-motor observado

clinicamente também está presente em roedores (Nestler & Hyman, 2010). O aumento da atividade locomotora, que mimetiza os sintomas positivos da esquizofrenia, bem como a diminuição da sociabilidade, relacionada ao embotamento emocional comumente observado em indivíduos que apresentam os sintomas negativos da doença, também são observados (Jones et al., 2011).

A falta de diversidade de modelos animais pré-clínicos, entretanto, pode ter retardado a produção de descobertas que se traduzem em abordagens terapêuticas de fato bem-sucedidas para distúrbios neurodegenerativos e neuropsiquiátricos (Nestler & Hyman, 2010; Yartsev, 2017). Estima-se que 75% das pesquisas de neurociência comportamental sejam feitas em ratos, camundongos e humanos (Manger et al., 2008). Embora o uso de roedores ainda seja popular, organismos alternativos como o peixe-zebra têm ganhado notoriedade (Stewart et al., 2014). Apesar das diferenças entre humanos e peixes-zebra, os receptores, tipos celulares e arquitetura neuronal que compõem o sistema nervoso central são altamente conservados entre espécies (Wolman et al., 2011; Baraban et al., 2013). A utilização de mais de um organismo modelo em estudos pré-clínicos é, portanto, uma alternativa necessária, pois a mesma aumenta a validade externa dos estudos, evitando achados espécie-específicos e potencialmente aumentando a reprodutibilidade (Würbel, 2000; Bruni et al., 2016; Burrows & Hannan, 2016; Yartsev, 2017).

O uso de peixes-zebra especificamente como um modelo animal para doenças neuropsiquiátricas vem sendo discutido (Khan et al., 2017; Langova et al., 2020). Atualmente, a espécie tem sido utilizada para avaliar os efeitos de fatores genéticos e ambientais sobre a neurobiologia da esquizofrenia, principalmente focando no desenvolvimento de novas abordagens terapêuticas (Langova et al., 2020). Devido à sua transparência e tamanho pequeno, larvas de peixes-zebra se mostram como ferramentas úteis para manipulação e visualização da atividade neural e a triagem de novos alvos moleculares terapêuticos, bem como de genes candidatos (Brennan, 2011; Stewart et al., 2015). Por fim, peixes-zebra já são modelos utilizados para avaliar a toxicidade de psicotrópicos, (Akande et al., 2010; Kanungo et al., 2013), o que pode os tornar uma alternativa interessante para aumentar o valor preditivo de estudos pré-clínicos.

Poucos estudos experimentais bem conduzidos com peixes-zebra e esquizofrenia foram publicados até o momento, mas os efeitos farmacológicos de antagonistas NMDA já foram relatados e, em alguns aspectos, são similares aos

observados em roedores (Benvenutti et al., 2021), além de serem bloqueados e prevenidos por antipsicóticos (Seibt et al., 2010, 2011). Devido ao grande potencial do modelo, novos estudos comportamentais focados em entender o papel dessa espécie na descoberta de novas evidências que auxiliem no entendimento da doença são necessários.

1.5 Modelos animais de esquizofrenia induzidos por antagonistas glutamatérgicos

Diversas hipóteses relacionam a patofisologia da esquizofrenia com receptores glutamatérgicos do tipo N-Metil-D-Aspartato (NMDA) (Olney & Farber, 1995; Goff & Coyle, 2001; Coyle & Tsai, 2004). Estas hipóteses ganharam força devido aos efeitos observados em indivíduos saudáveis após a administração do antagonista não-competitivo do receptor NMDA fenciclidina, o qual induziu psicose similar à observada em indivíduos com esquizofrenia (Luisada & Brown, 1976a; Allen & Young, 1978a). Posteriormente, outros antagonistas não competitivos do receptor NMDA, como a cetamina e MK-801, mostraram ter efeitos complexos similares aos sintomas positivos e negativos, bem como os déficits cognitivos da doença (Adler et al., 1999; Bondi et al., 2012; Buffalo et al., 1994; Newcomer & Krystal, 2001).

O MK-801, um antagonista não-competitivo dos receptores glutamatérgicos NMDA, foi utilizado pela primeira vez em 1999 como modelo farmacológico de psicose em ratos (Andiné et al., 1999), e ainda hoje é amplamente utilizado como modelo animal de esquizofrenia (Bondi et al., 2012a; Rung et al., 2005; Svoboda et al., 2015). Apesar de não mimetizar o curso da doença, a administração aguda de MK-801 consegue induzir correlatos comportamentais relevantes à esquizofrenia, tais como distúrbios motores, déficit de inibição por pré-pulso e alterações no comportamento social de roedores, além de diminuir a plasticidade sináptica hipocampal por até quatro semanas após uma única administração, o que pode explicar os déficits de memória e cognição também observados no modelo (Bardgett et al., 2003; Goff & Coyle, 2001; Howes et al., 2015; Manahan-Vaughan et al., 2008; Rung et al., 2005). Assim, a administração de MK-801 para induzir comportamentos tipo-esquizofrenia em modelos animais apresenta validade de face relevante, uma vez que modelar os

sintomas do transtorno, ao invés de sua totalidade, pode aumentar a especificidade, utilidade e validade do mesmo (Fernando & Robbins, 2011).

2 OBJETIVOS

2.1 Objetivo Geral

O objetivo desse estudo foi avaliar os efeitos da taurina em modelos agudos de esquizofrenia induzidos por MK-801 em camundongos e peixes-zebra adultos.

2.1.1 Objetivos Específicos

a) Testar os efeitos da taurina (50, 100 e 200 mg/kg) na hiperlocomoção induzida por MK-801 em camundongos;
b) Testar os efeitos da taurina (50, 100 e 200 mg/kg) no déficit de inibição por pré-pulso da resposta de sobressalto induzido por MK-801 em camundongos;
c) Testar os efeitos da taurina (50, 100 e 200 mg/kg) na alteração do comportamento social induzida por MK-801 em camundongos;
d) Testar os efeitos da taurina (42, 150 e 400 mg/L) no déficit de interação social e hiperlocomoção induzidos por MK-801 em peixes-zebra.

3 ARTIGO CIENTÍFICO

Effects of taurine in mice and zebrafish behavioral assays with translational relevance to schizophrenia

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Effects of taurine in mice and zebrafish behavioral assays with translational relevance to schizophrenia

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Keywords: schizophrenia, taurine, MK-801, C57BL/6, zebrafish, behavior

Abstract

Background: Altered redox state and developmental abnormalities in glutamatergic and GABAergic transmission during development are linked to the behavioral changes associated with schizophrenia. As an amino acid that exerts antioxidant and inhibitory actions in the brain, taurine is a potential candidate to modulate biological targets relevant to this disorder. Here, we investigated in mice and zebrafish assays whether taurine prevents the behavioral changes induced by acute administration of MK-801 (dizocilpine), a glutamate NMDA receptor antagonist.

Methods: C57BL/6 mice were intraperitoneally administered with saline or taurine (50, 100 and 200 mg/kg) followed by MK-801 (0.15 mg/kg). Locomotor activity, social interaction and prepulse inhibition of the acoustic startle reflex were then assessed in different sets of animals. Zebrafish were exposed to tank water or taurine (42, 150 and 400 mg/L) followed by MK-801 (5 μM); social interaction and locomotor activity were evaluated in the same test.

Results: MK-801 induced hyperlocomotion and disrupted sensorimotor gating in mice; in zebrafish, it reduced sociability while increased locomotion. Taurine was mostly devoid of effects and did not counteract NMDA antagonism in mice or zebrafish.

Discussion: Contradicting previous clinical and preclinical data, taurine did not show antipsychotic-like effects in the present study. However, it still warrants consideration as a preventive intervention in animal models of relevance to the prodromal phase of schizophrenia; further studies are thus necessary to evaluate whether and how taurine might benefit patients.

INTRODUCTION

Schizophrenia is a serious mental illness that remains as one of the main challenges in modern psychiatry. With an incidence slightly higher in men than women (Jongsma et al., 2019), schizophrenia affects approximately one in a hundred people (McGrath et al., 2008) and dramatically changes the individual's life course. Psychotic symptoms, social isolation, cognitive impairment, and stigma are only a few of the obstacles that contribute to the poor prognosis of this condition. Hyperdopaminergic activity in subcortical areas is associated with the onset of psychotic symptoms (McCutcheon et al., 2018), and might be linked to the loss of fast-spiking parvalbumin-positive GABAergic interneurons, hypofunction of glutamate NMDA receptors and oxidative stress (Cabungcal et al., 2013; Grace, 2016; Hardingham and Do, 2016). Furthermore, several studies have indicated increased frequency of smoking (Lasser et al., 2000), alcohol or illegal substances misuse (Hjorthøj et al., 2015), sedentary lifestyle (Stubbs et al., 2016) and poor dietary habits (Dipasquale et al., 2013) among individuals with schizophrenia, which results in a 13-15 years reduction in life expectancy, currently averaged at 65 years (Hjorthøj et al., 2017).

Early and continuous administration of antipsychotic drugs is the main pharmacological strategy; unfortunately, however, the currently available drugs do not provide a full recovery and a third of the patients are unresponsive to treatment (Jääskeläinen et al., 2013; Bruijnzeel et al., 2014). Antipsychotic drugs, which act by blocking dopamine D_2 receptors, ameliorate mainly the positive symptoms (e.g., hallucinations and delusions), with little to no effect on negative symptoms (e.g., social isolation, depression, avolition) and cognitive impairment (e.g., poor long-term memory, sustained attention and cognitive performance) (Shafer and Dazzi, 2019). Pharmacological interventions pose yet another challenge: side effects such as extrapyramidal symptoms, metabolic syndrome and weight gain further impact quality of life and compromise adherence to treatment (Ellenbroek, 2012; Bruijnzeel et al., 2014).

Since the observation that phencyclidine induces in healthy individuals a psychotic state that resembles schizophrenia (Luisada and Brown, 1976; Allen and Young, 1978), NMDA antagonists have been used to recapitulate relevant behavioral alterations in animal models (Jones et al., 2011). Other non-competitive NMDA antagonists, such as MK-801 (dizocilpine) and ketamine, also trigger complex effects

similar to the positive, negative and cognitive symptoms experienced by individuals with schizophrenia (Buffalo et al., 1994; Adler et al., 1999; Bondi et al., 2012). Here, we used acute administration of MK-801 in mice and zebrafish to study the antipsychotic-like properties of taurine in behavioral assays with translational relevance to schizophrenia.

Taurine, also known as 2-aminoethanesulfonic acid, is the most abundant free amino acid in the human body (Huxtable, 1992) and acts as an inhibitory neuromodulator in the brain (Oja and Saransaari, 1996); it also has neuroprotective, antioxidant and immunomodulatory properties (Almarghini et al., 1991; Redmond et al., 1998). The effects of taurine in the central nervous system are likely mediated by agonism at GABAergic and glycinergic receptors, yet it is still unknown whether taurine-specific receptors could be involved (Oja and Saransaari, 2015). Several studies have shown altered levels of taurine in the brain and plasma of schizophrenia patients and in animal models. Increased taurine levels were observed in the prefrontal cortex of schizophrenia patients, as well as a correlation between increased taurine and disease duration (Shirayama et al., 2010). A recent study also observed elevated taurine levels in serum samples of first psychotic episode and early stage patients (Parksepp et al., 2020). In contrast, decreased taurine levels were observed in the cerebrospinal fluid of drug-naïve individuals (Do et al., 1995). In a mice model of prenatal immune activation, taurine was decreased in the hippocampus, striatum, temporal and parietal cortex (Winter et al., 2009; Yang et al., 2019). Finally, a recent double-blind randomized trial on 86 individuals with first-episode psychosis showed a significant improvement in schizophrenia symptoms in patients who received taurine as an adjuvant treatment as compared to placebo (O'Donnell et al., 2016), while preclinical zebrafish data further support the potential benefits of taurine in this context (Franscescon et al., 2020, 2021).

Considering the above-cited evidence, this study aimed to test the hypothesis that taurine prevents schizophrenia-relevant behavioral alterations induced by acute administration of MK-801 in mice and zebrafish assays.

MATERIALS AND METHODS

Animals

Mice

C57BL/6 male mice (7 to 14-week-old, 20-30 g) were obtained from an external vendor (Centro de Cardiologia Experimental – Instituto de Cardiologia, RS, Brazil). Upon arrival at Unidade de Experimentação Animal (Hospital de Clínicas de Porto Alegre), animals were housed in groups of 3-5 animals per cage (20 × 30 × 13 cm) for at least two weeks before experiments. Different sets of animals were used for each of the behavioral assays. The animals were maintained under controlled environmental conditions (reversed 12-h light/dark cycle with lights on at 7:00 a.m. and constant temperature of 22 ± 1 °C) with free access to food (Nuvilab CR-1®, PR, Brazil) and water. All procedures were approved by the animal welfare and ethical review committee of Hospital de Clínicas de Porto Alegre (approval #180498) and were performed in accordance with the relevant guidelines on care and use of laboratory animals and the Brazilian legislation.

Zebrafish

Experiments were performed using male and female (50:50 ratio) short-fin wild-type zebrafish (6-month-old, 400-500 mg). Adult animals were obtained from a local commercial supplier (Delphis, RS, Brazil). The animals were maintained at Instituto de Ciências Básicas da Saúde in a light/dark cycle of 14/10 h with lights on at 7:00 a.m for at least two weeks before tests. Fish were kept in 16-L (40 × 20 × 24 cm) unenriched glass tanks with nonchlorinated water at a maximum density of two animals per liter. Tank water satisfied the controlled conditions required for zebrafish (26 ± 2 °C; pH 7.0 ± 0.3; dissolved oxygen at 7.0 ± 0.4 mg/L; total ammonia at <0.01 mg/L; total hardness at 5.8 mg/L; alkalinity at 22 mg/L $CaCO_3$; conductivity of 1,500–1,600 µS/cm) and was constantly filtered by mechanical, biological, and chemical filtration systems (Altamar®, SP, Brazil). Food was provided twice a day as commercial flake food (Poytara®, SP, Brazil) plus brine shrimp (*Artemia salina*). The sex of the animals was confirmed after euthanasia by dissecting and analyzing the gonads. Animals were euthanized by hypothermic shock according to the AVMA Guidelines for the Euthanasia of Animals (Leary and Johnson, 2020). For all experiments, no sex effects were observed, so data were pooled together. All procedures were approved by the animal welfare and ethical

review committee at the Universidade Federal do Rio Grande do Sul (approval #35525).

Drugs

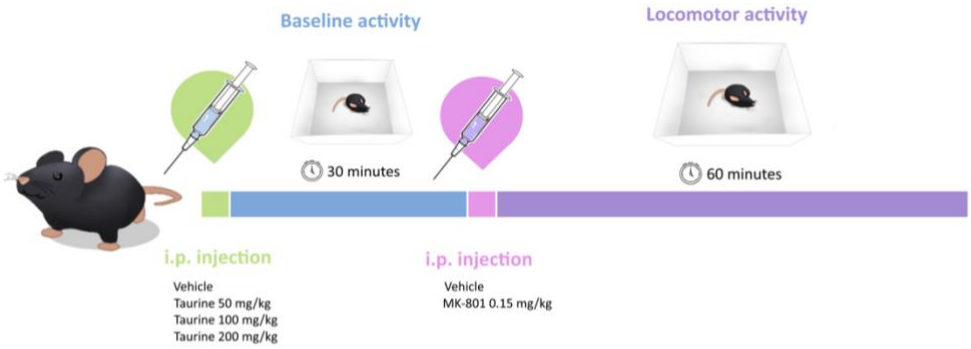
Taurine and MK-801 (dizocilpine) were purchased from Sigma-Aldrich (St. Louis, MO, USA). For rodent experiments, drugs were dissolved in saline (0.9% NaCl) and solutions were freshly prepared and injected intraperitoneally (i.p.) at a volume of 5 mL/kg. Animals were manually contained for drug administration. For the zebrafish assay, MK-801 and taurine were dissolved in tank water; solutions were freshly prepared and renovated halfway through the experiment.

Experimental design

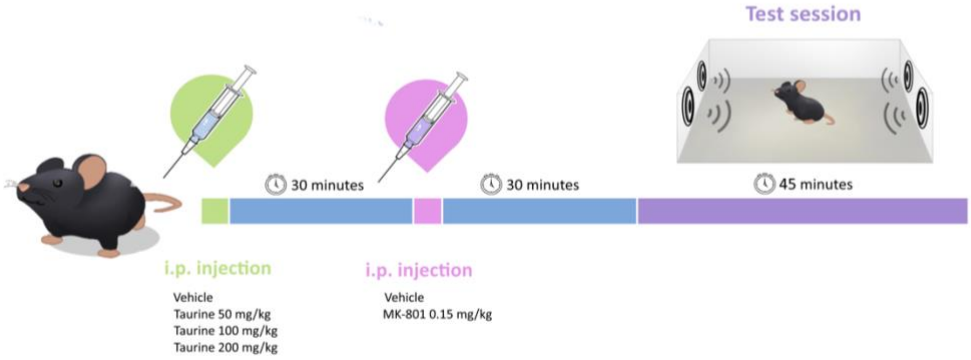
Different sets of animals were used for each experiment, totaling 288 mice and 96 zebrafish in the study. The animals were allocated to the experimental groups following block randomization procedures to counterbalance for litter and cage in mice experiments, and sex and home tank in zebrafish experiments. The order for outcome assessment was also randomized and care was taken to counterbalance the test apparatuses across the experimental groups. Outcome assessors were blind to the experimental groups, as well as the experimenters responsible for taking the animal and placing it in the test apparatus. An overview of the experimental design is illustrated in Figure 1.

We report how we determined our sample size, all data exclusions, all manipulations, and all measures in the study. Raw data and analyses outputs were deposited in the Open Science Framework and are openly available at https://osf.io/qy2uw (Giongo et al., 2022).

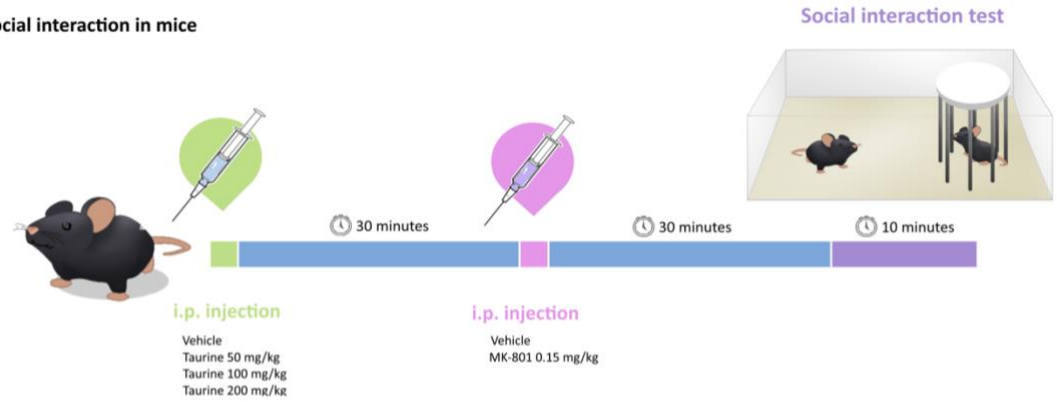
A) Locomotor activity in mice



B) Prepulse inhibition in mice



C) Social interaction in mice



D) Social interaction in zebrafish

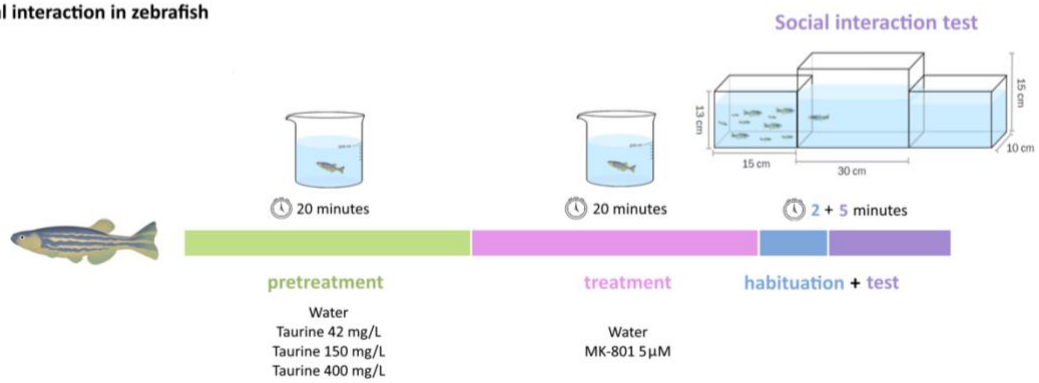


Figure 1. Overview of the experimental design. (A) Locomotor activity in mice, (B) prepulse inhibition of the startle reflex in mice, (C) social interaction in mice, (D) social interaction in zebrafish.

Locomotor activity in the open field in mice

The protocol for assessing the locomotor response to MK-801 was adapted from Meyer et al. (2008). Mice were injected with saline (0.9% NaCl) or taurine (50, 100, or 200 mg/kg, i.p.) and then placed in the center of an open field arena (40 × 40 × 40 cm) for baseline activity measurement during 30 min. Animals were then briefly removed to receive an i.p. injection of MK-801 (0.15 mg/kg); they were returned to the open field and locomotor activity was recorded for 60 min. The distance traveled in segments of 5 min was automatically scored using ANY-Maze software (Stoelting Co., Wood Dale, IL, USA).

Prepulse inhibition of the acoustic startle reflex in mice

Sensorimotor gating was assessed by measuring the prepulse inhibition (PPI) of the acoustic startle reflex, which refers to the attenuation of the reaction to a startling stimulus (pulse) when it is shortly preceded by a weaker stimulus (prepulse). The protocol was adapted based on the methodology fully described elsewhere (Meyer et al., 2005). The apparatus consisted of two sound-attenuated startle chambers (Insight®, SP, Brazil) equipped with a movement-sensitive platform above which an acrylic enclosure was placed. One day prior to the experiment, subjects were habituated for 10 min to the apparatus with background noise (65 dB_A). On the experiment day, animals received an i.p. injection of either saline (0.9% NaCl) or taurine (50, 100, or 200 mg/kg), followed 30 min later by an i.p. injection of saline or MK-801 (0.15 mg/kg). Testing started 30 min after the last drug administration. During a 45-min session, animals were presented to a series of stimuli comprising a mixture of four trial types: pulse-alone, prepulse-plus-pulse, prepulse-alone and no-stimulus (background noise, 65 dB_A). The startle program consisted of three different intensities of a 40-ms white noise pulse (100, 110, and 120 dB_A) combined or not with three different intensities of a 20-ms prepulse (71, 77, and 83 dB_A, which corresponded to 6, 12, and 18 dB above background, respectively). The stimulus-onset asynchrony of the prepulse and pulse stimuli on all prepulse-plus-pulse trials was 100 ms (onset-to-

onset). Each session began with a 2-min acclimation period in the enclosure, followed by 6 consecutive pulse-alone trials to habituate and stabilize the startle response. Subsequently, each stimulus was presented 12 times in a pseudorandom order with an average interval between successive trials of 15 ± 5 s. The session was concluded with 6 consecutive pulse-alone trials. Boxes were cleaned with water and dried between sessions. For each subject, PPI was indexed as mean percent inhibition of startle response obtained in the prepulse-plus-trials compared to pulse-alone trials by following the expression: [1-(mean reactivity on prepulse-plus-pulse trials / mean reactivity on pulse-alone trials) × 1/100]. The first and last six trials were not included in the calculation of percent PPI. In addition to PPI, reactivity to prepulse- and pulse-alone trials were also analyzed.

Social interaction in mice

The social interaction protocol was adapted from Jeevakumar et al. (2015). Experimental and stimulus mice were isolated for 24 h prior to testing. On the day of the experiment, mice received an i.p. injection of either saline (0.9% NaCl) or taurine (50, 100, or 200 mg/kg) followed by another i.p. injection of saline or MK-801 (0.15 mg/kg) 30 min later. Testing began 30 min after the last injection. A stimulus mouse was placed inside a cylindrical custom-built container (20 cm high, steel bars separated by 1 cm, acrylic lid) and then introduced to the home cage of experimental mice for 10 min. All sessions were video-recorded and interaction time (defined as sniffing and investigating at close proximity) were scored offline using Behavioral Observation Research Interactive Software (BORIS; Friard & Gamba, 2016).

Social interaction in zebrafish

The protocol for the social interaction test in zebrafish followed the method described by Benvenutti et al. (2021). Animals were individually exposed to water or taurine solutions at 42, 150 or 400 mg/L in 500-mL beakers containing 200-mL solution for 20 min. They were then transferred to another beaker containing either water or MK-801 at 5 μM for another 20 minutes. After exposure, animals were placed for 7 min in a tank (30 × 10 × 15 cm) flanked by two identical tanks (15 × 10 × 13 cm) either

empty (neutral stimulus) or containing 10 unknown zebrafish (social stimulus). All three tanks were filled with water in standard conditions at a level of 10 cm. The position of the social stimulus (right or left) was counterbalanced throughout the tests. The water in the test tanks was changed between every animal. To assess social behavior, the test apparatus was virtually divided into three vertical zones (interaction, middle, and neutral). Animals were habituated to the apparatus for 2 min and then analyzed for the last 5 min. Videos were recorded from the front view and time spent in the interaction zone was quantified as a proxy for social interaction. Additionally, total distance traveled, number of crossings between the vertical zones of the tank and immobility time were quantified as secondary locomotor parameters. All outcomes were automatically scored using ANY-Maze software (Stoelting Co., Wood Dale, IL, USA).

Statistical analysis

Outliers were defined following the rule of mean ± 2 standard deviations. This resulted in five outliers removed from the PPI test (one TAU 50/CTRL, one TAU 100/CTRL, two TAU 50/MK and one TAU 100/MK), five outliers removed from the social interaction test in mice (one TAU 50/CTRL, one TAU 100/CTRL, one TAU 200/CTRL, one TAU 50/MK and one TAU 200/MK), and two outliers removed from the social interaction test in zebrafish (one CTRL/CTRL and one TAU 42/CTRL). No outliers were removed from the hyperlocomotion test (mean distance traveled after MK-801 was the outcome used for the check). Two mice from the social interaction set died of unknown causes after allocation but before testing (one CTRL/MK and one TAU 100/MK).

The sample size to detect a 0.5 effect size with 0.95 power and 0.05 alpha was calculated using Minitab (version 21.1) for Windows; this resulted in n=10 for locomotor activity (4 groups) and n=12 for all other assays (8 groups). GraphPad Prism 8 (version 8.4.3) for macOS was used to run the statistical analyses and plot the results. For locomotor activity, distance traveled as a function of 5-min time segments was analyzed using repeated measures ANOVA, with time as the within-subjects factor, and taurine pretreatment as the between-subjects factor; the two phases of the experiment (i.e., baseline and MK-801 treatment) were analyzed separately. The data from the remaining experiments were analyzed by two-way ANOVA, with taurine pretreatment and MK-801 treatment as the main factors. Bonferroni post hoc test was

applied as appropriate. The significance level was set at $p<0.05$. Data were expressed as mean ± standard deviation.

RESULTS

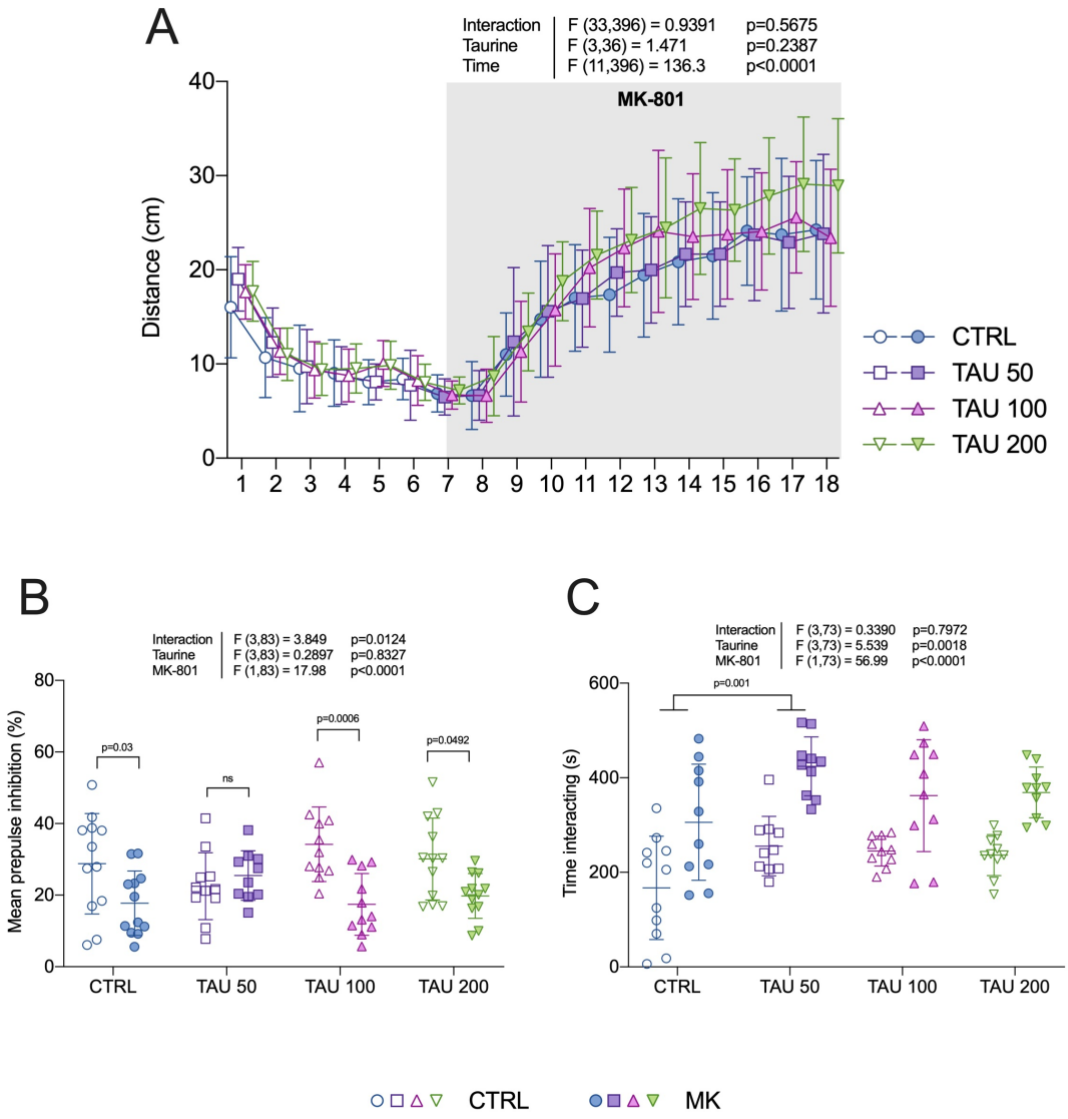


Figure 2. Effects of taurine on behavioral abnormalities induced by MK-801 in mice. (A) Locomotor activity (in segments of 5 min), (B) prepulse inhibition of the startle reflex and (C) social interaction were evaluated as measures relevant to the positive, cognitive, and negative symptoms of schizophrenia, respectively. Two-way ANOVA followed by Bonferroni post hoc test. Data are presented as mean ± standard deviation. n=10-12. CTRL: control, TAU: taurine (doses are denoted in mg/kg).

Locomotor activity in response to MK-801

The hyperlocomotion in response to an MK-801 challenge was assessed as an outcome related to the positive symptoms of schizophrenia (Powell and Geyer, 2007). Figure 2A shows that distance travelled by the mice in the open field increased after the MK-801 challenge in all experimental groups (time effect: $F_{11,396}$ = 136.3, p<0.0001). Taurine did not prevent the effects of MK-801 (taurine effect: $F_{3,36}$ = 1.471, p=0.2387; interaction effect: $F_{33,396}$ = 0.9391, p=0.5675). In the baseline phase (first 30 min), locomotion decreased as animals habituated to the environment (time effect: $F_{5,180}$ = 102.0, p<0.0001), and no differences between the groups were observed (taurine effect: $F_{3,36}$ = 0.1631, p=0.9205; interaction effect: $F_{15,180}$ = 1.140, p=0.3239).

MK-801-induced prepulse inhibition deficits

The effects of MK-801 on sensorimotor gating were assessed by the paradigm of prepulse inhibition (PPI) of the acoustic startle reflex, which is a translational measure related to the cognitive symptoms of schizophrenia (Powell et al., 2009). Mice treated with MK-801 showed lower levels of PPI when compared to controls (MK-801 main effect: $F_{1,83}$ = 17.98, p<0.0001), indicating deficits in sensorimotor gating (Figure 2B). Two-way ANOVA also revealed a significant interaction effect ($F_{3,83}$ = 3.849, p=0.0124) in the absence of a taurine main effect ($F_{3,83}$ = 0.2897, p=0.8327). Bonferroni post hoc tests comparing MK-801 groups to their respective controls resulted in significant differences for all comparisons except for the groups pretreated with taurine at 50 mg/kg (lowest dose), indicating an attenuation of the PPI deficit induced by MK-801.

Social interaction in mice

The social behavior towards an unfamiliar mouse introduced in the home cage was evaluated as a phenotype relevant to the negative symptoms of schizophrenia (Jones et al., 2011). Subject mice were manually scored according to their interest in sniffing or investigating at proximity the enclosed stimulus mouse. Figure 2C shows that groups treated with MK-801 spent more time interacting with the social stimulus (MK-801 main effect: $F_{1,73}$ = 56.99, p<0.0001). Two-way ANOVA also revealed a taurine main effect ($F_{3,73}$ = 5.539, p=0.0018) without a significant interaction ($F_{3,73}$ = 0.339, p=0.7972). Bonferroni post hoc comparisons restricted to the pretreatment

factor (taurine main effect) indicated that interaction time was significantly higher in groups pretreated with taurine at 50 mg/kg in comparison to control groups (p=0.001).

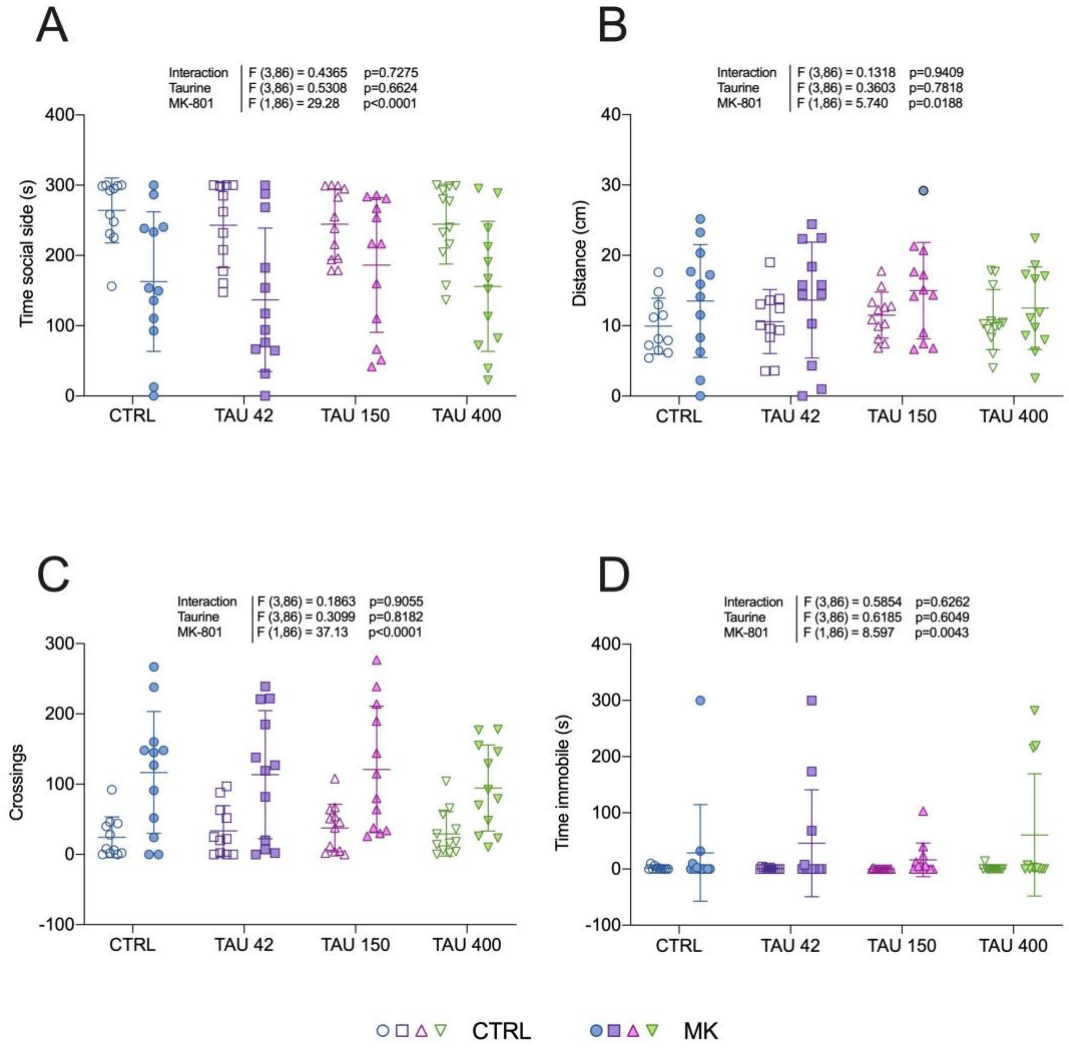


Figure 3. Effects of taurine in the social interaction test in zebrafish. (A) Time spent in the social stimulus side was measured as a proxy for social interaction, while (B) total distance traveled, (C) number of crossings and (D) time spent immobile were quantified as secondary locomotor parameters. Two-way ANOVA. Data are presented as mean ± SD. n=11-12. CTRL: control, TAU: taurine (exposure concentrations are denoted in mg/L).

Social interaction in zebrafish

Zebrafish is increasingly considered as a model organism suitable to study drug-induced behavioral phenotypes relevant to schizophrenia (Gawel et al., 2019; Benvenutti et al., 2021). Figure 3A shows that zebrafish exposed to MK-801 spent less time in the side of the tank where conspecifics were presented, denoting decreased social preference in comparison to control groups (MK-801 main effect: $F_{1,86}$ = 29.28,

p<0.0001). The other outcomes evaluated in this test were also altered by MK-801, as shown by increases in total distance travelled ($F_{1,86}$ = 5.74, p=0.0188; Fig. 3B), number crossings between the zones of the tank ($F_{1,86}$ = 37.13, p<0.0001; Fig. 3C) and immobility time ($F_{1,86}$ = 8.597, p=0.0043; Fig. 3D). Taurine was devoid of effects in all parameters, as no main effects for drug pretreatment or interaction effects were observed.

DISCUSSION

Animal models provide a unique opportunity to understand how genetic, molecular, and environmental factors might lead to the development of schizophrenia. In this study, we used MK-801 to acutely induce behavioral alterations of translational relevance to schizophrenia in C57BL/6 mice and zebrafish, and ultimately evaluate the preventive effects of taurine against the deficits caused by NMDA antagonism in a two-species approach.

Taurine's role has been studied in several conditions, including depression, cardiac failure, retina degeneration and growth problems (Lourenço and Camilo, 2002). Here, we found that taurine largely failed to prevent MK-801-induced behavioral alterations. Although a significant interaction was found in the prepulse inhibition (PPI) test and post hoc analysis showed that the group pretreated with taurine at 50 mg/kg before MK-801 administration was not significantly different than its respective control, this should be interpreted with caution as taurine at this dose seems to buffer PPI to intermediate levels instead of fully preventing the deficit induced by MK-801. In the social interaction test in mice, both groups treated with taurine at 50 mg/kg spent more time interacting when compared to pretreatment controls. This agrees with a previous study in which taurine at a similar dose (42 mg/kg) was shown to increase social interaction in Wistar rats (Kong et al., 2006); such increases in social interaction may be explained by the anxiolytic effects reported for taurine in several studies (Kong et al., 2006; El Idrissi et al., 2009; Mezzomo et al., 2016, 2019; Jung and Kim, 2019; Neuwirth et al., 2019; Fontana et al., 2020).

As expected, MK-801 increased the total distance traveled and caused a PPI deficit in mice. In zebrafish, we observed reduced time in the social side as well as hyperlocomotion in all groups exposed to MK-801. Curiously, mice treated with MK-801 spent more time interacting with the stimulus mice when compared to controls.

This was unexpected since in most studies NMDA antagonism leads to decreased levels of social interaction (Morales and Spear, 2014; Zoicas and Kornhuber, 2019). Jeevakumar *et al.* (2015), for example, used a similar home cage protocol and observed a significantly reduced investigation time in adult mice exposed to ketamine in the second postnatal week. Although both ketamine and MK-801 are NMDA antagonists, differences might be related to MK-801 being a more specific NMDA antagonist, while ketamine also interacts with dopaminergic and serotoninergic systems (Kapur and Seeman, 2002; Stone et al., 2007). Drug administration regimen and protocol adaptations also might contribute to this difference in social behavior. Moreover, MK-801 showed a fast-acting but nonsustainable antidepressant response in control mice (Autry et al., 2011; Zanos et al., 2016), which could explain the increased social behavior in our experiment as mice were tested 30 minutes after the MK-801 injection.

It is well known that excessive stimulation of glutamatergic receptors causes excitotoxicity due to increased intracellular levels of calcium. Previous studies have demonstrated that taurine may act as a neuroprotector either by decreasing intracellular free calcium or by counterbalancing glutamatergic transmission via voltage-gated calcium channels (Lidsky et al., 1995; El Idrissi and Trenkner, 1999; Saransaari and Oja, 2000). Acamprosate, a synthetic analog of taurine, is hypothesized to decrease NMDA receptor activity by modulating the expression of NMDA receptor subunits in specific brain regions (Rammes et al., 2001; Heilig and Egli, 2006). In addition, Chan et al (2015) showed that taurine binds to GluN2B subunit of the NMDA receptor and causes a prolonged inhibition of excitatory synaptic transmission in an *ex vivo* model.

Although taurine was not able to prevent the deficits observed in our study, it still might have beneficial effects in psychosis models that better mimic the course of schizophrenia, such as neurodevelopmental models. Various studies linked behavioral and neurobiological dysfunctions of schizophrenia to neurodevelopment, which translates into symptoms that appear mainly during late adolescence (Brown, 2006; Knuesel et al., 2014; Volk and Lewis, 2014; Hantsoo et al., 2019). Interventions that aim to act in the prodrome period, preventing the first psychotic episode, are thought to have better outcomes than antipsychotic treatment, once they have been ultimately unsuccessful in preventing disease onset in individuals with schizophrenia (McGlashan et al., 2003; McGorry et al., 2013; Woods et al., 2017). Therefore, continuous taurine

administration in vulnerability periods might prevent the abnormalities that emerge in early adulthood. With antioxidant and neuroprotector properties, taurine might be able to normalize the altered redox state and parvalbumin-positive interneurons loss found in animal models and patients with schizophrenia (Fung et al., 2010; Gill and Grace, 2014; Salim, 2014; Steullet et al., 2017; Kaar et al., 2019; Goh et al., 2022). Grace *et al.* (2016) hypothesized that the dysfunction of the dopaminergic system might be a consequence of the loss of a large number of fast-spiking parvalbumin-positive GABAergic interneurons in the ventral subiculum of the hippocampus, causing hyperactivation and dysrhythmic behavior of pyramidal neurons. Taurine might ameliorate this hyperactivation by compensating the inhibitory loss at parvalbumin-positive interneurons and preventing the onset of symptoms in a neurodevelopmental model. Since this dysregulation is postulated to occur in late adolescence or early adulthood, taurine should be administered prior to this period to prevent the disruption of basolateral amygdala, nucleus accumbens and prefrontal cortex activity and rhythmicity, all of which participate in circuits interconnected with the ventral subiculum.

In regards to zebrafish, it has been demonstrated that MK-801 induces hyperlocomotion, although it is not clear which neuronal mechanisms might be involved (Menezes et al., 2015; Tran et al., 2016; Benvenutti et al., 2021; Franscescon et al., 2021). Zebrafish increasing use is a great solution to avoid species biases and focus on a robust cross-species approach, aside from being an accessible way to screen for potential novels treatments (Bruni et al., 2016; Burrows and Hannan, 2016; Gawel et al., 2019). Taurine has been demonstrated to prevent MK-801 hyperlocomotion and memory impairment in zebrafish (Franscescon et al., 2020, 2021), a finding that we could not replicate in our study. Here, taurine was not able to counteract MK-801 effects on locomotor activity or social interaction. In our protocol, zebrafish were exposed to taurine and MK-801 in a beaker for 20 minutes, and time spent on the social side and distance traveled were assessed. Differences in drug administration route and exposure time might contribute to the divergent outcomes. Considering that we also observed a lack of a clear antipsychotic effect of taurine in rodents, we reckon that our findings are robust and consistent across species, which does not necessarily rule out taurine antipsychotic effect in other treatment regimens.

A limitation of our study is that it remains to be established whether taurine can prevent the neuropathological events of schizophrenia in preclinical models that better simulate the course of the disease. Our study was not designed to act in the prodromal

phase of schizophrenia, which we believe is a key opportunity window to prevent the alterations that emerge in early adulthood. Another limitation is that preclinical models of schizophrenia likely do not reflect the neurobiology underlying the positive symptoms of the disease, making it difficult to be accurately assessed in behavior tests (Kesby et al., 2018). Because hallucinations are false percepts perceived subjectively as true, a valid assessment of this behavior in rodents can require extensive training, and thus are incompatible with time-sensitive analysis, such as MK-801 acute administration (Schmack et al., 2021). Therefore, other rodent preclinical models of schizophrenia that have a more long-lasting endophenotype, and wherefore allow this type of assessment, may appraise positive-like symptoms with a better predictive validity than the locomotory response to MK-801.

The frequent failure in translating preclinical findings to clinical settings has been increasingly discussed, and strategies to overcome this loss in translation have been suggested (Seyhan, 2019). The strength of our study lies in including two model organisms from different phylogenetic classes, which increases the external validity of preclinical studies. Though more studies are necessary to evaluate taurine's role in schizophrenia, our two-species approach contradicts previous studies by showing that, at least acutely, taurine is not able to prevent the behavioral alterations induced by antagonism of NMDA receptors.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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4 CONCLUSÃO

Apesar dos resultados negativos que obtivemos quanto ao potencial preventivo da taurina em um modelo agudo de psicose, não podemos descartar que ela possa agir de maneira preventiva em uma janela de vulnerabilidade neurodesenvolvimental em modelos que melhor mimetizam o curso natural da doença. Evidências corroboram que o aparecimento dos primeiros sintomas da esquizofrenia deve-se à perda de atividade GABAérgica no hipocampo, principalmente por redução de interneurônios GABAérgicos parvalbumina-positivos no subículo ventral. Quando administrada em um período anterior à instalação dos sintomas psicóticos – e, portanto, à desregulação dos sistemas GABérgicos, dopaminérgicos e glutamatérgicos –, a taurina pode ser capaz de compensar a perda inibitória, uma vez que atua em receptores GABAérgicos e glicinérgicos.

5 PERSPECTIVAS

Neste estudo, o antagonista NMDA MK-801 mimetizou de maneira transitória sintomas relevantes à esquizofrenia, embora com menor valor de face em comparação com modelos de ativação imunológica, por exemplo, os quais conseguem inclusive reproduzir em roedores alterações como a perda dos interneurônios GABAérgicos de parvalbumina e o aparecimento dos sintomas após a adolescência. Deste modo, a administração crônica da taurina em caráter preventivo nestes modelos é uma perspectiva lógica desse estudo. Além disso, estes modelos possibilitariam testes comportamentais que requerem treinos repetidos e, portanto, mais tempo para detectar os sintomas positivos da doença em camundongos.

Outra observação que merece maior escrutínio é o aumento do interesse social pelo animal estímulo induzido por MK-801 em camundongos. Esse achado contradiz evidências anteriores da literatura, e novos experimentos são necessários para investigar os fatores que poderiam modular a resposta diferencial do MK-801 sobre o comportamento social.

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ANEXO A – CARTAS DE APROVAÇÃO DA COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA)

HOSPITAL DE CLÍNICAS DE PORTO ALEGRE

Grupo de Pesquisa e Pós Graduação

Carta de Aprovação

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

Projeto: 2018/0498

Título: AVALIAÇÃO DOS EFEITOS DA TAURINA EM MODELOS PRÉ-CLÍNICOS DE ESQUIZOFRENIA

Pesquisador Responsável: **ADRIANE RIBEIRO ROSA**

Equipe de Pesquisa:

MATHEUS GALLAS LOPES	SILVIA AMORETTI	ADRIELI SACHETT
RADHARANI BENVENUTTI	ANA PAULA HERRMANN	FRANCIELE KICH GIONGO

Data de Aprovação: 19/12/2018

Data de Término: 16/09/2019

Espécie/Linhagem	Sexo/Idade	Quantidade
CAMUNDONGO ISOGÊNICO	M/55 Dia(s)	110

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

Impresso do sistema AGHUse-Pesquisa por RAFAEL LEAL ZIMMER em 14/03/2019 13:35:09

GRUPO DE PESQUISA E PÓS-GRADUAÇÃO

PARECER VALIDADO

Projeto: 2018-0498

AVALIAÇÃO DOS EFEITOS DA TAURINA EM MODELOS PRÉ-CLÍNICOS DE ESQUIZOFRENIA

Parecer
O pesquisador apresenta relatório com a exposição dos resultados do estudo piloto, confirmando reprodutibilidade do modelo experimental agudo de esquizofrenia nas condições de laboratório atuais (110 animais já utilizados), e solicita a liberação dos demais animais para dar seguimento ao estudo principal (248 animais). Liberação aprovada. Tamanho amostral: 358 camundongos C57Bl6 machos, sendo 110 animais (piloto) + 248 animais (estudo principal). Validado em 11/12/2019.

Gerado no sistema AGHUse-Pesquisa em 11/12/2019

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UNIVERSIDADE FEDERAL
DO RIO GRANDE DO SUL

PRÓ-REITORIA DE PESQUISA

Comissão De Ética No Uso De Animais

CEUA UFRGS

CARTA DE APROVAÇÃO

Comissão De Ética No Uso De Animais analisou o projeto:

Número: 35525
Título: Estabelecimento e validação farmacológica de modelos de esquizofrenia em peixes-zebra

Vigência: 19/06/2018 à 19/06/2022

Pesquisadores:

Equipe UFRGS:

ÂNGELO LUIS STAPASSOLI PIATO - coordenador desde 19/06/2018
Ana Paula Herrmann - coordenador desde 19/06/2018
RADHARANI BENVENUTTI - Aluno de Doutorado desde 19/06/2018
Matheus Felipe Marcon - Aluno de Doutorado desde 19/06/2018

Comissão De Ética No Uso De Animais aprovou o mesmo , em reunião realizada em 06/08/2018 - Sala 330 do Anexo I do Prédio da Reitoria - Campus Centro/UFRGS, em seus aspectos éticos e metodológicos, para a utilização de 2720 Peixes-zebra da linhagem AB, machos e fêmeas de diferentes idades, oriundos da colônia proveniente do Biotério da PUCRS; de acordo com os preceitos das Diretrizes e Normas Nacionais e Internacionais, especialmente a Lei 11.794 de 08 de novembro de 2008, o Decreto 6899 de 15 de julho de 2009, e as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), que disciplinam a produção, manutenção e/ou utilização de animais do filo Chordata, subfilo Vertebrata (exceto o homem) em atividade de ensino ou pesquisa.

Porto Alegre, Quinta-Feira, 4 de Julho de 2019

ALEXANDRE TAVARES DUARTE DE OLIVEIRA
Coordenador da comissão de ética

1

ANEXO B – CERTIFICADO DE PERMISSÃO PARA REPRODUÇÃO DE CONTEÚDO PROTEGIDO POR COPYRIGHT®

SPRINGER NATURE LICENSE
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Number of figures/tables/illustrations	1
High-res required	no
Will you be translating?	no