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AVALIAÇÃO DE ENDOFENÓTIPOS RELACIONADOS À ESQUIZOFRENIA EM PEIXES-ZEBRA

Porto Alegre
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Orientador: Prof. Dr. Angelo Piato
Coorientadora: Prof. Dra. Ana Paula Herrmann

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RESUMO

A esquizofrenia é um transtorno psiquiátrico neurodesenvolvimental, crônico e complexo, associada à hiperatividade dopaminérgica, hipofunção de receptores NMDA e estresse oxidativo. Apesar da dizocilpina (MK-801) e da anfetamina, serem as drogas mais utilizadas em roedores para mimetizar a hipofunção de receptores NMDA e a hiperatividade dopaminérgica na esquizofrenia, respectivamente, pouco se sabe sobre seus efeitos em peixes-zebra. Assim, o objetivo principal desta tese foi investigar os efeitos do MK-801 e da anfetamina sobre parâmetros comportamentais e neuroquímicos em peixes-zebra. Primeiramente, foi realizada uma revisão da literatura científica, integrando os estudos sobre os efeitos de antagonistas de receptores NMDA em parâmetros comportamentais com relevância para a esquizofrenia nessa espécie. Foram identificados 44 artigos que atenderam aos critérios de inclusão, a partir de 590 artigos selecionados nas bases de dados MEDLINE (PubMed) e Web of Science. O MK-801 e a cetamina foram utilizados em 29 e 10 estudos, respectivamente. O uso de outros antagonistas de receptor NMDA, como a fenciclidina, ácido DL-2-amino-5-fosfonopentanóico (APV), memantina e tiletamina, foi descrito em 6 estudos. Achados frequentemente relatados são déficits de interação social e de memória induzidos por MK-801, e alteração do comportamento circular induzida pela cetamina. No entanto, resultados divergentes foram descritos para vários parâmetros locomotores e exploratórios. Portanto, na segunda etapa dessa tese, foram realizados experimentos com o objetivo de comparar os efeitos do MK-801 e da anfetamina sobre parâmetros comportamentais e neuroquímicos em peixes-zebra. Os animais foram expostos a MK-801 (1, 5 e 10 μM) ou anfetamina (0,625, 2,5 e 10 mg/L) e avaliados em testes de atividade locomotora e comportamento social; parâmetros oxidativos foram quantificados em tecido encefálico. O MK-801 diminuiu a interação social, um efeito semelhante aos sintomas negativos da esquizofrenia, e alterou a locomoção de forma dependente de contexto, induzindo hiperatividade quando os peixes foram testados na presença de pistas sociais e hipoatividade quando testados isolados. Por outro lado, a exposição à anfetamina não causou efeitos na locomoção e no comportamento social, mas aumentou a peroxidação lipídica no encéfalo. Com esse estudo concluímos que, o MK-801 recapitulou as alterações comportamentais com valor translacional observadas em roedores, corroborando com o uso de antagonistas NMDA para estudar

endofenótipos relacionados à esquizofrenia em peixes-zebra. Ainda assim, mais estudos são necessários para avaliar a validade preditiva de paradigmas pré-clínicos em peixes-zebra e otimizar a triagem de novos tratamentos.

ABSTRACT

Schizophrenia is a chronic and complex neurodevelopmental psychiatric disorder associated with dopaminergic hyperactivity, hypofunction of NMDA receptors and oxidative stress. Although dizocilpine (MK-801) and amphetamine are the most commonly used drugs in rodents to mimic NMDA receptor hypofunction and dopaminergic hyperactivity in schizophrenia, respectively, little is known about their effects in zebrafish. Thus, this thesis aimed to investigate and review the effects of these drugs on the behavioral and neurochemistry of zebrafish (*Danio rerio*). First, we conducted an extensive review of the scientific literature to integrate the major findings reported in the zebrafish regarding the behavioral effects of NMDA receptor antagonists with relevance to schizophrenia. We identified 44 research articles that met our inclusion criteria from 590 studies retrieved from MEDLINE (PubMed) and Web of Science databases. MK-801 and ketamine were employed in 29 and 10 studies, respectively. The use of other NMDA receptor antagonists, such as phencyclidine (PCP), DL-2-Amino-5-phosphonopentanoic acid (APV), memantine, and tiletamine, was described in 6 studies. Frequently reported findings are the social interaction and memory deficits induced by MK-801 and circling behavior induced by ketamine. However, mixed results were described for several locomotor and exploratory parameters. Therefore, we performed a study to compare the effects of MK-801 and amphetamine in several zebrafish behavioral and neurochemical assays. Briefly, adult zebrafish were exposed to MK-801 (1, 5, and 10 μM) or amphetamine (0.625, 2.5, and 10 mg/L) and observed in paradigms of locomotor activity and social behavior; oxidative parameters were quantified in brain tissue. MK-801 decreased social interaction, an effect similar to negative symptoms of schizophrenia, and altered locomotion in a context-dependent manner, inducing hyperactivity when the fish were tested in the presence of social cues and hypoactivity when tested alone. On the other hand, exposure to amphetamine had no effect on locomotion and social behavior, but increased lipid peroxidation in the brain. With this study, we conclude that MK-801 recapitulated the behavioral changes with translational value observed in rodents, corroborating the use of NMDA antagonists to study schizophrenia-related endophenotypes in zebrafish. Still, more studies are needed to assess the predictive validity of preclinical paradigms in zebrafish and to optimize the screening of new treatments.

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LISTA DE ABREVIATURAS

APV: ácido DL-2-amino-5-fosfonopentanóico

CID: Classificação estatística internacional de doenças e problemas relacionados à saúde

DSM-5: Manual diagnóstico e estatístico de transtornos mentais, quinta edição

GSH: glutationa

IPSCs: células tronco pluripotentes induzidas

MHC: sistema complexo principal de histocompatibilidade

NMDA: N-metil-D-aspartato

NPSH: tióis não proteicos

PCP: fenciclidina

PPI: inibição do pré-pulso

SNC: Sistema Nervoso Central

SNP: Sistema Nervoso Periférico

TBARS: substâncias reativas ao ácido tiobarbitúrico

VTA: área tegmental ventral

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1. INTRODUÇÃO

1.1. Esquizofrenia

1.1.1. Epidemiologia e critérios de diagnóstico

A esquizofrenia é um transtorno psiquiátrico neurodesenvolvimental heterogêneo e crônico, com prevalência de cerca de 1% na população mundial (Kahn et al. 2015). Esse transtorno é caracterizado por três classes de sintomas, os sintomas positivos (alucinações, delírios, desorganização de pensamento e de discurso), os sintomas negativos (embotamento emocional, isolamento social, anedonia e avolia) e os sintomas cognitivos (prejuízo de funções executivas, déficit de atenção e de memória de trabalho) (American Psychiatric Association 2013). A Tabela 1 apresenta os principais sintomas da esquizofrenia de acordo com o Manual Diagnóstico e Estatístico de Transtornos Mentais (DSM-5).

Os pacientes com esquizofrenia geralmente apresentam prejuízo em vários domínios da vida cotidiana, incluindo dificuldade de manter relacionamentos sociais e emprego, viver de forma independente e, além disso, alguns desses déficits geralmente persistem mesmo após os pacientes alcançarem a remissão dos sintomas psicóticos (Harvey 2014). Por causar prejuízo crônico em múltiplos domínios da vida, a esquizofrenia está entre as 10 principais causas de incapacidade (Marder e Cannon 2019), e estima-se que a expectativa de vida dos pacientes é de 15 a 20 anos menor do que da população em geral (Chesney, Goodwin, e Fazel 2014; McCutcheon, Reis Marques, e Howes 2020). Além disso, pessoas com esquizofrenia possuem um risco de morte por suicídio 5% a 10% maior em comparação com a população em geral (Hjorthøj et al. 2017).

Tabela 1. Sintomas da esquizofrenia de acordo com o DSM-5 (American Psychiatric Association 2013)

Sintomas		**Características dos sintomas**
Positivos	Delírios	Distúrbios de pensamento; Crenças fixas e irreais, não passíveis de mudança à luz de evidências.
	Alucinações	Distúrbios de percepção; Experiências vívidas e claras, semelhantes às percepções normais, não estando sob controle voluntário; Podem ocorrer em qualquer modalidade sensorial, embora as alucinações auditivas sejam as mais comuns.
	Pensamento e discurso desorganizado	Pensamento e discurso incoerentes e inespecíficos; Descarrilamento ou afrouxamento das associações.
	Comportamento desorganizado ou catatônico	Comportamento motor grosseiramente desorganizado ou anormal; Agitação imprevisível; Redução acentuada na reatividade ao ambiente; Manutenção de postura rígida, inapropriada ou bizarra ou falta total de respostas verbais e motoras; Atividade motora sem propósito.
Negativos	Alogia	Diminuição do discurso e da comunicação verbal.
	Avolia	Redução de atividades motivadas, autoiniciadas e com uma finalidade.

	Anedonia	Diminuição da capacidade de sentir prazer resultante de estímulos positivos.
	Expressão emocional diminuída	Redução na expressão de emoções pelo rosto; Redução do contato visual e dos movimentos das mãos, da cabeça e da face.
	Isolamento social	Ausência ou diminuição no interesse em interações sociais.
Cognitivos	Déficit de atenção	Dificuldade para realizar atividades que exigem maior atenção e concentração; Dificuldade de manter a atenção por um longo período de tempo.
	Déficit de cognição social	Diminuição da capacidade de inferir as intenções dos outros.
	Déficit de memória	Diminuições de memória declarativa; Diminuições de memória de trabalho; Velocidade de processamento mais lenta.

O diagnóstico da esquizofrenia é realizado com base na avaliação clínica do paciente e em critérios operacionais, geralmente baseados no DSM-5 (American Psychiatric Association 2013) ou na Classificação Estatística Internacional de Doenças e Problemas Relacionados à Saúde (CID) (Kahn et al. 2015). Esses critérios levam em consideração a apresentação de sintomas positivos, negativos e cognitivos, juntamente com a duração em que os sintomas estão presentes, seu efeito no funcionamento social e ocupacional e a contribuição potencial de outras condições psiquiátricas, como transtorno de humor bipolar, depressão ou abuso de substâncias (Kahn et al. 2015) (tabela 2).

Tabela 2. Critérios diagnósticos da esquizofrenia de acordo com o DSM-5 (American Psychiatric Association 2013).

Critério A: devem estar presentes dois ou mais dos sintomas a seguir, durante no mínimo um mês. Pelo menos um dos sintomas deve ser (1), (2) ou (3):
1. Delírios
2. Alucinações
3. Discurso desorganizado
4. Comportamento grosseiramente desorganizado ou catatônico
5. Sintomas negativos
Critério B: O nível de funcionamento do indivíduo em áreas como trabalho, relações interpessoais ou autocuidado está acentuadamente abaixo do nível alcançado antes do início dos sintomas.
Critério C: Os sinais de perturbação persistem durante, pelo menos, seis meses.
Critério D: Outros transtornos psiquiátricos devem ser descartados.
Critério E: A perturbação não pode ser atribuída aos efeitos fisiológicos de uma substância ou a outra condição médica.

1.1.2. Bases neurobiológicas

Nas últimas décadas, diversos estudos investigaram os complexos mecanismos neurobiológicos da esquizofrenia, como a existência de possíveis assinaturas moleculares e fatores genéticos. Outros estudos descreveram alterações no estado funcional de populações neurais específicas e na arquitetura da comunicação entre as células neurais (Kahn et al. 2015). Embora alguns achados importantes tenham sido descritos e replicados, esse transtorno é caracterizado por mecanismos patofisiológicos e etiológicos extremamente complexos, e a compreensão das bases neurobiológicas da esquizofrenia permanece limitada e incerta.

Apesar da etiologia da esquizofrenia não estar completamente elucidada, sabe-se

que se trata de uma condição neurodesenvolvimental multifatorial que resulta de uma combinação complexa entre fatores genéticos, ambientais e comportamentais (Zamanpoor 2020). O neurodesenvolvimento precoce é caracterizado pela migração neural e formação de conexões sinápticas entre os neurônios, que continuam a se desenvolver durante a infância e adolescência. A hipótese mais aceita atualmente é de que fatores de risco genéticos e/ou ambientais que ocorrem em estágios iniciais do neurodesenvolvimento embrionário produzem alterações em circuitos neurais (Grace e Gomes 2019; Kesby et al. 2018; McCutcheon, Krystal, e Howes 2020). Acredita-se que essas alterações durante o neurodesenvolvimento tornem o encéfalo mais vulnerável a futuros eventos de risco, geralmente na adolescência ou no início da idade adulta, capazes de desencadear o aparecimento dos sintomas (Kahn et al. 2015; Marder e Cannon 2019; Owen, Sawa, e Mortensen 2016). Diversos fatores de risco foram associados ao desenvolvimento da esquizofrenia, entre os principais estão: complicações pré-natais e perinatais (desnutrição, exposição materna a infecções virais, pré-eclâmpsia, parto por cesárea), idade parental avançada, residir em ambiente urbano, migração, adversidades sociais, abuso físico e psicológico e abuso de substâncias (Kahn et al. 2015).

Os sintomas da esquizofrenia geralmente iniciam durante a adolescência ou início da idade adulta. Além disso, uma fase prodrômica pode anteceder o aparecimento dos sintomas psicóticos em até 10 anos (Kahn e Keefe 2013). Essa fase é caracterizada por sintomas mais brandos, que muitas vezes passam despercebidos, como por exemplo leve déficit cognitivo, ansiedade ou depressão (Kahn e Keefe 2013). Por essa razão, geralmente os pacientes não são encaminhados para consulta psiquiátrica até que a psicose se apresente na adolescência ou início da idade adulta. De fato, é justamente nesse período que processos fisiológicos importantes para a maturação cerebral ocorrem, mediados pela poda sináptica, ou seja, a eliminação de sinapses desnecessárias pela micróglia (Cornell et al. 2022).

Na esquizofrenia, a poda sináptica ocorre de forma desproporcional, resultando na disfunção de algumas populações neuronais e, provavelmente, no aparecimento dos sintomas (Cardozo et al. 2019; Sellgren et al. 2019). Estudos em modelos animais de esquizofrenia e em células tronco pluripotentes induzidas (IPSCs) derivadas de pacientes, sugerem que fatores de risco genéticos e ambientais resultam em disfunções no funcionamento da microglia e do complexo principal de histocompatibilidade

(MHC). Considerando que o MHC possui um papel essencial na sinalização durante a poda sináptica, enquanto a microglia é a responsável pela eliminação de sinapses, essas disfunções resultam em uma poda sináptica disfuncional e exagerada (Sellgren et al. 2019; Cardozo et al. 2019). Assim, a poda sináptica disfuncional, principalmente em regiões do córtex pré-frontal e hipocampo, poderia explicar as principais alterações neuronais identificadas na patofisiologia da esquizofrenia.

Nesse contexto, disfunções complexas em vias neurais GABAérgicas, glutamatérgicas e dopaminérgicas parecem exercer um papel chave na patofisiologia da esquizofrenia (Grace e Gomes 2019; McCutcheon, Krystal, e Howes 2020). Estudos pré-clínicos e clínicos sugerem que os sintomas psicóticos surgem da hiperatividade dopaminérgica em regiões encefálicas subcorticais, mais especificamente na via que se projeta da área tegmental ventral (VTA) para o estriado associativo (Kesby et al. 2018; McCutcheon et al. 2018). Estudos *post-mortem* em encéfalo de pacientes e estudos em modelos animais (Krystal et al. 1994; Mohn et al. 1999; Weickert et al. 2013) demonstraram que há uma função aberrante dos interneurônios GABAérgicos *fast-spiking* positivos para parvalbumina, acompanhado por um déficit funcional de receptores N-metil-D-aspartato (NMDA) que são expressos na membrana desses interneurônios, o que leva a alterações do equilíbrio de excitação-inibição em áreas corticais e subcorticais, resultando em hiperativação dopaminérgica (Insel 2010; Korotkova et al. 2010; Cabungcal et al. 2013; Hardingham e Do 2016; Konradi et al. 2011).

A Figura 1 apresenta os principais fatores e características associados à patofisiologia da esquizofrenia.

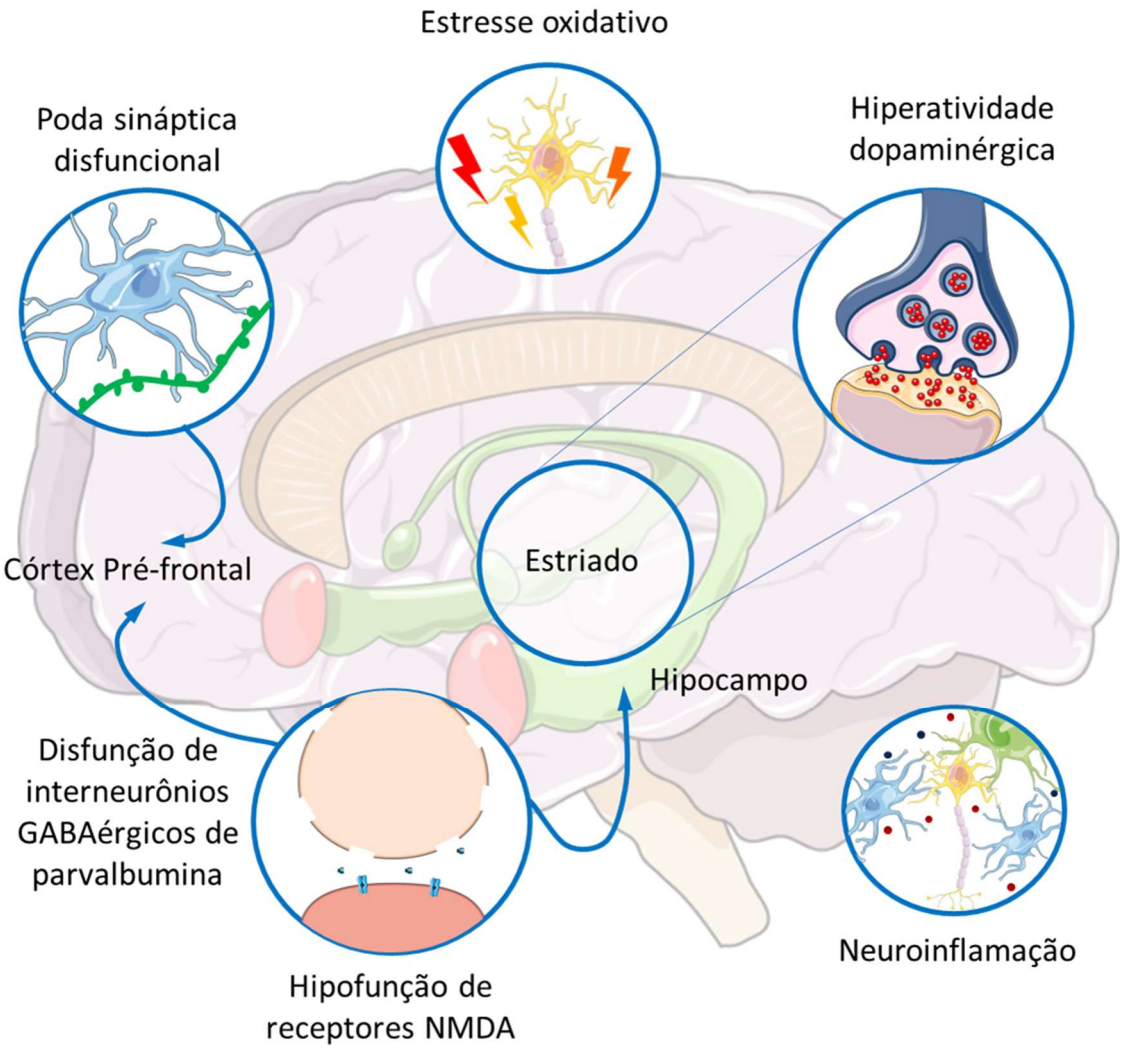

Figura 1. Principais fatores associados à patofisiologia da esquizofrenia (baseado em Grace e Gomes 2019; Sonnenschein, Gomes, e Grace 2020; Cabungcal et al. 2013; Hardingham e Do 2016).

1.1.3. Tratamentos farmacológico e não farmacológicos da esquizofrenia

Até o momento, não existe cura para a esquizofrenia e o tratamento farmacológico é paliativo. De acordo com as principais diretrizes, o tratamento tanto na fase aguda quanto na fase crônica ou de manutenção é feito principalmente com fármacos da classe dos antipsicóticos (Huhn et al. 2019; Chisholm et al. 2008). De forma geral, os antipsicóticos são divididos em duas classes: os típicos, ou de primeira geração, e os atípicos, ou de segunda geração. Todos os antipsicóticos disponíveis até o momento são

antagonistas do receptor D_2 de dopamina; no entanto, os atípicos também são antagonistas do receptor 5-HT_{2A} de serotonina (McCutcheon, Reis Marques, e Howes 2020).

Os antipsicóticos são eficazes apenas no tratamento dos sintomas positivos, não apresentando eficácia sobre os sintomas negativos e cognitivos do transtorno (Keefe et al. 2007; McCutcheon, Reis Marques, e Howes 2020). Além disso, os antipsicóticos estão associados a efeitos adversos como hipotensão postural, efeitos anticolinérgicos (boca seca, visão borrada, constipação), ganho de peso e sedação (Lally e MacCabe 2015; Huhn et al. 2019; Kahn et al. 2015; Owen, Sawa, e Mortensen 2016). Além disso, em alguns casos os antipsicóticos podem produzir efeitos adversos graves como efeitos extrapiramidais, discinesias, distúrbios cardiometabólicos (diabetes, eventos cardiovasculares, obesidade), hiperprolactinemia e até mesmo agranulocitose, um efeito adverso potencialmente fatal (Kahn et al. 2015). Ainda, muitos pacientes são refratários aos tratamentos farmacológicos disponíveis, não respondendo ou respondendo pouco aos antipsicóticos (Tiihonen et al. 2017).

Alguns tipos de intervenções psicossociais têm demonstrado eficácia quando utilizadas em conjunto com o tratamento antipsicótico, incluindo treinamento de habilidades sociais (Kurtz e Mueser 2008), terapia cognitivo-comportamental (Jauhar et al. 2014), tratamento comunitário assertivo (Coldwell e Bender 2007), intervenção em crises (Joy, Adams, e Rice 2000) e emprego apoiado (Campbell, Bond, e Drake 2011). Estudos demonstram que o exercício físico pode melhorar consideravelmente o estado mental e os sintomas negativos em pacientes (Girdler, Confino, e Woesner 2019; Ho, Dahle, e Noordsy 2018; Gorczynski e Faulkner 2010). Além disso, as intervenções familiares podem ajudar a reduzir as taxas de recaída (Claxton, Onwumere, e Fornells-Ambrojo 2017; Pharoah et al. 2010). Contudo, o tratamento farmacológico é considerado o tratamento padrão de primeira escolha (Lally e MacCabe 2015; Huhn et al. 2019; Correll 2020).

Nesse contexto, há necessidade de desenvolvimento de novos fármacos para tratamento da esquizofrenia, com melhor perfil de eficácia e menos efeitos adversos. Com esse objetivo, um grande esforço científico tem sido empregado na busca por uma maior compreensão dos mecanismos etiológicos e patofisiológicos da esquizofrenia, assim como para a descoberta e desenvolvimento de novos tratamentos farmacológicos.

1.2. Modelos animais de esquizofrenia

Modelos animais são uma ferramenta pré-clínica importante para o estudo dos mecanismos fisiopatológicos dos transtornos neuropsiquiátricos, assim como para o desenvolvimento e caracterização de novos fármacos. Os modelos animais possibilitam realizar monitoramento invasivo de alterações estruturais e moleculares subjacentes à doença, assim como testar o efeito de novos fármacos ou intervenções, o que, por questões éticas, seria impossível realizar em humanos (Powell e Miyakawa 2006). No entanto, o principal desafio científico em modelos animais de transtornos psiquiátricos é como avaliar alguns dos principais sintomas, como por exemplo os sintomas relacionados a pensamentos e percepção, que são características exclusivamente humanas.

Nas últimas décadas, diversos modelos de esquizofrenia foram desenvolvidos e validados, tornando possível o estudo de diferentes endofenótipos desse complexo transtorno. Um endofenótipo é um traço biológico que reflete a função de um sistema biológico (razoavelmente hereditário) e está mais relacionado à causa da doença do que o fenótipo clínico amplo (Meyer-Lindenberg et al. 2006). Ou seja, é a conexão entre os sintomas comportamentais e os fenótipos estruturais neurobiológicos. De forma geral, existem três classes de modelos animais de esquizofrenia: modelos desenvolvimentais, modelos farmacológicos e modelos genéticos (Winship et al. 2019). Os modelos animais farmacológicos de esquizofrenia são utilizados há décadas e possuem diferentes graus de validade de face, construção ou preditiva (Jones, Watson, e Fone 2011; Winship et al. 2019). A principal razão por trás de um número tão vasto de modelos é que não existe um único modelo animal que mimetize todos os aspectos de um transtorno psiquiátrico, sendo necessário o uso de diferentes modelos capazes de mimetizar diferentes aspectos do transtorno, com diferentes graus de validade.

A Figura 2 apresenta um esquema dos diferentes tipos de validade (face, construção e preditiva) que foram descritas em modelos animais de esquizofrenia (Jones, Watson, e Fone 2011; Winship et al. 2019).

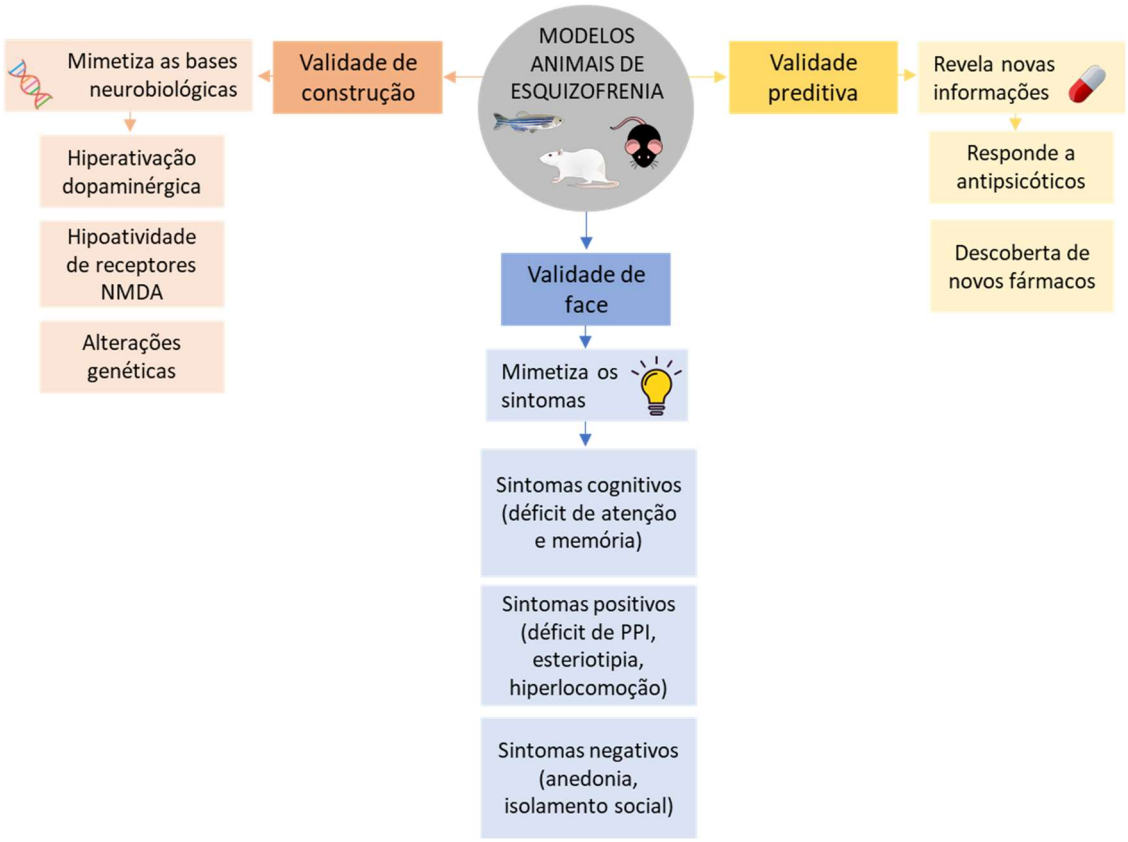

Figura 2. Esquema dos diferentes tipos de validade (face, construção e preditiva) descritas em modelos animais de esquizofrenia (baseado em Jones, Watson, e Fone 2011; Winship et al. 2019). PPI, inibição por pré-pulso da resposta de sobressalto.

Alguns sintomas da esquizofrenia, como os delírios e as alucinações, dependem exclusivamente da descrição e percepção do paciente e tornam desafiadora a interpretação dos resultados obtidos em modelos animais (Canetta e Kellendonk 2018). Uma alternativa é avaliar outras respostas relacionadas com o aumento da atividade dopaminérgica, como por exemplo o aumento da atividade locomotora ou de comportamentos estereotipados em resposta à exposição a drogas como anfetaminas ou antagonistas do receptor NMDA. Outras alterações frequentemente observadas em modelos animais farmacológicos de esquizofrenia são o déficit na inibição por pré-pulso da resposta de sobressalto (PPI), que mimetiza sintomas sensoriomotores, e a diminuição de interação social, que mimetiza o embotamento social, como correlato de sintoma negativo (Jones, Watson, e Fone 2011). Alguns sintomas negativos e cognitivos, como isolamento social e déficit de memória e de atenção também podem ser avaliados em modelos farmacológicos de esquizofrenia (Jones, Watson, e Fone 2011; Winship et al.

2019). Esses testes geralmente fornecem uma avaliação comportamental com validade de face e preditiva, através do bloqueio dos sintomas com a administração de antipsicóticos (Jones, Watson, e Fone 2011; Winship et al. 2019). Assim, as drogas usadas com mais frequência em modelos animais de esquizofrenia são os agonistas dopaminérgicos, como anfetamina e apomorfina, e os antagonistas não competitivos do receptor NMDA, como fenciclidina, cetamina e MK-801 (Winship et al. 2019). Alguns modelos farmacológicos também apresentam validade de construção, apresentando estresse oxidativo e neuroinflamação (Aguilar-Valles, Rodrigue, e Matta-Camacho 2020; Cabungcal et al. 2013; Hardingham e Do 2016).

Apesar do avanço constante nessa área de estudo, é preciso ter em mente que os modelos disponíveis ainda apresentam uma série de limitações e desafios de replicabilidade, tornando necessário o desenvolvimento de novos modelos e critérios mais rigorosos de pesquisa (Kafkafi et al. 2018).

1.2.1. Antagonistas do receptor NMDA

Os receptores NMDA são um subtipo de receptor ionotrópico de glutamato amplamente distribuídos pelo encéfalo (Paoletti, Bellone, e Zhou 2013). Esses receptores são acoplados a canais permeáveis a sódio e cálcio e, quando ativados, geram potenciais pós-sinápticos excitatórios. Os receptores NMDA tem um papel importante em uma série de funções encefálicas, como aprendizado, memória e plasticidade sináptica (Paoletti, Bellone, e Zhou 2013).

Compostos que possuem ação antagonista dos receptores NMDA, como o MK-801, a cetamina e a fenciclidina, induzem estado psicótico (alucinações e delírios) e déficits cognitivos em indivíduos saudáveis (Adler et al. 1999; Allen e Young 1978), e exacerbam sintomas psicóticos em pacientes com esquizofrenia, o que sugere o papel central desses receptores na patofisiologia da esquizofrenia (Lahti et al. 1995; Lahti et al. 2001). Modelos farmacológicos de esquizofrenia baseados na exposição a antagonistas de receptores NMDA mimetizam a hipofunção dos receptores NMDA e a disfunção dos interneurônios GABAérgicos onde estão localizados, levando a uma desinibição da via e consequente hiperativação dopaminérgica subcortical (Nakazawa e Sapkota 2020). Dessa forma, os antagonistas de receptores NMDA têm sido amplamente utilizados como importantes agentes farmacológicos em modelos animais de

esquizofrenia.

Comportamentos como hiperlocomoção e estereotipia são alterações robustas observadas após exposição a antagonistas de receptores NMDA. Essas alterações comportamentais são frequentemente relacionadas aos sintomas positivos, pois resultam da hiperatividade nos neurônios dopaminérgicos (Jones, Watson, e Fone 2011). Além disso, os antagonistas de receptores NMDA induzem mudanças comportamentais relacionadas a sintomas negativos e cognitivos, como comprometimento da memória, déficit sensório-motor e disfunção da interação social (Winship et al. 2019). Essas drogas, portanto, levam a mudanças comportamentais que se assemelham ao espectro de sintomas positivos, negativos e cognitivos observados em pacientes com esquizofrenia.

1.2.2. Agonistas dopaminérgicos

A dopamina é um neurotransmissor com papel modulador complexo, relacionada a comportamentos de motivação, predição de recompensas, atenção, aprendizado e controle motor (Berke 2018). A dopamina é sintetizada tanto no Sistema Nervoso Central (SNC) como no sistema nervoso periférico e, até o momento, foram identificados 5 tipos de receptores (D_1, D_2, D_3, D_4 e D_5) (Klein et al. 2019). Os receptores D_1 e D_5 são membros da família de receptores de dopamina do tipo D_1. Apesar de terem expressão generalizada em todo o encéfalo, os receptores D_1 tem especial importância em regiões corticais do encéfalo. Os receptores D_2, D_3 e D_4 são membros da família tipo D_2. Os receptores D_2 estão localizados em áreas subcorticais, como o estriado, e possuem especial importância no estudo da esquizofrenia (Klein et al. 2019). Os neurônios dopaminérgicos se propagam através do encéfalo dos mamíferos em vias específicas, sendo as principais a via mesolímbica, a via mesocortical, a via tuberoinfundibular e a via nigroestriatal (Klein et al. 2019). Por atuarem como agonistas dopaminérgicos, as anfetaminas atuam aumentando a transmissão dopaminérgica, sendo frequentemente utilizadas na pesquisa pré-clínica no estudo de condições relacionadas a alterações dopaminérgicas, como adição e transtornos psicóticos.

Estudos farmacológicos e exames de imagem em pacientes com esquizofrenia demonstram que há uma hiperatividade da via mesolímbica, e isso está relacionado principalmente aos sintomas positivos (Kesby et al. 2018; McCutcheon, Krystal, e Howes 2020). A administração de anfetaminas em seres humanos induz psicose

semelhante aos sintomas positivos da esquizofrenia e em modelos animais está relacionada a alterações comportamentais e bioquímicas que mimetizam aspectos importantes desses sintomas resultantes da hiperatividade dopaminérgica (Connell 1957; Bell 1973; Bramness et al. 2012; Chen et al. 2003; Jones, Watson, e Fone 2011). Além disso, a administração repetida e intermitente de anfetaminas em roedores resulta em uma sensibilização persistente caracterizada por hiperatividade exagerada em resposta a uma exposição aguda de anfetamina (Featherstone et al. 2008). Por outro lado, fármacos que bloqueiam ou diminuem a transmissão dopaminérgica têm atividade antipsicótica (Lally e MacCabe 2015), que é o caso dos antagonistas do receptor D_2 de dopamina.

1.3. Peixes-zebra como organismo modelo para estudar fenótipos relacionados à esquizofrenia

Camundongos e ratos são os organismos modelo mais utilizados na neurociência comportamental e, da mesma forma, a maioria dos estudos em modelos de esquizofrenia disponíveis na literatura científica foram realizados em roedores. Apesar da importância desses modelos na pesquisa em neurociências, (Burrows e Hannan 2016; Weber-Stadlbauer e Meyer 2019), utilizar apenas camundongos e ratos como animais modelo para estudar aspectos relacionados à esquizofrenia, que possuem características específicas da espécie, acaba sendo uma fonte de viés que pode limitar a validade externa dos estudos na área (Weber-Stadlbauer e Meyer 2019; Gerlai 2019). Isso poderia ser evitado por abordagens entre espécies e com um foco em aspectos de comportamento evolutivamente conservados (Burrows e Hannan 2016; Weber-Stadlbauer e Meyer 2019). Ainda, utilizar apenas modelos em roedores pode levar a descobertas superinterpretadas ou simplificadas quando os dados de outros animais não estão disponíveis, afetando a translabilidade dos resultados obtidos em animais para as condições humanas (Lambert, Kent, e Vavra 2019). Por essas razões, fica clara a necessidade de desenvolvimento de novos modelos animais em organismos alternativos, como o peixe-zebra.

O peixe-zebra se apresenta como uma opção efetiva de animal modelo vertebrado no estudo de endofenótipos relacionados a transtornos neuropsiquiátricos, permitindo uma análise comportamental de alto rendimento para a investigação de novos fármacos na área da neuropsiquiatria (Khan et al. 2017; Kalueff, Stewart, e Gerlai 2014). Apesar

das diferenças existentes entre o ser humano e o peixe-zebra, a arquitetura neuronal, incluindo receptores e tipos celulares presentes no SNC, permanece conservada (Bruni et al. 2016). Diversas classes de fármacos que atuam em alvos moleculares no SNC afetam padrões comportamentais no peixe-zebra (Rihel et al. 2010; Bruni et al. 2016). Dessa forma, o peixe-zebra tem sido amplamente utilizado em diversos modelos de transtornos neuropsiquiátricos visando uma rápida identificação preditiva de compostos neuroativos (Khan et al. 2017; Kalueff, Stewart, e Gerlai 2014).

As semelhanças na arquitetura neural no SNC apontam para a possibilidade de induzir fenótipos semelhantes à esquizofrenia nessa espécie e investigar os efeitos de drogas psicotrópicas no comportamento e neuroquímica. Neurônios catecolaminérgicos e receptores dopaminérgicos com estrutura e função semelhantes aos dos mamíferos são encontrados em diferentes regiões do encéfalo do peixe-zebra, como o diencéfalo e o telencéfalo (Panula et al. 2010; Rink e Guo 2004; Rink e Wullimann 2001; Parker et al. 2013). As vias glutamatérgicas e GABAérgicas, importantes para regular a atividade dos neurônios dopaminérgicos, também estão presentes, integrando tanto sistemas de interneurônios quanto vias neurais longas (Panula et al. 2006). O peixe-zebra apresenta famílias conservadas de receptores NMDA (Cox, Kucenas, e Voigt 2005), alvo de drogas como o MK-801 e a cetamina. Além disso, o GABA é amplamente produzido no encéfalo e na medula espinhal de peixes-zebra por interneurônios, ajudando a modular diferentes sistemas neurais relacionados à esquizofrenia e outros transtornos neuropsiquiátricos (McCarroll et al. 2019).

Existem algumas diferenças importantes entre o sistema glutamatérgico e dopaminérgico no encéfalo dos peixes-zebra em comparação com os mamíferos, principalmente no número de genes que codificam proteínas, como resultado do evento de duplicação de genes em teleósteos (Horzmann e Freeman 2016). Na maioria dos casos, o peixe-zebra tem vários parálogos para cada gene humano e tem 13 genes putativos que codificam receptores ionotrópicos do tipo NMDA, enquanto os humanos têm 7 (Horzmann e Freeman 2016). O sistema glutamatérgico, incluindo seus principais componentes, vias metabólicas e funções, é compartilhado e análogo entre peixes-zebra e mamíferos (Horzmann e Freeman 2016). Até onde se sabe, não há estudos na literatura que investiguem os mecanismos farmacodinâmicos da ligação de antagonistas do receptor NMDA e de agonistas dopaminérgicos no encéfalo do peixes-zebra. No entanto, as semelhanças glutamatérgicas e dopaminérgicas apontam para um mecanismo

de ação semelhante de antagonistas do receptor NMDA e de agonistas dopaminérgicos entre peixes-zebra e mamíferos.

Neurônios catecolaminérgicos e receptores dopaminérgicos com estrutura e função semelhantes aos dos mamíferos são encontrados em diferentes regiões do encéfalo do peixe-zebra, como o diencéfalo e o telencéfalo (Panula et al. 2010; Panula et al. 2006; Rink e Guo 2004; Rink e Wullimann 2001). No entanto, diferenças nos circuitos neurais de regulação de sistema motores centrais do peixe-zebra em relação a outros mamíferos já foram descritas (Ryczko et al. 2017). Uma diferença óbvia é o fato de que os peixes estão nadando a maior parte do tempo. Acredita-se que a contribuição glutamatérgica é essencial para a locomoção dos peixes, enquanto a contribuição dopaminérgica fornece modulação adicional, mas não é essencial para a locomoção (Ryczko et al. 2017). Essa intrincada rede de neurônios, neurotransmissores e receptores preservados em todos os vertebrados permite que os pesquisadores criem e adaptem modelos relevantes para a esquizofrenia usando organismos alternativos, como o peixe-zebra, para impulsionar o conhecimento no campo das neurociências.

Publicações recentes endossaram o uso de peixe-zebra como um organismo modelo para estudar esquizofrenia e realizar a triagem de novos potenciais tratamentos (Bruni et al. 2016; Gawel et al. 2019; Leung e Mourrain 2016); no entanto, existem poucos estudos nessa área quando comparado com a grande quantidade de modelos e testes existentes em roedores. Além disso, os poucos estudos disponíveis na literatura científica que avaliam os efeitos de antagonistas de receptores NMDA e/ou agonistas dopaminérgicos em peixes-zebra muitas vezes apresentam resultados inconclusivos, conflitantes ou não têm replicação. Assim, são necessários mais estudos para determinar se os modelos em peixes-zebra podem fornecer resolução comportamental e bioquímica suficiente para avaliar traços complexos associados a transtornos psiquiátricos como a esquizofrenia.

Assim, a hipótese dessa tese é de que o MK-801 e a anfetamina, por mimetizarem aspectos patofisiológicos dessa condição, são capazes de induzir alterações comportamentais e neuroquímicas relevantes para o estudo da esquizofrenia.

2. OBJETIVOS

2.1. Objetivo geral

O objetivo geral foi investigar os efeitos do MK-801 e da anfetamina sobre parâmetros comportamentais e neuroquímicos relevantes à esquizofrenia em peixes-zebra.

2.2. Objetivos específicos

a. Realizar uma revisão da literatura dos estudos que avaliam os efeitos comportamentais dos antagonistas de receptores NMDA com relevância no estudo da esquizofrenia em peixes-zebra;

b. Avaliar os efeitos do MK-801 em parâmetros comportamentais relevantes à esquizofrenia;

c. Avaliar os efeitos do MK-801 em parâmetros de estresse oxidativo;

d. Avaliar os efeitos da anfetamina em parâmetros comportamentais relevantes à esquizofrenia;

e. Avaliar os efeitos da anfetamina em parâmetros de estresse oxidativo.

3. COLETÂNEA DE ARTIGOS

A tese está organizada no formato de coletânea de artigos científicos.

3.1. Capítulo 1:

Glutamate NMDA Receptor Antagonists With Relevance To Schizophrenia: A Review Of Zebrafish Behavioral Studies

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REVIEW ARTICLE

Glutamate NMDA Receptor Antagonists with Relevance to Schizophrenia: A Review of Zebrafish Behavioral Studies

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Abstract: Schizophrenia pathophysiology is associated with hypofunction of glutamate NMDA receptors (NMDAR) in GABAergic interneurons and dopaminergic hyperactivation in subcortical brain areas. The administration of NMDAR antagonists is used as an animal model that replicates behavioral phenotypes relevant to the positive, negative, and cognitive symptoms of schizophrenia. Such models overwhelmingly rely on rodents, which may lead to species-specific biases and poor translatability. Zebrafish, however, is increasingly used as a model organism to study evolutionarily conserved aspects of behavior. We thus aimed to review and integrate the major findings reported in the zebrafish literature regarding the behavioral effects of NMDAR antagonists with relevance to schizophrenia. We identified 44 research articles that met our inclusion criteria from 590 studies retrieved from MEDLINE (PubMed) and Web of Science databases. Dizocilpine (MK-801) and ketamine were employed in 29 and 10 studies, respectively. The use of other NMDAR antagonists, such as phencyclidine (PCP), APV, memantine, and tiletamine, was described in 6 studies. Frequently reported findings are the social interaction and memory deficits induced by MK-801 and circling behavior induced by ketamine. However, mixed results were described for several locomotor and exploratory parameters in the novel tank and open tank tests. The present review integrates the most relevant results while discussing variation in experimental design and methodological procedures. We conclude that zebrafish is a suitable model organism to study drug-induced behavioral phenotypes relevant to schizophrenia. However, more studies are necessary to further characterize the major differences in behavior as compared to mammals.

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1. INTRODUCTION

Schizophrenia is a chronic and heterogeneous neurodevelopmental mental disorder with a lifetime prevalence of 1% [1]. This condition is characterized by a series of complex positive (delusions, hallucinations, disorganized speech), negative (reduction in spontaneous speech, affective flattening, anhedonia, and social withdrawal), and cognitive symptoms (deficits in memory, attention, and executive function) [1]. These symptoms are disabling and it is estimated that the life expectancy of patients with schizophrenia is reduced by 20 years compared with the general population [2]. Antipsychotic drugs are relatively effective in treating positive symptoms; however, they are not effective against the negative and cognitive symptoms, strongly associated with functional impairment [1]. The mechanisms underlying schizophrenia pathophysiology are not completely understood, but the involvement of glutamatergic, GABAergic, and dopaminergic dysfunction has been noted in clinical and preclinical studies [3, 4]. The hypofunction of NMDA receptors (NMDAR) in fast-spiking parvalbumin-positive GABAergic interneurons may trigger an imbalance in brain circuits resulting in dopaminergic hyperactivity in subcortical areas [5, 6].

Animal models of schizophrenia enable the investigation of schizophrenia neurobiology and the search for new therapeutic interventions [7]. Administration of NMDAR antago-

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nists, such as ketamine [8] or PCP [9], induces delusions and hallucinations in healthy individuals and exacerbates negative and cognitive symptoms in patients with schizophrenia [10, 11]. NMDAR antagonists have thus been used to mimic NMDAR hypofunction and serve as an important pharmacological tool in animal models of schizophrenia. Hyperlocomotion and stereotypic behavior are robust behavioral changes observed after exposure to NMDAR antagonists, such as MK-801, ketamine, and PCP. They are often related to positive symptoms as they result from hyperactivity in dopaminergic neurons [11]. Moreover, NMDAR antagonists induce behavioral changes related to negative and cognitive symptoms, such as memory impairment, sensorimotor deficit, and social interaction dysfunction [12]. These drugs thus lead to behavioral changes that resemble the full spectrum of positive, negative, and cognitive symptoms observed in patients with schizophrenia [5, 6, 13, 14].

Together with statistical and methodological inconsistencies, species-specific characteristics of the animal models are another extremely relevant source of bias in behavioral neuroscience [15, 16]. Researchers have focused on a few species to conduct their experiments throughout the years, even though a vast range of behavioral responses are singularly displayed by some species. Mice and rats are the most frequently used model organisms in behavioral science. However, this overwhelming reliance on rodents may lead to species-specific biases and poor translatability, which could be avoided by cross-species approaches and a focus on evolutionarily conserved aspects of behavior [15, 17]. It may also lead to overinterpreted or simplified findings when data from other animals are not available, impacting the translation of animal results to human conditions [18].

In the last years, a series of publications have endorsed the use of zebrafish as an alternative model animal to study brain disorders and conduct high-throughput drug screening for potential novel treatments [19-21]. Although several studies investigated the effects of NMDAR antagonism in zebrafish, some findings are inconclusive, conflicting, or lack replication. We thus aimed to review and integrate the major findings reported in the zebrafish literature regarding the behavioral effects of NMDAR antagonists with relevance to schizophrenia.

We conducted a literature search for "zebrafish AND ('glutamatergic antagonist' OR 'NMDA antagonist' OR dizocilpine OR MK-801 OR ketamine OR phencyclidine OR PCP OR memantine)" limited to January 1st 2000 and September 30th 2020 in MEDLINE *via* PubMed (n=293) and Web of Science (n=345) databases. After the removal of duplicates, the total number of records was n=393. We then excluded 266 entries based on title and abstract screening for the following reasons: review articles, retracted articles, conference abstracts, book chapters, not available in English, no use of zebrafish as a model organism, no report of behavioral outcomes, and no administration of an NMDA antagonist drug. The full-texts were analyzed from the remaining 127 studies, and we excluded 83 based on the same criteria. A final 44 articles were included in this review. A detailed overview of the studies is presented in Table **1**.

2. ZEBRAFISH AS A MODEL ORGANISM FOR MODELING SCHIZOPHRENIA

The use of zebrafish as a model organism is quite recent as compared to other animals. It has been increasingly recognized that zebrafish is suitable as an alternative model to broaden the number of species used in behavioral science, allowing the comparison of interventions on species with distinct natural histories [22-24]. Although zebrafish are simple organisms making them an easy target for genetic manipulation [25], they present highly genetic homology to humans [25, 26]. Thus, a series of complex behaviors can be readily analyzed in this experimental model [27, 28]. Zebrafish also offer numerous other advantages, such as their low cost for acquisition and maintenance, easy reproduction, and rapid development compared to other animals [27]. Taken together, these characteristics reinforce the potential of zebrafish as a tool to study mechanisms and treatments for brain disorders such as anxiety, epilepsy, and schizophrenia [19-21, 23, 29].

Zebrafish possess a conserved neuronal architecture, with cells, neurotransmitters, and receptors analogous to those present in mammals [30]. These similarities point to the possibility of inducing schizophrenia-like phenotypes in zebrafish and investigating the impact of psychotropic drugs on the behavior and neurochemistry of zebrafish. Catecholaminergic neurons and dopaminergic receptors with structure and function similar to those of mammals can be found in different zebrafish brain regions like the diencephalon and telencephalon [30-33]. Glutamatergic and GABAergic pathways, important for regulating the activity of dopaminergic neurons, are present as well, integrating both interneuron systems and long pathways [34]. Zebrafish present conserved families of NMDAR [35], the target of drugs such as dizocilpine (MK-801), and ketamine. On the other hand, GABA is extensively produced in the brain and spinal cord of zebrafish by interneurons, helping modulate different neural systems related to schizophrenia and several other conditions [36].

There are some important differences between the glutamatergic system of zebrafish brain compared to mammals, mainly in the number of genes encoding proteins, as a result of the teleost gene duplication event [37]. In most cases, zebrafish have multiple paralogs for each human gene and have 13 putative genes that code for NMDA-type ionotropic receptors, while humans have 7 [37]. Some genetic manipulation studies highlight the importance of the GluN1 receptor subunits. CRISPR-mediated manipulations to the genes grin1a and grin1b, which encode obligatory GluN1 receptor subunits, have been reported in zebrafish [38]. The knockout of both these genes directly impairs NMDAR-mediated synaptic transmission and the mutant animals present some atypical behaviors such as hyperactivity, deficits in prey capture, and abnormal responses to light and acoustic stimuli [38]. The glutamatergic system, including its main components, metabolic pathways and function, is shared and analogous between zebrafish and mammals [37]. To our knowledge, there are no studies in the literature that investigated the pharmacodynamic mechanisms of the binding of NMDAR antagonists in the zebrafish brain. However,

Table 1. Published studies reporting zebrafish behavioral analysis involving NMDA antagonists.

Drug	Delivery/Duration	Concent/Dose	Age	Behavioral Domain	Main Findings	Refs.
APV	Water, acute	100, 200 µM	6-8 dpf	Sensory response	**Rapid escape reflex** ↑ startle response	[121]
Ketamine	Water, acute	0.2% (v/v)	4-8 mpf	Locomotion; stress response	**Locomotor activity** ↑ circling behavior **Ventilatory response to hypoxia** ↓ gill movements **Response to hypoxic stress** ↓ response (body pulses)	[93]
		2, 20, 40 mg/L	5-7 mpf	Locomotion; anxiety; social behavior	**NTT** ↑ rotations ↑ time in the upper zone ↓ latency and transitions to the upper zone = distance traveled **OTT** = circling behavior = distance traveled and speed **LDT** ↑ entries and time in the white zone **SI** ↑ inter-fish distance ↑ entries in all arms	[94]
		5, 20, 40, 60 mg/L	6-8 mpf	Anxiety	**LDT** ↓ latency to enter the white zone ↑ time in the white zone and crossings = average entry duration	[96]
		2, 20, 40 mg/L	4-6 mpf	Aggressiveness; locomotion	**Aggressiveness** ↑ aggressive episodes (latency to attack and time spent in the mirror zone) ↓ absolute turn angle ↑ distance traveled and rotations	[97]
		1 g/mL	Adult	Locomotion	**Locomotor activity** ↑ erratic, acceleration, and circular movements	[98]
		0.1, 1, 10, 50 µM	5 dpf	Locomotion	**Locomotor activity** ↑ total distance	[99]
		72.9 µM	28 dpf	Social behavior; aggressiveness	**SI** ↑ inter-fish and nearest neighbor distance **Aggressiveness** ↓ aggressive episodes (time and distance in the mirror zone)	[102]
		0.2, 0.4, 0.8 mg/mL	2, 5, 10 hpf	Locomotion; anxiety; social behavior	**Locomotor activity** ↑ distance traveled in the center zone ↑ time in the upper zone = distance traveled and speed **SI** = inter-fish and nearest neighbor distance	[128]

(Table 1) contd….

Drug	Delivery/Duration	Concent/Dose	Age	Behavioral Domain	Main Findings	Refs.
Ketamine	-	50, 70, 90 mg/L	2 hpf	Locomotion; anxiety; social behavior	**Locomotor activity** ↑ distance traveled in the center zone ↑ avoidance behavior **SI** = inter-fish and nearest neighbor distance	[101]
	Water, chronic	0.2% (v/v)	4-8 mpf	Locomotion, stress response	**Locomotor activity** ↑ circling behavior Ventilatory response to hypoxia ↓ gill movements Response to hypoxic stress ↓ response (body pulses)	[93]
		20 mg/L	> 90 dpf	Anxiety	**NTT** = time in the upper zone	[95]
Memantine	Water, acute	5, 10, 20, 50, 100 µM	5 dpf	Locomotion	**Locomotor activity** = speed	[78]
		3, 10, 30 µM	6 dpf	Sensory response	**Acoustic startle response** ↑ distance traveled in response to auditory stimuli	[119]
MK-801	i.c.v. injection	8-12 nL of 100 µM/fish	24 hpf	Sensory response	**Spontaneous coiling** = duration of spontaneous coiling ↑ touch response	[83]
	i.m. injection	10 µg/fish	Adult	Learning and memory	**Active avoidance** = memory retention (association between light and shock)	[64]
	i.p. injection	0.1, 0.3, 0.5, 1, 2 mg/kg	4 mpf	Learning and memory	**Inhibitory avoidance** ↓ latency to enter the dark zone (memory impairment) **NTT** ↑ distance and time in the upper = male and female Taurine prevented these effects	[61]
		0.2, 2 mg/kg	4 mpf	Anxiety	**LDT** ↑ time, entries, and distance in the white zone ↑ thigmotaxis	[81]
	r.o. injection	4 µL of 12.5 µM/fish	6-12 mpf	Locomotion; anxiety; social behavior	**NTT** = distance, speed, meander, freezing, latency to the upper zone **SI** = inter-fish distance	[72]
	Water, acute	0.1, 1 µM	10 dpf	Learning and memory	**NOR** = postures and eye movement towards the novel objects	[58]
		20 µM	Adult	Learning and memory	**Inhibitory avoidance** ↓ latency to enter the dark zone (memory impairment)	[59]
		5 µM	< 8 mpf	Learning and memory; social behavior	**Inhibitory avoidance** ↓ latency to enter the dark zone (memory impairment) **SI** ↓ time in the interaction zone Sulpiride and olanzapine, but not haloperidol, reversed these effects	[60]

(Table 1) contd….

Drug	Delivery/ Duration	Concent/ Dose	Age	Behavioral Domain	Main Findings	Refs.
MK-801	-	20 µM	3-6 mpf	Learning and memory; locomotion	**Inhibitory avoidance** ↓ latency to enter the deep zone (memory impairment) **Locomotor activity** = distance and mean speed	[63]
		20 µM	3-12 mpf	Learning and memory	**Contextual fear conditioning** ↓ distance associated with shock (memory impairment)	[62]
		2, 20, 100 µM	6-8 mpf	Locomotion; anxiety; social behavior	**OTT** ↑ circling behavior = creeping, thrashing on the side, thrashing on the bottom, floating, foraging, freezing, sinking, swimming, tilting, jumping **LDT** = time in the white zone **SI** ↓ time in the interaction zone	[68]
		5, 10, 20 µM	> 8 mpf	Learning and memory; locomotion	**Y-maze** ↓ time in the novel arm (memory impairment) **Locomotor activity** = distance, speed, absolute turn angle, crossings	[65]
		5, 10 µM	6-7 mpf	Learning and memory	**NOR** ↓ time exploring new object (memory impairment) ↑ distance traveled ↓ immobile time	[67]
		100 µM	21 dpf	Social behavior	**SI** ↓ time in the social zone = locomotor activity	[69]
		5 µM	6-8 mpf	Social behavior; aggressiveness	**SI** ↓ time in the social zone **Aggressiveness** ↓ time in the opponent zone Oxytocin and carbetocin prevented these effects	[70]
		1, 2, 5 µM	6 mpf	Social behavior	**SI** ↓ inter-fish distance ↑ distance	[71]
		100, 200, 400 nM	3 dpf	Locomotion	**NTT** = distance and speed	[129]
		5, 10, 15, 20 µM	12 mpf	Locomotion	**NTT** ↑ distance	[73]
		20 µM	Adult	Locomotion; anxiety	**NTT** ↑ total distance, speed, and time in the upper zone Haloperidol and olanzapine reversed these effects	[74]
		2, 20 µM	Adult	Locomotion; preference	**OTT** ↑ circling behavior ↓ crossings ↑ time in the upper zone **Place preference** ↓ time in the enriched chamber = male and female	[75]

(Table 1) contd….

Drug	Delivery/ Duration	Concent/ Dose	Age	Behavioral Domain	Main Findings	Refs.
-	-	20 μM	6 dpf	Locomotion; predatory behavior	**Locomotor activity** ↓ spontaneous activity ↑ swim length and speed **Predatory behavior** ↓ paramecium captured	[38]
		1, 5, 20 μM	30, 60, 120 dpf; 2 ypf	Locomotion; anxiety	**NTT** ↑ distance and time in the upper zone	[76]
		5, 20, 100, 200 μM	7 dpf	Locomotion	**Locomotor activity** ↑ AB strain distance traveled ↓ TU strain distance traveled	[77]
		1, 5, 10, 20, 50, 100 μM	5 dpf	Locomotion	**Locomotor activity** ↑ spontaneous locomotion SKF-83566 and sulpiride did not alter these effects	[78]
		1, 5, 20 μM	15 dpf	Locomotion	**NTT** ↓ distance	[79]
		5, 10, 15, 20 μM	6-8 mpf	Anxiety	**OTT** ↑ time in the upper zone Caffeine and DPCPX prevented this effect	[80]
		10, 20, 50, 100, 200 μM	7 dpf	Anxiety	**LDT** ↓ distance traveled under light and dark	[82]
		20 μM	Adult	Learning and memory	**Passive avoidance** ↓ latency to enter the dark zone (memory impairment)	[130]
		20 μM	6-8 mpf	Learning and memory	**Plus maze** ↓ latency to enter and time in the conspecifics chamber (memory impairment)	[66]
PCP	Water, acute	0.5, 1; 3 mg/L	5-8 mpf	Locomotion; anxiety; social behavior	**NTT** ↓ latency to the upper zone, freezing, and immobility ↑ erratic movements = distance traveled and speed **OTT** ↑ circling **SI** = inter-fish and nearest neighbor distance	[111]
		3 mg/L	5-7 mpf	Locomotion; anxiety	**NTT** ↑ circling behavior	[112]
Tiletamine	Water, acute	1, 5, 10 mg/L	5-7 mpf	Locomotion; anxiety	**NTT** ↓ entries to the upper zone ↑ erratic movements and freezing	[120]

Abbreviations: APV, DL-2-amino-5-phosphonopentanoic acid; concent, concentration; dpf, days post-fertilization; DPCPX, selective adenosine A1 receptor antagonist; hpf, hours post-fertilization; i.c.v., intracerebroventricular; i.p., intraperitoneal; LDT, light/dark test; MK-801, dizocilpine; mpf, months post-fertilization; NOR, novel object recognition; NTT, novel tank test; OTT, open tank test; PCP, phencyclidine; r.o., retro-orbital; SI, social interaction test; SKF-83566, selective D1 receptor antagonist; water, water exposure; ↑, increased or higher as compared to control group; ↓, decreased or lower as compared to control group; =, no difference compared to control group.

the glutamatergic similarities point to a similar mechanism of action of NMDAR antagonists between zebrafish and mammals.

This intricate network of neurons, neurotransmitters, and receptors preserved throughout the vertebrates allows researchers to create and adapt models relevant to schizophrenia using different animals, like the zebrafish, to push forward the knowledge on this field. However, a full picture of the zebrafish behavioral repertoire that is reliably altered by interventions known to induce schizophrenia-like pheno-

types in mammals is needed to identify eventual differences and limitations of this species.

3. GLUTAMATE NMDA RECEPTOR ANTAGONISTS AS PHARMACOLOGICAL TOOLS TO MODEL SCHIZOPHRENIA

The ionotropic NMDAR is a cation channel that produces excitatory postsynaptic potentials when activated and has an important role in a series of brain functions, such as learning, memory, and synaptic plasticity [39]. Several studies have shown that NMDAR antagonists can induce transient schizophrenia-like symptoms in animals and humans [40-42]. Besides that, exposure to these drugs can exacerbate symptoms in patients [43]. Studies using imaging techniques and postmortem analysis of the brain of patients with schizophrenia, as well as results obtained from experiments with animal models, [13, 44, 45] have suggested there is a dysregulation of hippocampal and cortical fast-spiking GABAergic parvalbumin-positive interneurons and hypofunctional NMDAR expressed by these interneurons, which leads to altered excitation-inhibition balance in cortical and subcortical areas of the brain [46-48]. Studies in animal models of schizophrenia have an indispensable role in our understanding of the etiopathogenesis of this condition. Furthermore, animal models in scientific research are an important tool for developing new treatments with therapeutic potential.

Early administration of NMDAR antagonists to rodents [49] and marmoset [50, 51] can mimic aspects of the schizophrenia pathology, assuming that a disturbance during pre- or perinatal brain development results in the manifestation of schizophrenia-like phenotypes in early adulthood [49]. The behavioral changes observed are related to the positive, negative, and cognitive symptoms of schizophrenia, such as hyperlocomotion and stereotypy (related to dopaminergic hyperactivation), deficits in information processing, impairment of cognitive functions (working memory and attention), and social interaction deficits [52]. This pharmacological induction of NMDAR hypofunction leads to disinhibition of excitatory hippocampal neurons and, consequently, disruption of the firing of dopaminergic neurons in the mesolimbic and mesocortical pathways [5, 53]. Acute administration of NMDAR antagonists induces schizophrenia-like symptoms in animals, and this model is often used to predict the effects of drugs with potential antipsychotic properties [54]. Animal models based on repeated administration of NMDAR antagonists can change neurotransmitter systems in the long-term and better recapitulate the natural course and neurobiological alterations associated with schizophrenia.

3.1. Dizocilpine (MK-801)

MK-801 is a potent non-competitive NMDAR antagonist frequently used as a pharmacological tool in rodent schizophrenia models [5, 53]. It is also the more frequently used NMDAR antagonist in zebrafish behavioral studies, accounting for 64.5% of the publications reviewed here (Fig. **1**). In the rodent literature, experimental protocols employ repeated administration of MK-801 in neonatal [55, 56] or adult animals [11]. Acute administration is also used to assess sensitivity to drug-induced locomotor activity [57] in other rodent models of schizophrenia (*e.g*., neonatal lesion of the ventral hippocampus). Studies evaluating the effects of MK-801 on zebrafish behavior mainly assess behavioral paradigms such as learning and memory, social behavior, locomotion, and anxiety (Fig. **1**). Although most experimental protocols vary widely between studies and conflicting findings were reported, some effects such as social and memory deficits seem robust and replicated by different research groups (Fig. **3**).

Most studies have found that MK-801 impairs learning and memory in adult zebrafish, but not in larvae [58]; the inhibitory avoidance protocol was frequently used to assess learning and memory in these studies [59-63]. On the other hand, Xu *et al*. (2007) [64] did not find any effects induced by MK-801 in an active avoidance paradigm. MK-801 also impaired learning and memory in maze tasks [65, 66] and the novel object recognition test [67]. Interestingly, Cognato *et al*. (2012) [65] reports that the experiments were performed using only male zebrafish.

The effects of MK-801 on social behavior in adult zebrafish are robust as most of the reviewed studies observed social interaction deficits without locomotor changes. Seibt *et al*. (2011) [60], Sison *et al*. (2011) [68], Dreosti *et al*. (2015) [69] and Zimmermann *et al*. (2016) [70] observed that MK-801-treated animals present reduced social interaction as compared to controls in a test that measured the time fish spent near a tank containing conspecifics. Interestingly, Seibt *et al*. (2011) [60] performed the experiments using only males and observed that the atypical antipsychotics sulpiride and olanzapine, but not the typical antipsychotic haloperidol, reverse the social deficit. Zimmermann *et al*. (2016) [70] found that oxytocin and carbetocin prevent the social deficit induced by MK-801. In a study performed by Maaswinkel *et al*. (2013) [71], MK-801-induced social deficit was shown by reduced social cohesion in a test that evaluates inter-individual distance in a group of female fish. Only McCutcheon *et al*. (2017) [72] did not find any differences between control and MK-801-treated fish in a shoaling test. However, the administration route was a retro-orbital injection, unlike other studies that performed drug administration *via* aqueous exposure.

Regarding the effects of MK-801 on locomotor activity in adult zebrafish, Tran *et al*. (2016) [73] and Seibt *et al*. (2010) [74] found an increase in total distance traveled, mean speed, and time spent in the upper zone following MK-801 exposure in the novel tank test (NTT). Interestingly, Tran *et al*. [73] observed that hyperlocomotion induced by MK-801 was context-dependent, as it only occurs when the animal is subjected to a new environment (NTT), and no differences were observed when exposure to MK-801 occurs in the house tank. On the other hand, McCutcheon *et al*. (2017) [72], Sison *et al*. (2011) [68], Cognato *et al*. (2012) [65] and Ng *et al*. (2012) [63] failed to observe locomotor changes after MK-801 exposure. Swain *et al*. (2004) [75] observed that adult zebrafish exposed to MK-801 presented more circling behavior than control animals, along with increased time spent in the upper zone of the NTT. The results for MK-801 locomotor effects in larvae are equally conflicting as some articles report higher [38, 76-78], lower [79], or equal levels of locomotor activity as compared to control animals [72].

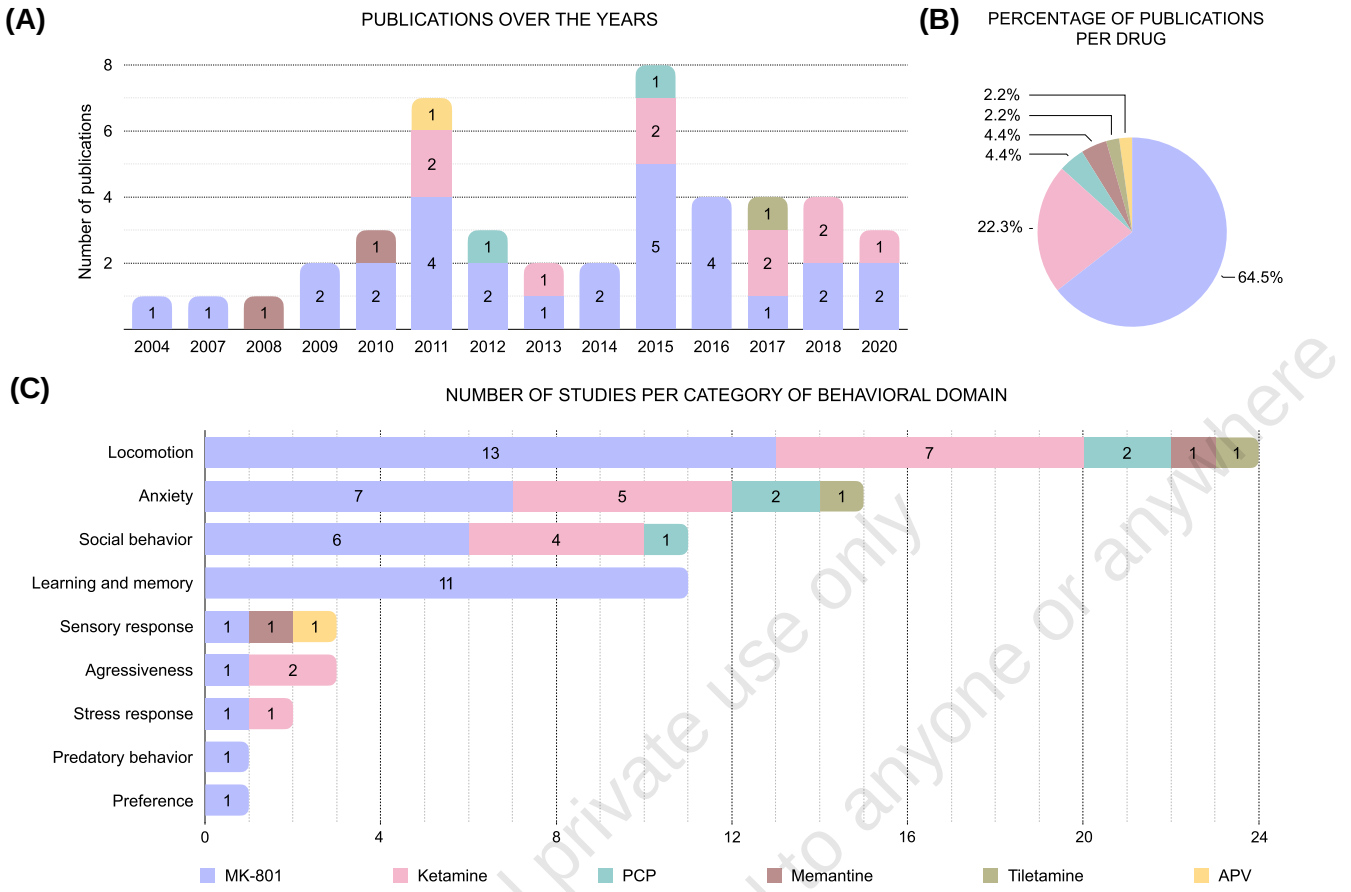

Fig (1). A quantitative overview of the studies reporting the use of NMDAR antagonist drugs (MK-801, ketamine, PCP, memantine, tiletamine, and APV) in zebrafish behavioral research published in peer-reviewed scientific journals between 2000 and 2020. (**A**) The number of publications per year with each drug. (**B**) Percentage of publications with each drug. (**C**) The number of publications per behavioral domain with each drug. APV, DL-2-amino-5-phosphonopentanoic acid, MK-801, dizocilpine; PCP, phencyclidine. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

The effects on other behavioral domains, such as anxiety, sensory response, aggressiveness, and predatory behavior, are less well characterized. However, in the NTT, fish exposed to MK-801 spent more time in the upper zone of the tank [74, 76, 80], which is typically considered an anxiolytic effect. However, as MK-801 altered locomotor activity, including stereotypy-related behaviors such as circling [68], the increased time spent in the upper zone may be better explained by modulation of locomotor instead of anxiety pathways. While Herculano *et al*. (2015) [81] found that MK-801 increases time spent in the white zone during the light/dark test, Sison *et al*. (2011) [68] and Li *et al*. (2018) [82] found no significant differences between treated animals and controls. Pietri *et al*. (2009) [83] found that larvae exposed to MK-801 had an increase of touch response, a sensory response. In the predatory behavior, MK-801 treated larvae captured fewer paramecia as compared to controls [38]. Furthermore, MK-801-treated zebrafish adults were less aggressive than controls in the mirror stimulus-response test [70].

3.2. Ketamine

Ketamine, a dissociative anesthetic, also acts as a non-competitive antagonist of NMDAR [84]. It is also a drug of abuse known as special K, popular in raves and clubs. This drug provokes pro-psychotic effects in healthy individuals [13] and exacerbates psychosis in schizophrenia patients [43, 85]. When administered to rodents, ketamine can generate a series of behavioral and neurochemical alterations that resemble the deficits shown by schizophrenia patients [86].

In rodents, acute administration of ketamine elicits cognitive impairment, disruption of prepulse inhibition of the startle reflex, hyperlocomotion, and social interaction deficits [87-89]. Repeated administration induces the same behavioral phenotypes [90] but also leads to loss of parvalbumin-expressing interneurons [91] and hippocampal hyperactivity [92].

Classical protocols used to evaluate zebrafish behavior, such as the NTT, have been used to understand the impacts of ketamine on fish behavior. When exposed to this drug, zebrafish show behavioral alterations similar to those seen in other animal models. Zakhary *et al*. (2011) [93] demonstrated that acute or repeated exposure (daily for five days) of adult zebrafish leads to increased circling behavior along with decreased gill movements and body pulses in response to stress. Riehl *et al*. (2011) [94] reported that ketamine-treated animals presented more circling behavior, fewer transitions to the upper area of the tank, and more time in this

zone than controls. Pittman and Hylton (2015) [95], on the other hand, reported that repeated ketamine exposure (daily for two weeks) had no impact on the time spent in the upper area of the tank. In the light/dark test, ketamine was associated with more transitions [94, 96] and more time spent in the white area [96]. In a shoaling, test ketamine was observed to increase the average inter-fish distance [94]. Aggressive behavior was also modulated by ketamine: Michelotti *et al*. (2018) [97] reported that lower doses of ketamine enhanced aggressive behavior while higher doses had the opposite effect, reducing the number and duration of aggressive episodes. Furthermore, when using this drug to anesthetize the fish, Martins *et al*. (2018) [98] described that, before the loss of reflexes, zebrafish presented phases of intense, erratic, and circular movements.

Experiments on early developmental stages can also recapitulate behavioral phenotypes observed on schizophrenia models. Suen *et al*. (2013) [99] showed that lower ketamine concentrations increase the distance traveled by larvae, while high concentrations had anesthetic effects. Félix *et al*. (2016 and 2017) [100, 101] reported alterations in exploratory behavior, with an increase of distance traveled in the center of the well and enhanced avoidance behavior towards an aversive stimulus. As seen in adults, Shen *et al*. (2020) [102] showed higher nearest neighbor distance and inter-individual distance in larvae exposed to ketamine than controls.

3.3. Phencyclidine (PCP)

Another non-competitive NMDAR antagonist widely used in schizophrenia research is phencyclidine [84]. Similar to ketamine, PCP is also a drug of abuse and is known to induce a psychotic-like state in humans [103]. Different animal models of schizophrenia using PCP have been developed, with either perinatal or adult acute or chronic drug administration [104]. In rodents, PCP treatment in early developmental stages can induce long-lasting schizophrenia-like phenotypes that manifest in the adult stage [105, 106]. In adults, both acute and chronic administration induce hyperlocomotion [107, 108] and cognitive impairment [109, 110]. Chronic adult treatment also elicits long-lasting behavioral abnormalities, as seen in perinatal models [104].

Only a couple of studies exposing adult zebrafish to PCP have been performed. Kyzar *et al*. (2012) [111] reported that exposure to PCP induced anxiety and locomotor changes in the NTT, including increased erratic movements, reduced latency to the upper zone of the tank, freezing bouts, and immobility time. In contrast, in the open tank test (OTT), PCP increased circling behavior. Stewart *et al*. (2015) [112] evaluated the locomotor activity of zebrafish in a three-dimensional setup and also observed more circling behavior in fish exposed to PCP as compared to controls. Although studies using PCP are still scarce, exposure to the drug evoked behavioral alterations compatible with the expected from other animal models of schizophrenia.

3.4. Other Glutamatergic Antagonists

Other NMDAR antagonists were also employed in zebrafish studies. They are less commonly used to recapitulate schizophrenia-like behaviors in animals, but as the pharmacological target is the same, they can nevertheless induce the relevant phenotypes and were thus included in this review. Memantine appeared in 2 publications, while tiletamine and DL-2-amino-5-phosphonopentanoic acid (APV) appeared in only one (Fig. **1**). As reviewed elsewhere [113], memantine, especially in lower doses, can induce similar effects as ketamine on locomotor and cognitive parameters in rodents. Tiletamine also generates effects comparable to ketamine, reducing social interaction time, and inducing learning deficits in the passive avoidance task [114]. In contrast, most of the research carried out using APV focuses on inhibiting long-term potentiation as a tool to understand key factors of learning and memory rather than to study schizo-

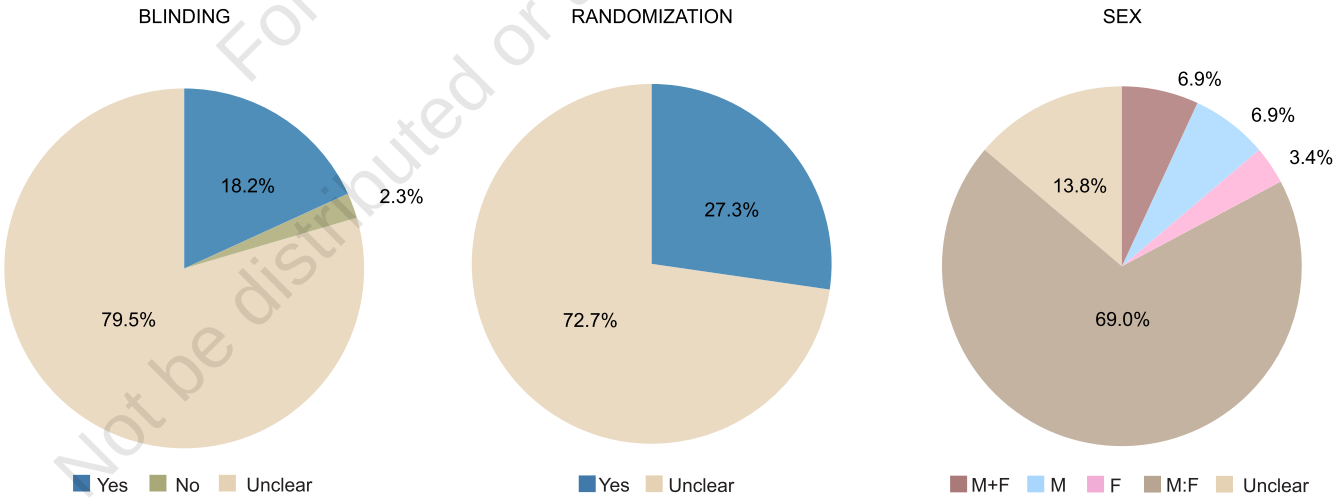

Fig (2). Description of relevant methodological details in the publications included in this review. For blinding and randomization, graphs depict the percentage of publications that reported implementation or not of these practices (unclear was computed when there was no mention). Sex of the animals used was computed as M:F when male and female were included but tested and analyzed as a mixed group, and M+F when male and female fish were discriminated in the experiments. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

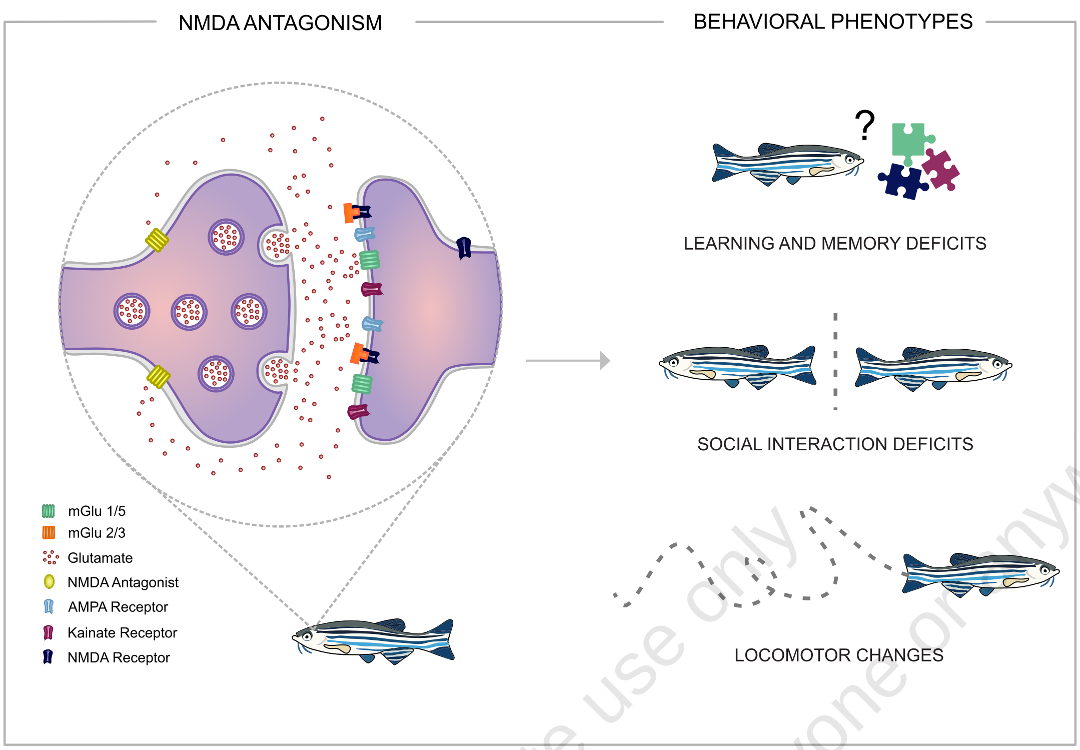

Fig (3). Schematic illustration of the mechanism of action of NMDAR receptor antagonists in the zebrafish brain and behavioral phenotypes induced by the administration of these drugs. AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; mGLU, metabotropic glutamate; NMDA, N-methyl-D-aspartate. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

phrenia [115, 116]. Unlike the other NMDAR antagonist reviewed here, memantine is shown to inhibit distinct subpopulations of receptors depending on the intensity and duration of its activation [117, 118]. Such selectivity may explain the divergent clinical effects compared to the other drugs and could implicate in contrasting outcomes in the behavior of animals.

Best *et al*. (2008) [119] reported that zebrafish larvae exposed to memantine display augmented startle response to an acoustic stimulus. Chen *et al*. (2010) [78] reported that memantine has no effects on the locomotor parameters of zebrafish larvae. In adult zebrafish, tiletamine reduced the upper zone entries and increased erratic movements and freezing frequency and duration in the NTT [120]. Roberts *et al*. (2011) [121] reported that APV reduces the habituation index after spaced training to an acoustic stimulus. Although research investigating these drug effects in zebrafish is still scarce and needs to be replicated, they are important to highlight the importance of NMDAR antagonism as a tool to investigate the mechanisms underlying zebrafish behavior in several domains.

4. REPRODUCIBILITY CHALLENGES

Reproducibility issues are increasingly discussed by the scientific community [122], including zebrafish neuroscience and behavioral researchers [16]. Although variation in protocols and methodologies adopted by different research groups have been pointed out as a source for inconsistent data in preclinical research, standardization is not likely to be a major solution for this problem [123, 124]. Nevertheless, several experimental protocols differed between the studies regarding the size or type of the apparatus used, zebrafish strain and age, drug delivery route, and time and frequency of exposure to drugs, among others (Table **1**). Such differences in methodology among the research groups may explain, at least in part, the conflicting results reported for behavioral parameters such as locomotion, exploratory behavior, and anxiety.

Furthermore, some replication challenges could be avoided by improved reporting of methodological procedures and open access to protocols, code, and data [125, 126]. The critical assessment of the validity of a study's findings also requires that relevant information regarding the experimental design and statistical analysis is reported in detail, including blinding, randomization, and sample size calculation procedures [125]. A matter of concern is that, with rare exceptions, the studies reviewed here did not describe the blinding and randomization procedures used, or if they were even used (Fig. **2**). Most of the studies did not state whether the sample size was calculated a priori or whether a criterion was used for removing outliers. This lack of information and description precludes the assessment of whether good research practices and experimental planning were followed.

As zebrafish have almost no prominent sexually dimorphic characteristics, it is quite hard for researchers to visually identify the sex of the animals *in vivo* and take this biological variable into account when evaluating behavioral parameters. The few studies that discriminate male and female zebrafish frequently fail to report how sexual characteriza-

tion was made. Most of the studies reviewed here used a mixed pool of male and female animals and, therefore, did not evaluate any possible sex influence on the effects of NMDAR antagonism (Fig. **2**). Reviews on the literature demonstrate the importance of studying both sexes in animal models of schizophrenia since there are clinical differences between men and women with schizophrenia. Male and female rodents also display significant differences in response to behavioral testing and treatment [127]. Considering sex as a biological variable is important to understand and delineate better schizophrenia-like endophenotypes in zebrafish and improve the reproducibility of findings across laboratories.

As the use of zebrafish as a model organism for the study of relevant aspects of schizophrenia is relatively recent, there are few studies investigating the predictive validity of zebrafish behavioral assays. Of the 44 articles described in this review, only 3 assessed the effects of antipsychotic drugs on reversing or preventing MK-801-induced changes (there are no data with the other NMDAR antagonists). As mentioned in the table, the antipsychotics haloperidol, sulpiride, and olanzapine [60, 74] were able to prevent locomotor, cognitive, and social alterations MK-801-induced, highlighting the predictive validity of the tests. This reinforces the need to further evaluate the effects of antipsychotic drugs in relevant zebrafish behavioral assays and better assess the predictive validity of pharmacological models of schizophrenia in zebrafish.

CONCLUSION

Deficits in social interaction and memory impairment induced by MK-801 and circular behavior induced by ketamine were the most frequently reported findings in the zebrafish literature reviewed here. However, mixed results have been observed for locomotor and anxiety parameters, which can be at least in part explained by a large variation in experimental design. This review integrated the most relevant findings reported in the zebrafish literature on schizophrenia-relevant behavioral effects of NMDAR antagonists. We conclude that zebrafish is a model organism suitable for studying drug-induced behavioral phenotypes relevant to schizophrenia, however further studies are needed to further characterize the main differences in behavior compared to mammals.

LIST OF ABBREVIATIONS

APV	=	DL-2-amino-5-phosphonopentanoic acid
DPCPX	=	Selective adenosine A_1 receptor antagonist
dpf	=	Days post-fertilization
hpf	=	Hours post-fertilization
i.c.v.	=	Intracerebroventricular
i.p.	=	Intraperitoneal
LDT	=	Light/dark test
MK-801	=	Dizocilpine
mpf	=	Months post-fertilization
NOR	=	Novel object recognition
NTT	=	Novel tank test
OTT	=	Open tank test
PCP	=	Phencyclidine
r.o.	=	Retro-orbital
SI	=	Social interaction tests
SKF-83566	=	Selective D_1 receptor antagonist

CONSENT FOR PUBLICATION

Not applicable.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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3.2. Capítulo 2:

How do zebrafish (Danio rerio) respond to MK-801 and amphetamine? Relevance for assessing schizophrenia-related endophenotypes in alternative model organisms

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RESEARCH ARTICLE

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How do zebrafish (*Danio rerio*) respond to MK-801 and amphetamine? Relevance for assessing schizophrenia-related endophenotypes in alternative model organisms

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Abstract

Schizophrenia pathophysiology has been associated with dopaminergic hyperactivity, NMDA receptor hypofunction, and redox dysregulation. Most behavioral assays and animal models to study this condition were developed in rodents, leaving room for species-specific biases that could be avoided by cross-species approaches. As MK-801 and amphetamine are largely used in mice and rats to mimic schizophrenia features, this study aimed to compare the effects of these drugs in several zebrafish (*Danio rerio*) behavioral assays. Male and female adult zebrafish were exposed to MK-801 (1, 5, and 10 μM) or amphetamine (0.625, 2.5, and 10 mg/L) and observed in paradigms of locomotor activity and social behavior. Oxidative parameters were quantified in brain tissue. Our results demonstrate that MK-801 disrupted social interaction, an effect that resembles the negative symptoms of schizophrenia. It also altered locomotion in a context-dependent manner, with hyperactivity when fish were tested in the presence of social cues and hypoactivity when tested alone. On the other hand, exposure to amphetamine was devoid of effects on locomotion and social behavior, while it increased lipid peroxidation in the brain. Key outcomes induced by MK-801 in rodents, such as social interaction deficit and locomotor alterations, were replicated in zebrafish, corroborating previous studies and reinforcing the use of zebrafish to study schizophrenia-related endophenotypes. More studies are necessary to assess the predictive validity of preclinical paradigms with this species and ultimately optimize the screening of potential novel treatments.

KEYWORDS

dextroamphetamine, dizocilpine maleate, locomotion, schizophrenia, social behavior, zebrafish

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1 | INTRODUCTION

Although the pathophysiology of schizophrenia is not completely understood, it has already been linked to GABAergic, glutamatergic, and dopaminergic dysfunction (Grace & Gomes, 2019; McCutcheon et al., 2020). Clinical and preclinical studies have suggested that psychotic symptoms arise from dopaminergic hyperactivity in subcortical regions (Kesby et al., 2018; McCutcheon et al., 2018), which may be due to the loss of fast-spiking parvalbumin-positive GABAergic interneurons, hypofunction of NMDA receptors (NMDARs), and oxidative stress (Cabungcal et al., 2013; Hardingham & Do, 2016; Konradi et al., 2011; Steullet et al., 2010). Animal models of schizophrenia overwhelmingly rely on rodents, which may lead to species-specific biases that could be avoided by cross-species approaches (Burrows & Hannan, 2016; Weber-Stadlbauer & Meyer, 2019). Recent publications have endorsed the use of zebrafish as an alternative model animal to study schizophrenia and screen for potential novel treatments (Bruni et al., 2016; Gawel et al., 2019; Leung & Mourrain, 2016). More studies, however, are necessary to determine whether zebrafish models can provide sufficient behavioral and biochemical resolution to assess complex traits associated with psychiatric disorders such as schizophrenia.

Zebrafish have a conserved neural architecture analogous to mammals, including cell types, neurotransmitters, and receptors. Despite the absence of midbrain dopaminergic neurons in zebrafish and other teleost fish, populations of tyrosine hydroxylase-immunoreactive cells have been identified in the diencephalon and telencephalon (Meek & Joosten, 1993; Parker et al., 2013; Rink & Guo, 2004; Rink & Wullimann, 2001), with structure and function similar to birds and mammals (Matsui, 2017; Rink & Wullimann, 2001). Furthermore, glutamatergic and GABAergic neurons exert an important regulatory role in the zebrafish brain (Parker et al., 2013). The presence of these neuronal systems and their organization indicate it is feasible to use zebrafish to investigate the impact of psychotropic drugs on behavior and neurochemistry.

Well-established animal models of schizophrenia use pharmacological tools to recapitulate aspects of the neurobiology and symptomatology of the condition. MK-801 (dizocilpine), for instance, is a noncompetitive NMDAR antagonist, while D-amphetamine (AMPH) inhibits dopamine uptake across the synaptic and vesicular membranes, leading to dopamine efflux. These drugs thus mimic the hypofunction of NMDARs and dopaminergic hyperactivity, respectively (Jones et al., 2011). The inhibition of NMDARs in humans and animals leads to behavioral effects that resemble the full spectrum of positive, negative, and cognitive symptoms of schizophrenia (Coyle et al., 2012; Hardingham & Do, 2016; Krystal et al., 1994; Morris et al., 1986). In mice and rats, MK-801 causes hyperlocomotion, stereotypic behavior, decreased social interaction, sensorimotor gating deficits, and cognitive impairment (Jones et al., 2011). In zebrafish, a few studies have shown that acute exposure to MK-801 causes hyperlocomotion (Franscescon et al., 2020; Menezes et al., 2015; Tran et al., 2016), social deficits (Zimmermann et al., 2016), and cognitive impairment (Cognato et al., 2012; Franscescon et al., 2020; Gaspary et al., 2018), with fish submitted individually to the behavioral tasks. There are, however, many differences regarding drug dose, administration route, and exposure duration, which hinders direct comparison of the phenotypes reported by different studies. The behavioral effects of AMPH administration, on the other hand, only recapitulate the positive symptoms of schizophrenia, which include delusions, hallucinations, and agitation in humans (Krystal et al., 2005), and stereotypic behavior and hyperlocomotion in rodents (Featherstone et al., 2007; Tenn et al., 2005). Although AMPH exposure in zebrafish is known to be anxiogenic (Kyzar et al., 2013), it has not been extensively investigated in the context of psychosis in this species. Furthermore, the dopaminergic hyperactivation induced by NMDA antagonists and dopaminergic agonists makes it relevant to evaluate neurochemical parameters related to oxidative stress, since the enzymatic metabolism of dopamine leads to increased production of reactive oxygen species (Bitanihirwe & Woo, 2011).

Although MK-801 and AMPH are widely used in rodent models, their effects on behavioral and neurochemical parameters in zebrafish need to be further investigated. This is an important step to develop high-throughput drug screening platforms that may facilitate the investigation of potential candidates. Thus, this study aimed to investigate the effects of acute exposure to MK-801 and AMPH in adult zebrafish by analyzing locomotor activity, stereotypy-related behaviors, social behavior, and neurochemical parameters of oxidative status as translatable markers.

Significance

Alternative model animals enable researchers to focus on behavioral features conserved across species, which may ultimately improve the translatability of preclinical findings with relevance to neuropsychiatric conditions. Zebrafish has been recently touted as a model animal to study schizophrenia neurobiology, though basic questions remain to be answered. We thus investigated the effects of known pro-psychotic agents, amphetamine and MK-801, in this species. While amphetamine did not increase locomotion as observed in mammals, fundamental outcomes induced by MK-801 were replicated in zebrafish. This species may be suitable as an alternative model animal to study schizophrenia, but further studies are needed.

2 | METHODS AND MATERIALS

2.1 | Experimental subjects

Experiments were performed using 288 male and female (50:50 ratio) short-fin wild-type zebrafish, 6 months old, and weighing 400 to 500 mg. Adult animals were obtained from a local commercial supplier (Delphis, RS, Brazil). The outbred population

used here is expected to possess a large genetic variability across individuals, which may be advantageous in this study as it mimics the variability of the general human population, adding translational value and external validity to our findings (Gerlai, 2019). The animals were maintained in our animal facility (Altamar, SP, Brazil) in a light/dark cycle of 14/10 hr for at least 15 days 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 the species (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 $CaCO_3$; and conductivity of 1,500–1,600 μS/cm) and was constantly filtered by mechanical, biological, and chemical filtration systems. Food was provided twice a day (commercial flake food (Poytara®, Brazil) plus the brine shrimp *Artemia salina*). After the tests, animals were euthanized by hypothermic shock according to the AVMA Guidelines for the Euthanasia of Animals (Leary et al., 2020). Briefly, animals were exposed to chilled water at a temperature between 2 and 4°C for at least 2 min after the loss of orientation and cessation of opercular movements, followed by decapitation as a second step to ensure death.

2.2 | Materials

(+)-MK-801 hydrogen maleate (CAS Number: 77086-22-7) and D-amphetamine hemisulfate salt (CAS Number: 51-63-8) were purchased from Sigma-Aldrich (St. Louis, MO, USA). All drugs were dissolved in water at the same conditions as the home tanks. Solutions were freshly prepared immediately before tests and were renovated half-way through the tests. Reagents used for biochemical assays were obtained from Sigma-Aldrich (St. Louis, MO, USA) and included 5,5′-dithiobis(2-nitrobenzoic acid) (CAS Number 69-78-3), thiobarbituric acid (CAS Number: 504-17-6), and trichloroacetic acid (CAS Number: 76-03-9). Absolute ethanol (CAS Number: 64-17-5) was obtained from Merck KGaA (Darmstadt, Germany).

2.3 | Experimental design and procedures

The animals were exposed to a concentration curve of MK-801 or AMPH for all tests. For MK-801 experiments, animals were randomly allocated to the following experimental groups: control (H_2O); 1 μM MK-801, and 5 μM MK-801, or 10 μM MK-801 ($n = 12$). These concentrations correspond to 0.337, 1.687, and 3.373 mg/L, respectively. For AMPH experiments, animals were randomly allocated as follows: control (H_2O); 0.625 mg/L AMPH; 2.5 mg/L AMPH and 10 mg/L AMPH ($n = 12$). These concentrations correspond to 3.392, 13.568, and 54.274 μM, respectively. We opted to express drug concentrations in the units routinely used in the literature for these drugs. The exposure time and concentration curves were based in the literature (Kyzar et al., 2013; Tran et al., 2016; Zimmermann et al., 2016) and pilot studies from our group. The control group was exposed only to water, under conditions of exposure identical to the treatment groups. The animals were allocated to the experimental groups following block randomization procedures to counterbalance the sex, the two different home tanks, and the test arenas between the groups. Different sets of animals were used for each experiment, meaning that 96 animals were used for each behavioral test, totaling 288 animals in the study. Animal behavior was video recorded and analyzed with the ANY-Maze tracking software (Stoelting Co., Wood Dale, IL, USA) by researchers blinded to the experimental groups. All tests were performed between 08:00 and 12:00 a.m. The sex of the animals was confirmed after euthanasia by dissecting and analyzing the gonads. For all experiments, no sex effects were observed, so data were pooled together.

According to the scientific literature on zebrafish behavior, certain innate behavioral patterns of zebrafish are related to broader behavioral domains, such as locomotion, anxiety, and social behavior (Kalueff et al., 2013). Regarding locomotion, frequently reported variables are total distance traveled, speed, absolute turn angle, rotations, and line crossings. Thigmotaxis is an anxiety-related behavior, which in zebrafish can be measured as time spent in the periphery or the bottom zone of the apparatus. Behaviors related to activation of the sympathetic nervous system (fight or flight response), such as freezing or immobility episodes, are also considered anxiety proxies. Social interaction can be evaluated by the preference to stay near a tank with conspecifics, that is, time spent in the interaction zone as compared to other zones of the tank. We submitted zebrafish to three different apparatuses to evaluate such behaviors, as described below.

2.4 | Locomotor activity test

The experimental design of the locomotor activity test is depicted in Figure 1a. To assess the effect of drug exposure across time on locomotor behavior, animals were individually and sequentially placed in (a) a beaker with 200 ml of water for 20 min, (b) the test aquarium for 30 min to analyze basal locomotor activity, (c) a beaker with 200 ml of water or drug solutions (MK-801 or AMPH) at different concentrations for 20 min, and (d) the test aquarium for 60 min. The test aquarium consisted of glass tanks (24 × 8 × 20 cm) filled with water at the optimal conditions at a level of 15 cm. The water in the tanks was changed between animals to avoid interference from drug traces or alarm substances released by previously tested fish. As the vertical position of the animal represents ethologically relevant information for assessing motor and anxiety-like behaviors (Levin et al., 2007), the test apparatus was virtually divided into three equal horizontal zones (bottom, middle, and top) for the front view video analyses (Marcon et al., 2018). The following locomotor and exploratory parameters were quantified across bins of 5 min: total distance traveled and time spent in the upper zone.

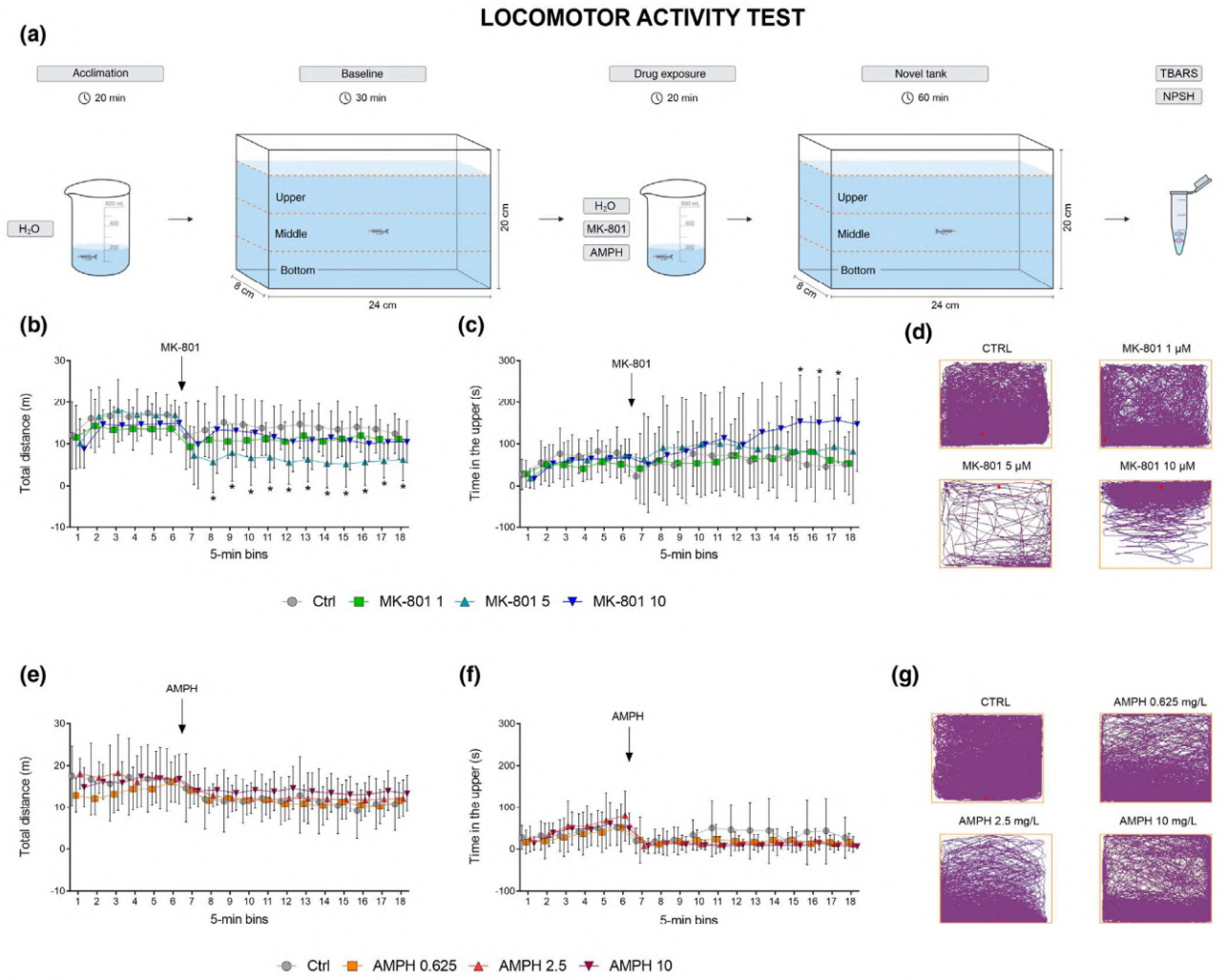

FIGURE 1 Effects of exposure to MK-801 and AMPH on locomotor activity across time. (a) Experimental design, (b, e) total distance traveled, (c, f) time spent in the upper zone, (d, g) representative track plots of one animal from each treatment group for 90 min. Data are expressed as mean ± standard deviation (*SD*). GEE analysis followed by Tukey's post hoc test. $^*p < 0.05$ versus control. $n = 10–12$. AMPH (amphetamine); MK-801 (dizocilpine)

2.5 | Open tank test

The experimental design of the open tank test (OTT) is depicted in Figure 2a. The apparatus used for the OTT consisted of a white circular arena (24 cm in diameter and 8 cm in height, 2 cm water level). The apparatus format and the acquisition of videos from the top view allows the evaluation of locomotor parameters related to stereotypic behavior (circular movements and absolute turn angle). For this test, the animals were individually exposed to water or different concentrations of the drugs in beakers filled with 200 ml of solution for 20 min. Following drug exposure, animals were placed in the center of the open tank arena and recorded for 10 min. The water was changed between every animal. For video analyses, the apparatus was virtually divided into two zones: the center zone of 12 cm in diameter and the periphery. As this experimental setup allows the acquisition of a great number of parameters, we performed a principal component analysis (PCA) to investigate this data set and selected for comparison only the parameters that contributed the most to total variation among animals (Figure 6). The following parameters are thus displayed: total distance traveled, absolute turn angle, immobility time, number of clockwise rotations, and the time spent in the center zone.

2.6 | Social interaction test

The experimental design of the social interaction test is represented in Figure 4a. In the social interaction test, animals were individually exposed to 200 ml of water or drug solutions in beakers for 20 min. 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 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

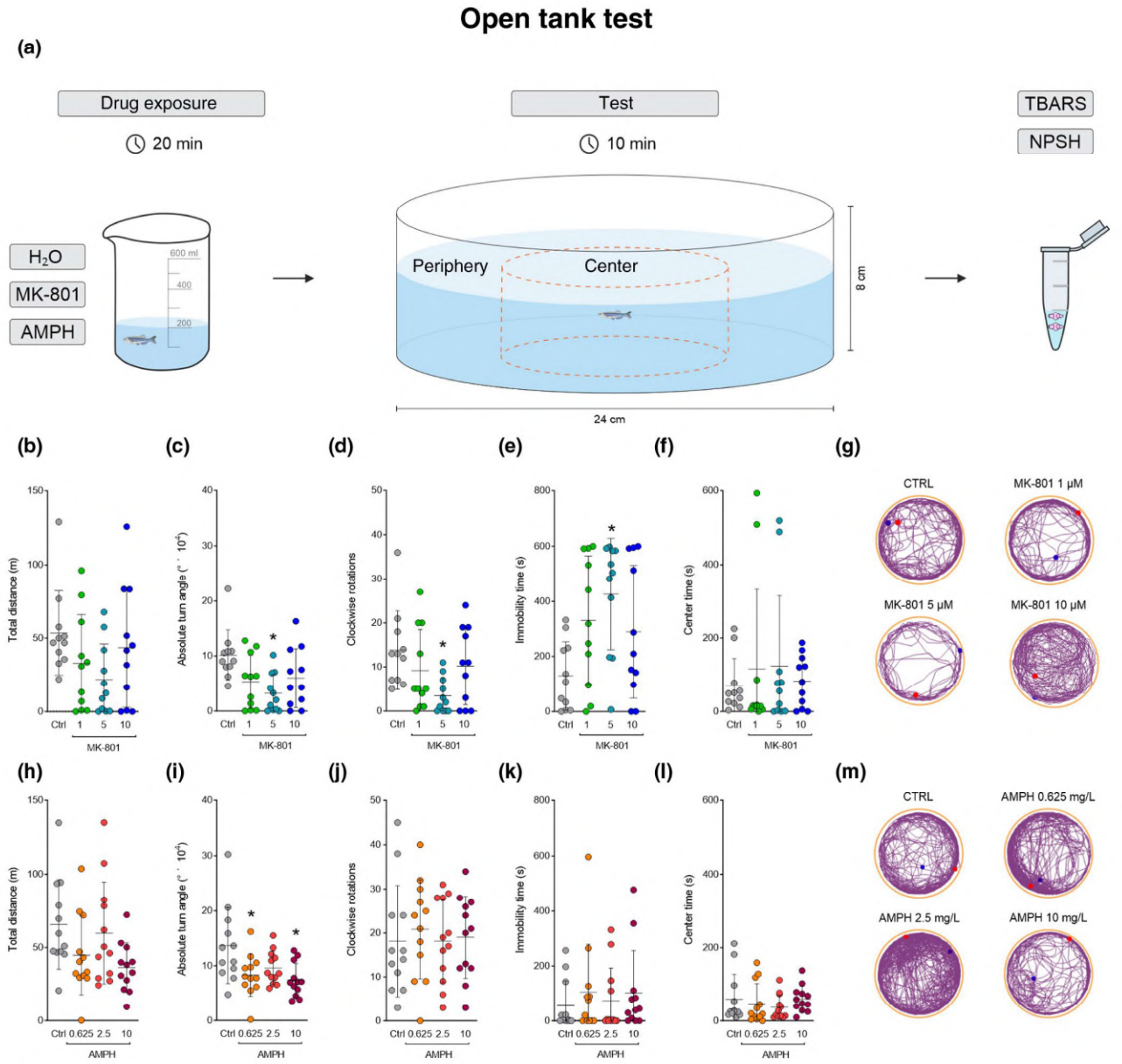

FIGURE 2 Effects of exposure to MK-801 and AMPH in the open tank test (OTT). (a) Experimental design, (b, h) total distance traveled, (c, i) absolute turn angle, (d, j) clockwise rotations, (e, k) immobility time, (f, l) and time spent in the center zone, (g, m) representative track plots of the behavior of one animal from each treatment group during 10 min. Data are expressed as mean ± standard deviation (*SD*). One-way ANOVA followed by Tukey's post hoc test. $^*p < 0.05$ versus control. $n = 11$–12. AMPH (amphetamine); MK-801 (dizocilpine)

left) was counterbalanced throughout tests. The water in the experimental tanks was changed between every animal. To assess social behavior, the test apparatus was virtually divided into three vertical zones (interaction, middle, and neutral). Videos were recorded from the front view. Animals were habituated to the apparatus for 2 min and then analyzed for 5 min. As for the OTT, a large number of parameters may be obtained from this setup, so we also performed a PCA to investigate this data set and selected for comparison only the parameters that contributed the most to total variation among animals (Figure 6). The following parameters were quantified: total distance traveled, time spent in the interaction zone (as a proxy for social interaction time), and number of line crossings.

2.7 | Oxidative stress parameters

The brain of animals submitted to the locomotor activity test and OTT was collected right after the euthanasia to assess oxidative stress parameters by thiobarbituric acid reactive substances (TBARS) and nonprotein thiol (NPSH) levels. A scalpel was used to remove the

cranium of the fish and to collect brain tissue. For each independent sample, two brains were pooled ($n = 6$) and homogenized in 300 μl of phosphate-buffered saline (PBS, pH 7.4, Sigma-Aldrich). Tissue preparation and biochemical analysis followed protocols described in the study by Sachett et al. (2018). More detailed step-by-step protocols are also publicly available at protocols.io (Sachett et al., 2020a, 2020b, 2020c, 2020d).

First, the homogenates were centrifuged at 2,400 g for 10 min at 4°C, and the supernatants were collected and kept in microtubes on ice until the assays were performed. Lipid peroxidation was evaluated by quantifying the production of TBARS. Fifty micrograms of proteins from the sample were mixed with thiobarbituric acid 0.5% and trichloroacetic acid 2% (150 μl). The mixture was heated at 100°C for 30 min. The absorbance of the samples was determined at 532 nm in a microplate reader. 1,1,3,3-Tetraethoxypropane 2 nmol/ml was used as the standard. The content of NPSH in the samples was determined by mixing equal volumes of the brain tissue preparation and 6% trichloroacetic acid, centrifuging the mix (2,400 g, 10 min at 4°C), and determining the absorbance of the supernatants at 432 nm.

2.8 | Statistical analyses

Data were expressed as mean ± *SD* For all comparisons, the significance level was set at $p < 0.05$. Data were analyzed using IBM SPSS Statistics version 27 for Windows and the graphs were plotted using GraphPad Prism version 8.0 for Windows.

The sample size was calculated as $n = 12$ for the primary outcome (total distance traveled) using MiniTab® software. We used the following parameters: number of experimental groups (4), alpha (5%), power (80%), minimum difference between the means (50%), and standard deviation estimate based on the literature (40%).

The normality and homogeneity of variance confirmation were analyzed using D'Agostino-Pearson and Levene tests, respectively. The locomotor activity across time was analyzed using Generalized Estimating Equation Model Analysis (GEE) to identify the main effects of treatment and time and their interaction, followed by Tukey post hoc test when appropriate. Data from the OTT, social interaction test, and biochemical assays were analyzed using one-way ANOVA followed by Tukey post hoc test when appropriate. The outliers were defined using the ROUT statistical test and were removed from the analyses. This resulted in two outliers (one from 5 μM MK-801 and one from 10 μM MK-801) removed from the social interaction test and four outliers (one control animal and one from each MK-801 groups) removed from the OTT. Also, four animals were excluded from locomotor activity test analyses due to technical problems in recording the videos (two animals from the control group and two from 0.625 mg/L AMPH) and three samples (one sample from the control group and two samples from 0.625 mg/L AMPH) were removed from oxidative stress analyses due to technical problems.

We performed PCA to integrate the multiple variable outputs obtained during the OTT and social interaction test. The new variables generated (principal components: PCs) are a paired weighed combination of the variables contained in the original data that individually explain the largest possible variation among the samples. The PCs were named in a decrescent manner, so that PC1 explains most variability, followed by the other PCs in an ordered fashion of reduced variability explanation. Based on the composition of the PCs and the individual contribution of each composing variable, it is possible to identify the most relevant and irrelevant variables to observe group differences. We also performed a K-means clustering analysis (MacQueen, 1967), to quantitatively assess the behavioral patterns observed in the PCA. Sampling adequacy was assessed before PCA through Bartlett's sphericity and Kaiser-Meyer-Olkin factor adequacy tests. Data frames presented $p < 0.05$ for Bartlett's sphericity test, and KMO of 0.62 and 0.66, respectively, indicating that both were adequate for PCA. We estimated correlations using Pearson's correlation test. We report correlations that, according to the literature (Akoglu, 2018; Mukaka, 2012; Schober et al., 2018), correspond to strong or very strong correlations ($r > 0.7$ or $r < -0.7$), and that also present $p < 0.05$. The statistical procedures were performed using base R-3.5.1 for MacOS (R Core Team, 2018), and R packages: "corrplot" (Wei & Simko, 2017), "psych" (Revelle, 2018), "circlize"(Gu et al., 2014), "ggplot2" (Wickham, 2016), "ggfortify" (Tang et al., 2016), and "devtools" (Wickham et al., 2019).

3 | RESULTS

3.1 | Effects of MK-801 and AMPH exposure in the locomotor activity test

In this experiment, we analyzed the effects of exposure to MK-801 (1, 5, and 10 μM) and AMPH (0.625, 2.5, and 10 mg/L) for 60 min, which allows a more detailed analysis of the locomotor activity and exploratory behavior of animals across time. As expected, no statistical differences were observed for baseline locomotor activity (Figure 1b,e). However, after drug exposure, we observed that 5 μM MK-801 decreased the total distance moved (Figure 1b) while 10 μM MK-801 increased the time spent in the upper zone (Figure 1c). No statistical differences were observed after AMPH exposure in the parameters analyzed (Figure 1e,f). Table 1 summarizes the GEE analyses.

3.2 | Effects of MK-801 and AMPH exposure in the open tank test

In this experiment, we analyzed the effects of exposure to MK-801 (1, 5, and 10 μM) and AMPH (0.625, 2.5, and 10 mg/L) in the OTT, which allows the analysis of stereotypy-related behaviors, such as circular swimming and absolute turn angle. Exposure to 5 μM

TABLE 1 Summary of the GEE analysis for the locomotor activity test

Test	Dependent variable	Effects	Wald Chi-square	DF	*p* value	Effect size
MK-801 locomotor activity	Total distance	Interaction	14.76	3	**0.002**	0.001
		Time	25.37	1	**<0.001**	0.253
		MK-801	2.78	3	0.436	0.165
	Upper time	Interaction	16.44	3	**<0.001**	0.113
		Time	17.70	1	**<0.001**	0.131
		MK-801	5.43	3	0.142	0.052
AMPH locomotor activity	Total distance	Interaction	4.34	3	0.227	0.075
		Time	30.20	1	**<0.001**	0.265
		AMPH	4.31	3	0.229	0.042
	Upper time	Interaction	5.34	3	0.148	0.094
		Time	7.26	1	**0.007**	0.200
		AMPH	1.42	3	0.700	0.055

Note: Significant effects ($p < 0.05$) are given in bold font. Effect sizes are expressed as partial eta^2.
Abbreviation: DF, degrees of freedom.

TABLE 2 Summary of the one-way ANOVAs for the open tank, social interaction, and oxidative status tests

Test	Dependent variable	*F*-value	DF	*p* value	Effect size
MK-801 open tank test	Total distance	1.00	3,40	0.130	0.070
	Absolute turn angle	4.41	3,40	**0.008**	0.249
	Clockwise rotations	3.14	3,40	**0.035**	0.191
	Immobility time	2.67	3,40	**0.014**	0.200
	Center time	0.31	3,40	0.815	0.690
AMPH open tank test	Total distance	2.78	3,44	0.051	0.160
	Absolute turn angle	4.46	3,44	**0.007**	0.239
	Clockwise rotations	0.69	3,44	0.559	0.110
	Immobility time	0.35	3,44	0.782	0.240
	Center time	0.54	3,44	0.651	0.036
MK-801 social interaction test	Interaction time	8.29	3,42	**<0.001**	0.372
	Total distance	11.55	3,42	**<0.001**	0.452
	Crossings	19.77	3,42	**<0.001**	0.582
AMPH social interaction test	Interaction time	0.36	3,44	0.778	0.240
	Total distance	1.91	3,44	0.141	0.150
	Crossings	0.11	3,44	0.949	0.008
MK-801 oxidative stress	TBARS 10 min	0.86	3,20	0.476	0.115
	TBARS 60 min	5.29	3,20	**0.007**	0.442
	NPSH 10 min	1.33	3,20	0.290	0.167
	NPSH 60 min	0.77	3,20	0.521	0.104
AMPH oxidative stress	TBARS 10 min	2.11	3,20	0.130	0.241
	TBARS 60 min	8.78	3,19	**<0.001**	0.581
	NPSH 10 min	0.22	3,18	0.879	0.036
	NPSH 60 min	0.93	3,20	0.441	0.155

Note: Significant effects ($p < 0.05$) are given in bold font. Effect sizes are expressed as partial eta^2.
Abbreviation: DF, degrees of freedom.

MK-801 decreased the absolute turn angle (Figure 2c) and clockwise rotations (Figure 2d) and increased the time spent immobile (Figure 2e). Exposure to 0.625 and 10 mg/L AMPH decreased the absolute turn angle (Figure 2i) but did not alter clockwise rotations (Figure 2j) or time spent immobile (Figure 2k). There was no statistical difference in experimental groups regarding total distance (Figure 2b,h) and time spent in the center area (Figure 2f,l). Pearson's correlation analysis shows that MK-801 (Figure 3b–d) exposure elicits a negative correlation between anxiety-related behaviors, namely freezing time, and locomotor and stereotypy-related behaviors, not observed in control animals (Figure 3a). Interestingly, stereotypy- and locomotor-related behaviors were positively correlated in animals exposed to 5 μM MK-801 (Figure 3c). A negative correlation between locomotor and stereotypy behaviors was only observed in animals exposed to 2.5 mg/L AMPH (Figure 3f). PCA shows that PC1, corresponding to 46.9% of the explained variation, is largely positively composed of locomotor and stereotypy behaviors, while negatively composed of anxiety behaviors (Figure 6a). A K-means analysis shows that three statistical clusters of similar behaviors are observed and that a cluster containing most of the animals treated with 5 μM MK-801 is observed, which is comprised of intense anxiety-like behaviors and reduced locomotor and stereotypy behaviors (Figure 6a). Table 2 summarizes the one-way ANOVAs.

3.3 | Effects of MK-801 and AMPH exposure in the social interaction test

To assess behavioral parameters related to the negative symptoms of schizophrenia, we investigated the effects of MK-801 and AMPH on zebrafish social behavior. Figure 4 shows the effects of exposure to MK-801 (1, 5, and 10 μM) and AMPH (0.625, 2.5, and 10 mg/L) in the social interaction test. MK-801 at 5 and 10 μM decreased the time spent in the interaction zone (Figure 4b), increased the total distance traveled (Figure 4c), and increased the number of line crossings (Figure 4d), while 1 μM MK-801 did not alter social and locomotor behaviors (Figure 4b–d). None of the AMPH concentrations altered social interaction (Figure 4f) or locomotor behavior (Figure 4g,h). Pearson's correlation analyses showed that MK-801 exposure at 5 (Figure 5c) and 10 μM (Figure 5d) lead to negative correlations between anxiety and locomotor behaviors. AMPH exposure (Figure 5e–g) abrogated positive correlations between social behavior and both locomotor and anxiety behaviors, also leading to negative correlations between anxiety and locomotor behaviors. Interestingly, only 10 mg/L AMPH-exposed animals presented a negative correlation between anxiety behavior (time freezing) and time spent in the interaction zone (Figure 5g). Further, PCA shows that PC1, corresponding to 56.1% of the variation observed, is positively composed of total distance, crossings and time spent in the

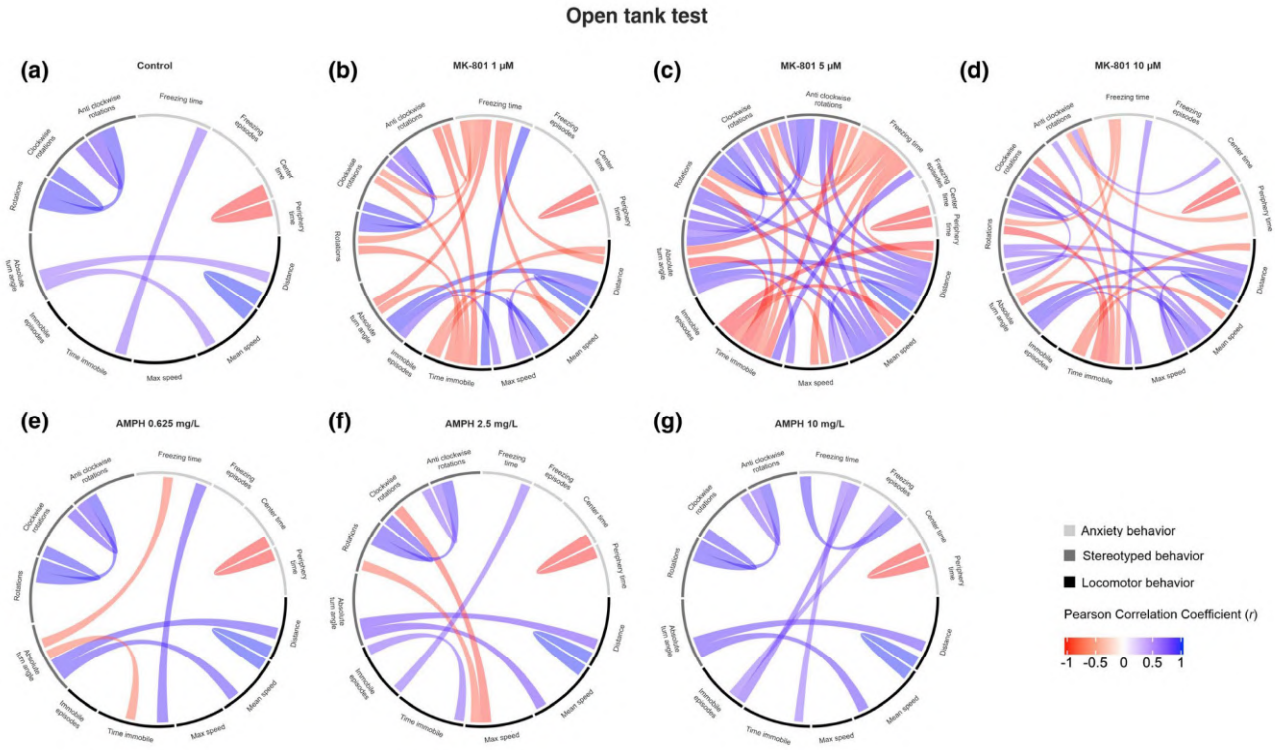

FIGURE 3 Mathematical analysis of the effects of MK-801 and AMPH on behavior during the open tank test (OTT). (a–g) Chord diagrams obtained from Pearson's correlation analysis between variables, links indicate strong positive (blue color) and negative (red color) significant correlations, with "*r*" values above 0.7 or below −0.7, respectively. Annotation track colors indicate variables with related behavioral outcomes, classified as locomotor, stereotypy, and anxious behaviors, in black, gray, and light gray colors, respectively

interaction zone, while negatively composed of time spent in the neutral zone and freezing time (Figure 6b). PC2, corresponding to 35.3% of the variation observed, is positively composed of time spent in the interaction zone and freezing time, while negatively composed of distance, crossings and time spent in the neutral zone (Figure 6b). K-means analysis shows three distinct clusters among animals that suggest social interaction analysis in zebrafish presents a resolution to distinct phenotypes following 5 μM and 10 μM MK-801 exposure, associated with a reduced social interaction and increased locomotion (Figure 6b). Table 2 summarizes the one-way ANOVAs.

3.4 | Effects of MK-801 and AMPH exposure on oxidative status

Figure 7 shows the effects of exposure to MK-801 (1, 5, and 10 μM) and AMPH (0.625, 2.5, and 10 mg/L) on TBARS and NPSH levels. MK-801 exposure did not alter lipid peroxidation (Figure 7a,b) and nonprotein thiol levels (Figure 7c,d) at any of the concentrations tested. AMPH, on the other hand, induced lipid peroxidation after 60 min of exposure (Figure 7f), but not after 10 min (Figure 7e), while it did not alter NPSH levels (Figure 7g,h). It should be noted, though, that postexposure time may be confounded here by the context of the apparatus, as 10-min and 60-min samples were collected from animals submitted to the OTT and NTT, respectively. Table 2 summarizes the one-way ANOVAs.

4 | DISCUSSION

In this study, we investigated the effects of exposure to MK-801 and AMPH on behavioral and biochemical outcomes in adult zebrafish. We demonstrate that MK-801 impaired social interaction, an endophenotype related to the negative symptoms of schizophrenia. Furthermore, we observed that MK-801 decreased the total distance traveled and increased the immobility time in locomotor assays, which differs from studies in rodents. Interestingly, MK-801 induced hyperlocomotion in the presence of social cues in the social interaction test. This suggests MK-801 elicits opposing effects that depend on the context, such as the presence of social stimulus. On the other hand, exposure to AMPH did not induce hyperlocomotion or social interaction deficit, but it was able to alter oxidative status, a relevant aspect related to schizophrenia pathophysiology.

Postmortem brain studies and animal models of schizophrenia (Krystal et al., 1994; Mohn et al., 1999; Weickert et al., 2013) have suggested there is an aberrant function of hippocampal fast-spiking GABAergic parvalbumin-positive interneurons and a functional deficit in NMDARs expressed by these interneurons, which leads to altered excitation–inhibition balance in cortical and subcortical areas (Balu et al., 2013; Insel, 2010; Korotkova et al., 2010). MK-801 exposure simulates the NMDAR hypofunction, leading to disinhibition of excitatory hippocampal neurons and, consequently, disrupting the firing of dopaminergic neurons in the mesolimbic and mesocortical pathways, causing positive (hyperlocomotion and stereotypy-related behaviors) and negative (social interaction deficits) schizophrenia-like symptoms in rodents (Carlsson & Carlsson, 1989; Hardingham & Do, 2016). Although chronic exposure to NMDA antagonists in rodent models leads to persistent behavioral alterations, acute administration is also informative. Clinical studies have shown that acute exposure to NMDA antagonists can recapitulate the full range of schizophrenia symptoms in healthy subjects and worsen the symptoms in patients with schizophrenia (Beck et al., 2020), highlighting the relevance of acute exposure to MK-801 in animal models. Exposure to AMPH increases dopamine release and is used as a pharmacological tool to model dopaminergic hyperactivity in the striatum (Featherstone et al., 2007; McCutcheon et al., 2020).

A behavioral assay widely evaluated in rodents is the distance traveled in an open field after intraperitoneal injection of MK-801 (Bygrave et al., 2016) or AMPH (Herrmann et al., 2014). Although the effects of these drugs on rodent locomotion are well known, the zebrafish literature is less straightforward. We thus performed in zebrafish a similar protocol to that used in rodents, in which locomotor activity is assessed across time. We also exposed the animals to an aquarium identical to the test aquarium before drug exposure to assess the basal locomotor activity, which in rodents declines and then stabilizes in the first 30 min. This decline in exploration was not observed for zebrafish in our study. We also surprisingly observed that 5 μM MK-801 decreased the total distance traveled, and 10 μM MK-801 increased time spent in the upper zone, which could indicate decreased anxiety or locomotor alterations. Contrary to our findings, some studies demonstrated that MK-801 exposure induced hyperlocomotion in adult zebrafish (Franscescon et al., 2020; Menezes et al., 2015); however, such protocols lacked the baseline period and animals were directly placed in the test apparatus only after drug exposure. Thus, the differences in context novelty may underlie the conflicting results, as suggested by a previous study by Tran et al. (2016). Furthermore, there are important differences in doses and drug routes (aqueous exposure vs. intraperitoneal), exposure duration (20 vs. 30 min), and test duration (1 hr vs. 5 or 6 min) that could explain the contrasting results reported by others (Franscescon et al., 2020; Menezes et al., 2015).

In our experiments, AMPH did not induce hyperlocomotion in any behavioral test, which contrasts with the well-known stimulating effects observed in rodents. Differences in fish central regulatory motor circuits as compared to rodents and other mammals may explain our findings (Ryczko et al., 2017). An obvious difference is the fact that fish are swimming most of the time. Studies performed in lampreys (*Petromyzon marinus*) have shown that there are dopaminergic and glutamatergic neurons that project to the mesencephalic locomotor region (MLR), indicating a close interaction between these neurotransmitters in the generation of the locomotor command. Besides, blocking dopamine receptors in the MLR resulted in reduced swimming movements without interrupting the gradual locomotion control, while blocking glutamatergic receptors almost abolished locomotion, indicating that glutamatergic contribution is essential to obtain locomotion in

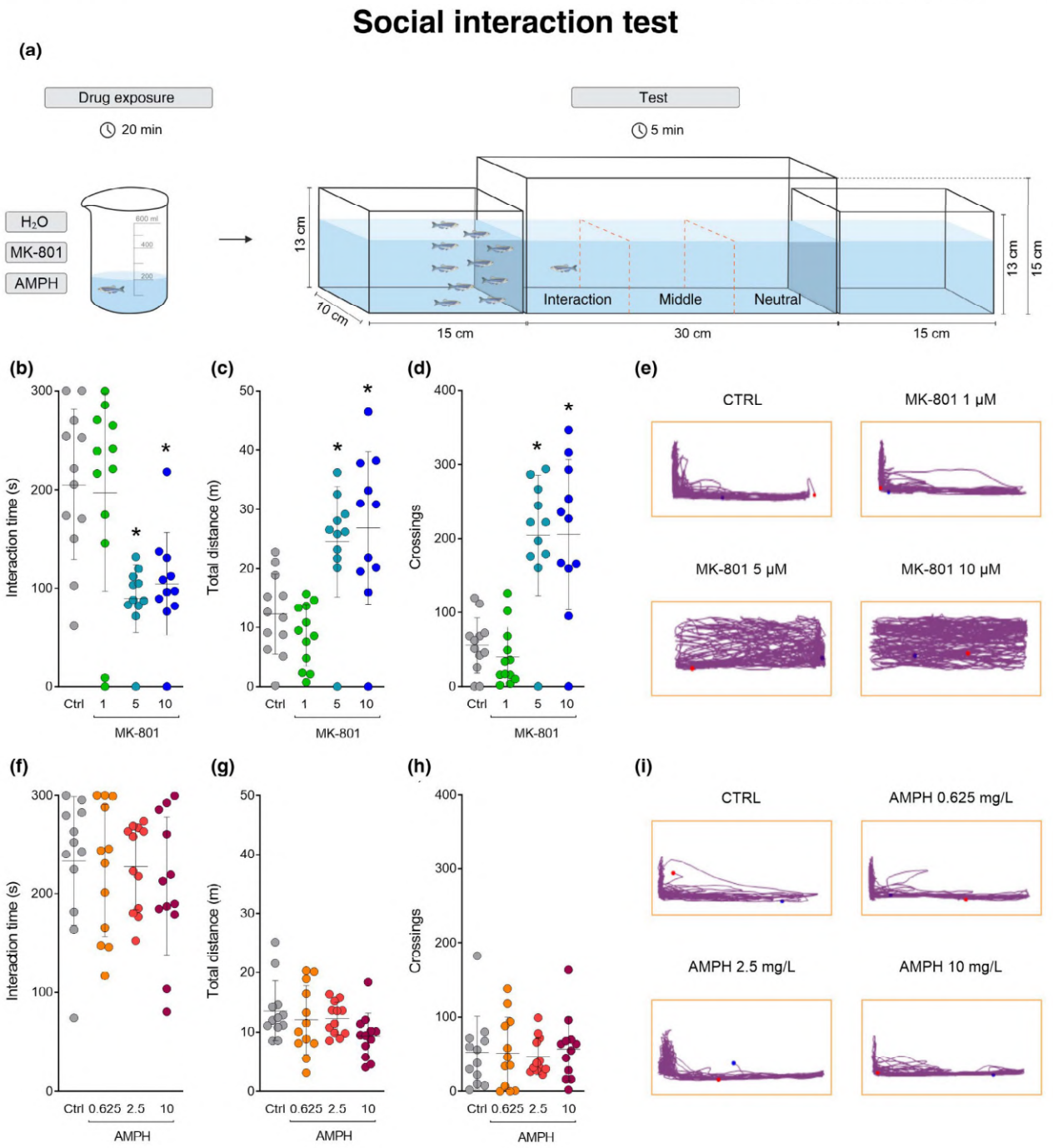

FIGURE 4 Effects of exposure to MK-801 and AMPH in the social interaction test. (a) Experimental design, (b, f) time spent in the interaction zone, (c, g) total distance traveled, (d, h) line crossings, (e, i) and representative track plots of the behavior of one animal from each treatment group during 5 min. Data are expressed as mean ± standard deviation (*SD*). One-way ANOVA followed by Tukey's post hoc test. $^*p < 0.05$ versus control. $n = 11$–12. AMPH (amphetamine); MK-801 (dizocilpine)

a graded fashion, while the dopaminergic contribution provides additional modulation, but is not essential to evoke locomotion (Ryczko et al., 2017). We hypothesized that, because of the different central circuits of motor regulation of fish, the results with MK-801 exposure on zebrafish locomotion parameters are more robust than with AMPH exposure.

Stereotypy is defined as repetitive and unvarying behavior (Morrens et al., 2006). Drug-induced stereotypic behaviors are not well characterized in zebrafish, but some authors suggest they may be displayed as altered rotation movements (Michelotti et al., 2018). To date, only a few studies explored the effects of MK-801 on stereotypy-related behaviors in zebrafish, and there is no study

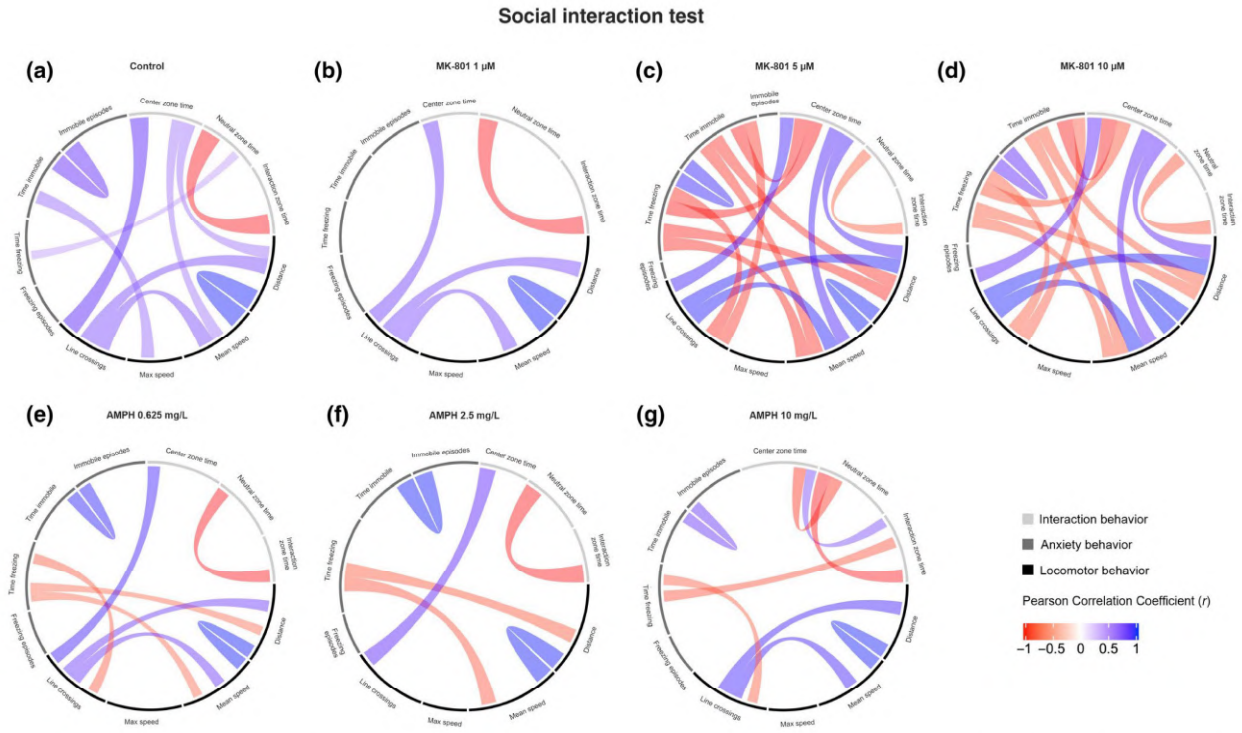

FIGURE 5 Mathematical analysis of the effects of MK-801 and AMPH on behavior during social interaction test. (a–g) Chord diagrams obtained from Pearson's correlation analysis between variables, links indicate strong positive (blue color) and negative (red color) significant correlations, with "*r*" values above 0.7 or below −0.7, respectively. Annotation track colors indicate variables with related behavioral outcomes, classified as locomotor, anxiety, and social behaviors, in black, gray, and light gray colors, respectively

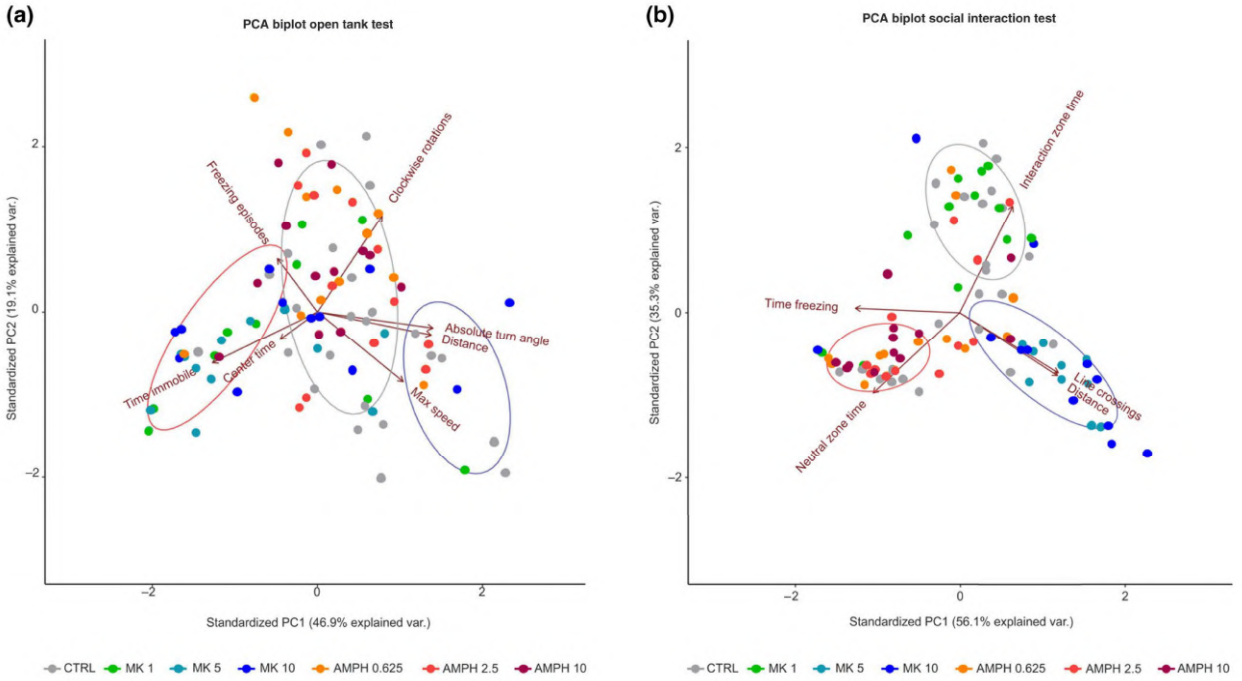

FIGURE 6 Mathematical analysis of MK-801 and AMPH effects on behavior during the open tank and social interaction tests. (a, b) Principal component analysis biplot displays arrows corresponding to observed variable eigenvectors relative to PC1 and PC2, grouping of animals in biplot is indicated in the legend. Ellipses indicate behavioral phenotype clustering model estimated through K-means clustering analysis

with AMPH. Franscescon et al. (2020) report a decrease in the absolute turn angle after exposure to MK-801; however, the test aquarium in this study was filmed from the front instead of the top, which hinders proper evaluation of circular movements in the horizontal plane of the fish. Another study found increased circular movements in zebrafish after exposure to a high concentration of MK-801 (100 μM) in the OTT (Sison & Gerlai, 2011). In our study, 5 μM MK-801 decreased the absolute turn angle and clockwise rotations,

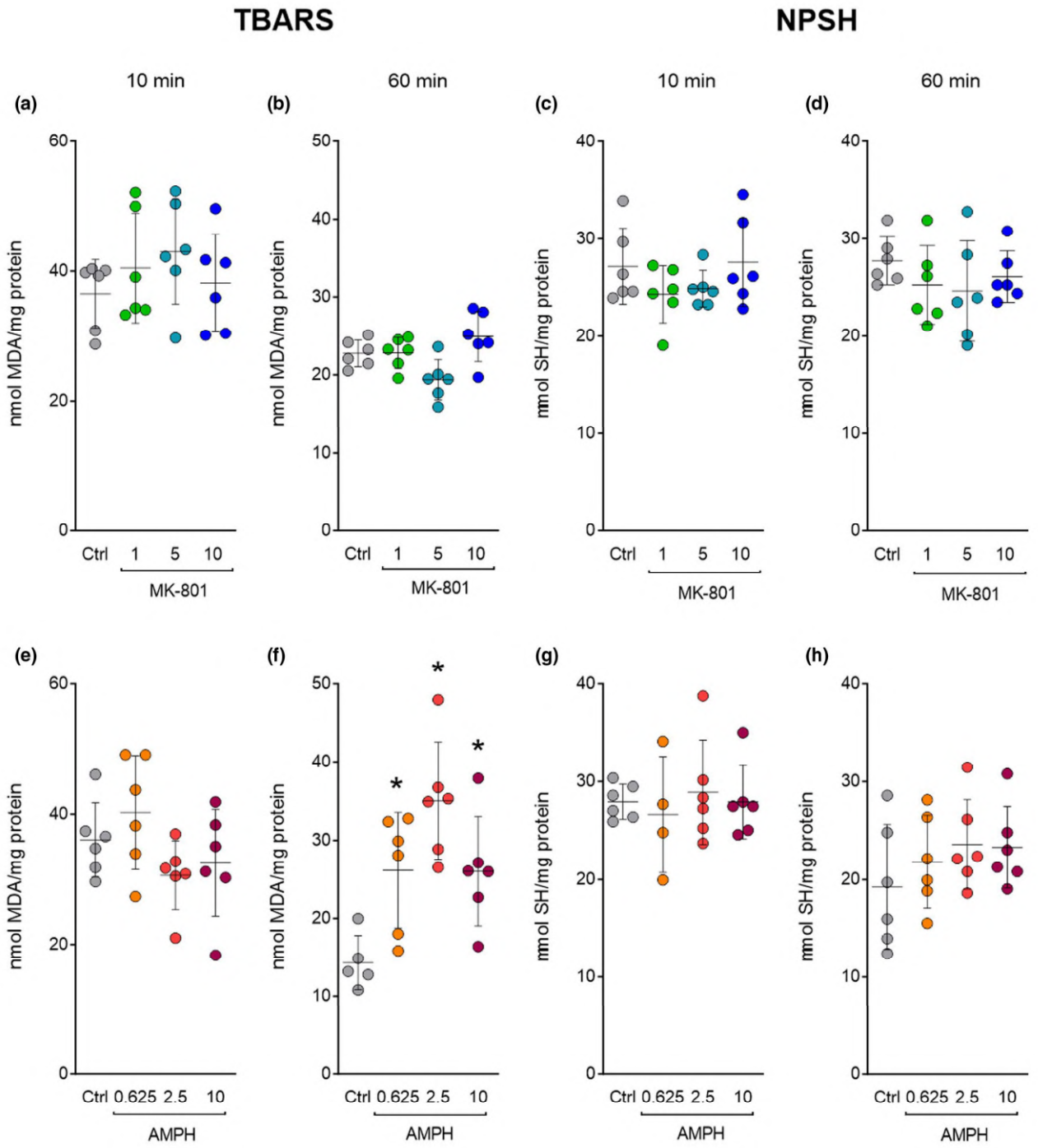

FIGURE 7 Effects of exposure to MK-801 and AMPH after 10 or 60 min on oxidative stress parameters (lipid peroxidation (TBARS) and nonprotein thiol (NPSH) levels). (a, e) Lipid peroxidation levels 10 min after drug exposure, (b, f) lipid peroxidation levels 60 min after drug exposure, (c, g) NPSH levels 10 min after drug exposure, (d, h) and NPSH levels 60 min after drug exposure. Data are expressed as mean ± standard deviation (*SD*). One-way ANOVA followed by Tukey's post hoc test. $^*p < 0.05$ versus control. $n = 6$, except for $n = 4$ in NPSH 10 min for 0.625 mg/L AMPH, and $n = 5$ in TBARS 1 hr for 0 mg/L AMPH. AMPH (amphetamine); MK-801 (dizocilpine)

and increased the time animals remained immobile, whereas 0.625 and 10 mg/L AMPH only decreased the absolute turn angle. It is also possible that zebrafish display other phenotypes related to stereotypic behavior that are not detectable by automated software, such as repetitive movements of the face and mouth commonly observed in humans and rodents exposed to AMPH or NMDAR antagonists (Kelley et al., 1988; Ridley & Baker, 1982). We observed that in both MK-801 and AMPH exposures, stereotypy-related behaviors were positively correlated with locomotor-related parameters in the OTT.

As social isolation is one of the main negative symptoms of schizophrenia, behavioral assays that model this aspect are critical for studying and developing treatments to this unmet clinical need. As zebrafish is a schooling animal with complex social behavior, such as hierarchical and breeding relationships (Dreosti et al., 2015), it is well-suited as a species for modeling social interaction. We observed that 5 and 10 μM MK-801 disrupted social interaction. This result corroborates previous findings in other zebrafish studies (Seibt et al., 2011; Zimmermann et al., 2016) and in rodent models. Interestingly, 5 and 10 μM MK-801 caused hyperlocomotion in the social interaction test, even though this drug was devoid of effects on the total distance traveled in the locomotor tests discussed earlier. This suggests that not only context novelty (Tran et al., 2016), but also the presence of social cues can differentially modulate the effects of MK-801. This idea, however, remains to be tested in experiments that isolate both variables. Although hyperlocomotion could be a confounder to social interaction, we observed that distance traveled did not correlate with time spent in the interaction zone, corroborating to the nonstochastic nature of this behavior. Regarding AMPH, it did not cause significant changes in zebrafish social behavior, replicating what is known for rodents. PCA and clustering analysis suggest that in fact, both OTT and social interaction test have sufficient resolution to identify distinct behavioral phenotypes related to schizophrenia in zebrafish models, especially following MK-801 exposure.

Studies in rodent models report that increased dopamine release induced by AMPH promotes oxidative stress, probably due to increased monoamine metabolism (Dichtl et al., 2018; El-Tawil et al., 2011; Frey et al., 2006). All tested concentrations of AMPH increased TBARS, a well-established marker of lipid peroxidation, after 60 min, but not after 10 min of exposure, indicating a time-dependent effect on the production of reactive species. This increase in TBARS levels, however, was not compensated by changes in NPSH levels, which indirectly represent reduced glutathione (GSH) levels and antioxidant activity. We cannot rule out, however, a decrease in NPSH or even a compensatory increase at a later time point. Further studies are necessary to untangle the kinetics involved in the pro-oxidant effects of amphetamine. These are novel data since there are no studies evaluating the effects of AMPH on oxidative stress parameters in zebrafish. On the other hand, MK-801 did not cause any change in the oxidative status after 10 or 60 min of exposure. This is in line with studies in rodents that demonstrate that only repeated exposures to MK-801 produce an increase in reactive oxygen species (Wang et al., 2012).

A limitation of our study is that we used a pool of whole-brain homogenates for the neurochemical analyses. Unfortunately, it is technically challenging to analyze specific regions considering the reduced size of the zebrafish brain, which also hinders the quantification of such biochemical markers for a single individual. For this reason, the neurochemical data must be interpreted with caution until confirmed in further studies. Another limitation is that we did not evaluate the effects of MK-801 and amphetamine on cognitive tests. This is certainly warranted in future studies, but the most relevant protocols often require several days for habituation and training sessions (Benvenutti et al., 2021), which makes the assessment of acute drug effects less straightforward.

With ongoing efforts in modulating schizophrenia-associated genes in zebrafish, straightforward behavioral readouts relevant to this condition need to be investigated. This is essential to advance the use of zebrafish in high-throughput drug screening assays. Our study corroborates the idea that schizophrenia endophenotypes may be modeled in zebrafish. Although more studies are needed to confirm our findings, it is reasonable to argue that MK-801, particularly at the 5 μM concentration, may be more useful than AMPH as a pharmacological tool to assess behavioral endophenotypes relevant to schizophrenia in adult zebrafish.

DECLARATION OF TRANSPARENCY

The authors, reviewers and editors affirm that in accordance to the policies set by the *Journal of Neuroscience Research*, this manuscript presents an accurate and transparent account of the study being reported and that all critical details describing the methods and results are present.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICAL APPROVAL

All procedures were approved by the institutional animal welfare and ethical review committee at the Universidade Federal do Rio Grande do Sul (approval #35525/2019). The animal experiments are reported in compliance with the ARRIVE guidelines 2.0 (Percie du Sert et al., 2020).

AUTHOR CONTRIBUTIONS

All authors had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. *Conceptualization*, R.B., A.P., and A.P.H.; *Methodology*, R.B.,

A.P., and A.P.H.; *Investigation*, R.B., M.G.L., A.S., C.G.R., and R.C.; *Formal Analysis*, R.B., N.R.S., M.M., A.P., and A.P.H.; *Resources*: A.P. and A.P.H.; *Writing – Original Draft*, R.B., M.G.L., and N.R.S.; *Supervision*, A.P. and A.P.H.; *Funding Acquisition*, A.P. and A.P.H.

PEER REVIEW

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This article has earned an Open Data badge for making publicly available the digitally-shareable data necessary to reproduce the reported results. The data is available at https://doi.org/10.17605/OSF.IO/76C59.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Open Science Framework at https://doi.org/10.17605/OSF.IO/76C59 (Benvenutti et al., 2021).

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the Supporting Information section.

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4. DISCUSSÃO GERAL

A hipótese inicial dessa tese foi de que o MK-801 e a anfetamina seriam capazes de induzir alterações comportamentais e neuroquímicas relevantes para o estudo da esquizofrenia. Essa hipótese foi confirmada parcialmente, pois o MK-801 foi capaz de induzir hiperlocomoção contexto-dependente e déficit de interação social nos peixes-zebra, endofenótipos relevantes no estudo da esquizofrenia e semelhantes aos achados observados em roedores. Porém, a exposição a anfetamina não induziu hiperlocomoção nos peixes-zebra como é observado em roedores. Por outro lado, a anfetamina induziu dano oxidativo, uma alteração patofisiológica observada em modelos de esquizofrenia.

O primeiro objetivo desta tese foi realizar uma ampla revisão da literatura, buscando investigar os efeitos de antagonistas de receptores NMDA em parâmetros comportamentais em peixes-zebra. Através dessa revisão da literatura foi possível integrar os principais achados relatados em peixes-zebra sobre os efeitos comportamentais dos antagonistas de receptores NMDA com relevância no estudo da esquizofrenia. Além disso, os resultados obtidos nessa revisão possibilitaram discutir as metodologias e os protocolos experimentais utilizados em peixes-zebra para avaliar o efeito de antagonistas de receptores NMDA nesse organismo modelo.

Através de uma busca nas bases de dados MEDLINE (PubMed) e Web of Science foram identificados 590 estudos. 44 artigos atenderam aos critérios de inclusão e foram selecionados para compor o artigo de revisão (capítulo I). Desses 44 artigos, MK-801 e cetamina foram as drogas utilizadas com maior frequência, sendo o MK-801 utilizado em 29 estudos e a cetamina em 10 estudos. O uso de outros antagonistas de receptor NMDA, como fenciclidina (PCP), ácido DL-2-amino-5-fosfonopentanóico (APV), memantina e tiletamina, foi descrito em apenas 6 estudos. Os achados mais frequentes nessa revisão foram os déficits na interação social e comprometimento da memória induzidos pelo MK-801 e comportamentos circulares induzidos pela cetamina (Figura 3). No entanto, foram observados resultados mistos e inconsistentes para os parâmetros locomotores e de ansiedade.

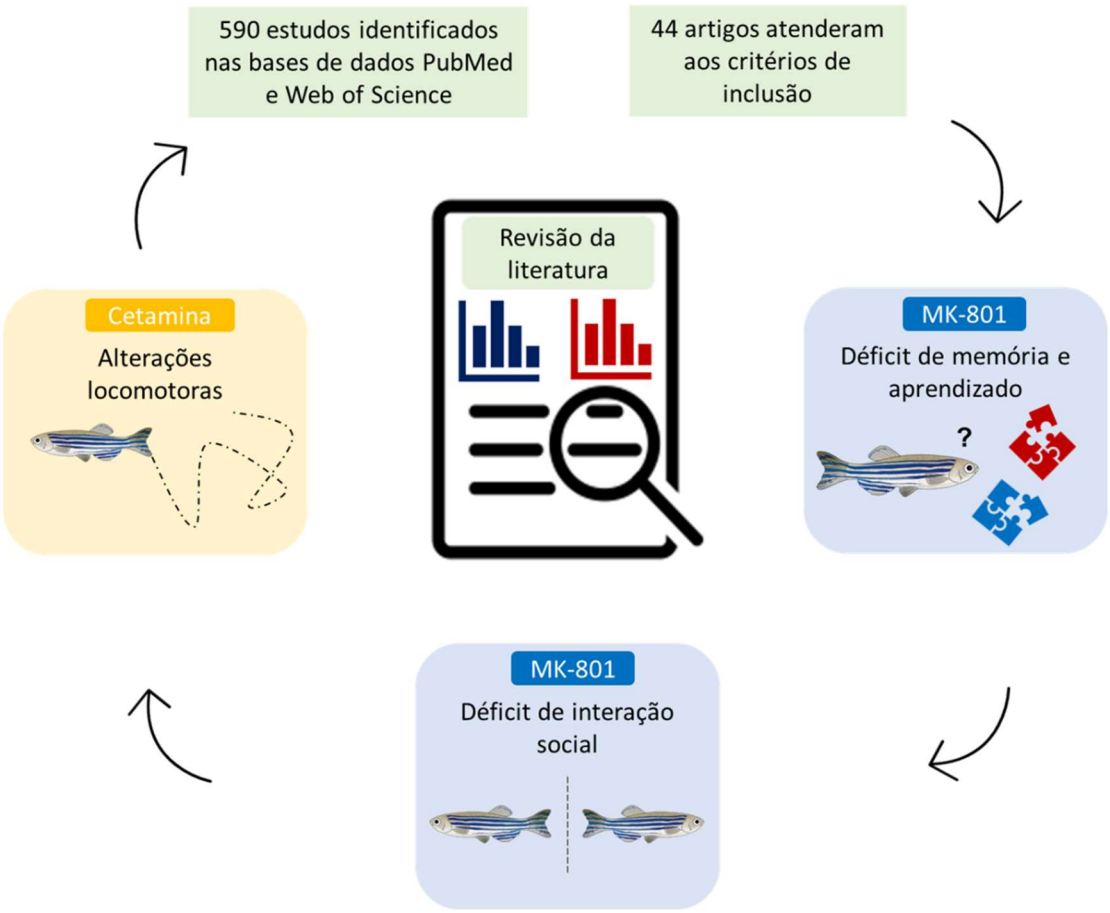

Figura 3. Esquema representando os principais resultados encontrados na revisão da literatura (Capítulo I).

O segundo objetivo dessa tese foi avaliar os efeitos do MK-801 e da anfetamina em parâmetros comportamentais e neuroquímicos com relevância na esquizofrenia (Figura 4). Para isso, os peixes-zebra adultos de ambos os sexos foram expostos ao MK-801 ou à anfetamina e avaliados em diversos testes comportamentais e parâmetros de estresse oxidativo. De forma geral, o MK-801 diminuiu a interação social em peixes-zebra adultos, um endofenótipo relacionado aos sintomas negativos da esquizofrenia. Além disso, o MK-801 diminuiu a distância total percorrida e aumentou o tempo de imobilidade em testes de atividade locomotora, o que difere do que é observado em estudos com roedores. Curiosamente, o MK-801 induziu hiperlocomoção na presença de estímulos sociais no teste de interação social, o que sugere que o MK-801 provoca efeitos opostos que são dependentes do contexto, como a presença de estímulo social. Por outro lado, a exposição à anfetamina não induziu hiperlocomoção ou déficit de

interação social em peixes-zebra adultos, mas foi capaz de alterar o estado oxidativo no encéfalo, um aspecto relevante relacionado à fisiopatologia da esquizofrenia.

Como discutido anteriormente, acredita-se que a disfunção de interneurônios GABAérgicos positivos para parvalbumina presentes no hipocampo, associada um déficit funcional dos receptores NMDA que são expressos por esses interneurônios, resultam em um desequilíbrio de excitação-inibição em áreas subcorticais (Balu et al. 2013; Insel 2010; Korotkova et al. 2010). Esse desequilíbrio resulta em uma hiperativação dopaminérgica no estriado, o que está associado aos sintomas da esquizofrenia. A exposição ao MK-801, um antagonista do receptor NMDA, mimetiza um aspecto importante da patofisiologia da esquizofrenia, que é a hipofunção do receptor NMDA. Estudos demonstram que esse bloqueio dos receptores NMDA resulta em uma desinibição dos neurônios piramidais excitatórios do hipocampo e, consequentemente, leva à alteração nos níveis de disparo de neurônios dopaminérgicos nas vias mesolímbica e mesocorticais, o que resulta em alterações comportamentais semelhantes à esquizofrenia em roedores (Hardingham e Do 2016). Embora a exposição crônica a antagonistas do receptor NMDA em modelos de roedores leve a alterações comportamentais persistentes, a administração aguda também é capaz de mimetizar aspectos importantes desse transtorno. Estudos clínicos mostraram que a exposição aguda a antagonistas de receptores NMDA pode recapitular toda a gama de sintomas de esquizofrenia em indivíduos saudáveis e piorar os sintomas em pacientes com esquizofrenia (Beck et al. 2020), destacando a relevância da exposição aguda ao MK-801 em animais modelo. Por outro lado, a exposição à anfetamina, um agonista dopaminérgico, mimetiza a hiperativação na transmissão dopaminérgica, relacionada aos sintomas positivos (Jones, Watson, e Fone 2011).

Um teste comportamental que é amplamente utilizado em modelos de esquizofrenia em roedores é a distância percorrida após uma injeção intraperitoneal de MK-801 (Bygrave et al. 2016) ou anfetamina (Herrmann et al. 2014). Embora os efeitos dessas drogas na locomoção de roedores sejam bem descritos e conhecidos, estudos nesse tipo de parâmetro em peixes-zebra são pouco descritos. Dessa forma, realizamos um protocolo experimental semelhante àquele usado em roedores, em que a atividade locomotora foi avaliada ao longo do tempo, durante 60 minutos após a exposição ao MK-801. Surpreendentemente, o MK-801, na concentração de 5 μM, diminuiu a distância total percorrida, já na concentração de 10 μM, aumentou o tempo na zona

superior, o que pode indicar diminuição da ansiedade ou alterações locomotoras. A partir desses resultados, nossa hipótese foi de que outros fatores poderiam estar influenciando essa resposta comportamental induzida por MK-801. A anfetamina não induziu hiperlocomoção nesse teste comportamental, o que contrasta com os efeitos estimulantes amplamente observados em roedores. Acreditamos que isso se deve às diferenças já descritas nos circuitos neurais motores centrais de peixes e de roedores e outros mamíferos (Ryczko et al. 2017). Uma diferença óbvia é o fato de que os peixes estão nadando a maior parte do tempo. Estudos realizados em lampreias (*Petromyzon marinus*) mostraram que existem neurônios dopaminérgicos e glutamatérgicos que se projetam para a região locomotora mesencefálica, indicando uma estreita interação entre esses neurotransmissores na geração do comando locomotor. Além disso, o bloqueio dos receptores dopaminérgicos nessa área resultou em movimentos de natação reduzidos sem interromper o controle gradual da locomoção, enquanto o bloqueio dos receptores glutamatérgicos quase aboliu a locomoção, indicando que a contribuição glutamatérgica é essencial para a locomoção dos peixes, enquanto a contribuição dopaminérgica fornece modulação adicional, mas não é essencial para a locomoção (Ryczko et al. 2017). Nossa hipótese é que, devido aos diferentes circuitos centrais de regulação motora dos peixes, os resultados com a exposição ao MK-801 nos parâmetros de locomoção do peixe-zebra são mais robustos do que com a exposição à anfetamina.

Realizamos então um teste comportamental para avaliar os efeitos do MK-801 e da anfetamina em comportamentos semelhantes a estereotipia. A estereotipia é definida como comportamentos repetitivos e invariáveis (Morrens et al. 2006). Comportamentos estereotipados induzidos por drogas não são bem caracterizados em peixes-zebra, mas alguns autores sugerem que eles podem ser exibidos como movimentos de rotação alterados (Michelotti et al. 2018). Realizamos o teste de tanque aberto que é capaz de avaliar movimentos circulares em peixes-zebra, já que os animais são filmados de cima. Os resultados demonstraram que o MK-801, na concentração de 5 μM, diminuiu o ângulo absoluto de virada e as rotações no sentido horário e aumentou o tempo que os animais permaneceram imóveis. A anfetamina também foi capaz de alterar um parâmetro relacionado à estereotipia. Nas concentrações de 0,625 e 10 mg/L, a anfetamina foi capaz de diminuir o ângulo absoluto de virada.

O terceiro teste realizado foi o teste de interação social, um teste capaz de avaliar a preferência social de peixes-zebra. Como o isolamento social é um dos principais

sintomas negativos da esquizofrenia, ensaios comportamentais que modelem esse aspecto são fundamentais para estudar e desenvolver tratamentos para essa necessidade clínica não atendida. Os peixes-zebra vivem em cardume e apresentam um comportamento social complexo, com relações hierárquicas e reprodutivas (Dreosti et al. 2015). Dessa forma, podem ser um organismo modelo adequado para modelar a interação social. Nesse teste, observamos que o MK-801, nas concentrações de 5 e 10 μM, diminuiu de forma robusta a interação social. Curiosamente, essas concentrações de MK-801 também causaram hiperlocomoção durante o teste de interação social, embora esta droga não tenha apresentado esse efeito nos testes anteriormente descritos. Isso sugere que não apenas a novidade de contexto (Tran et al. 2016), mas também a presença de pistas sociais, podem modular diferencialmente os efeitos do MK-801 em peixes-zebra. No teste de interação social, a anfetamina não causou mudanças significativas no comportamento motor ou social dos peixes-zebra, replicando o que é amplamente descrito em estudos com roedores.

Por fim, coletamos os encéfalos dos peixes-zebra expostos ao MK-801 e à anfetamina para avaliar parâmetros relevantes de estresse oxidativo. Estudos em modelos de roedores relatam que o aumento da liberação de dopamina induzida pela anfetamina promove estresse oxidativo, provavelmente devido ao aumento do metabolismo das monoaminas (El-Tawil et al. 2011; Dichtl et al. 2018; Frey et al. 2006). O MK-801 não causou nenhuma alteração no estado oxidativo após 10 ou 60 minutos de exposição, o que está de acordo com estudos em roedores, em que foi observado que apenas exposições repetidas ao MK-801 são capazes de produzir um aumento nas espécies reativas de oxigênio (Wang et al. 2013). Interessantemente, todas as concentrações testadas de anfetamina aumentaram os níveis de substâncias reativas ao ácido tiobarbitúrico (TBARS), um marcador bem estabelecido de peroxidação lipídica, após 60 minutos, mas não após 10 minutos de exposição, indicando um efeito dependente do tempo na produção de espécies reativas. Este aumento nos níveis de TBARS, no entanto, não foi compensado por alterações nos níveis de tióis não proteicos (NPSH), que indiretamente representam níveis reduzidos de glutationa (GSH) e atividade antioxidante. Não podemos descartar, no entanto, uma diminuição de NPSH ou mesmo um aumento compensatório em um momento posterior. Nesse contexto, mais estudos são necessários para desvendar a cinética envolvida nos efeitos pró-oxidantes da anfetamina em peixes-zebra. Estes são dados novos, uma vez que não há estudos avaliando os efeitos do anfetamina nos parâmetros de estresse oxidativo em peixes-

zebra.

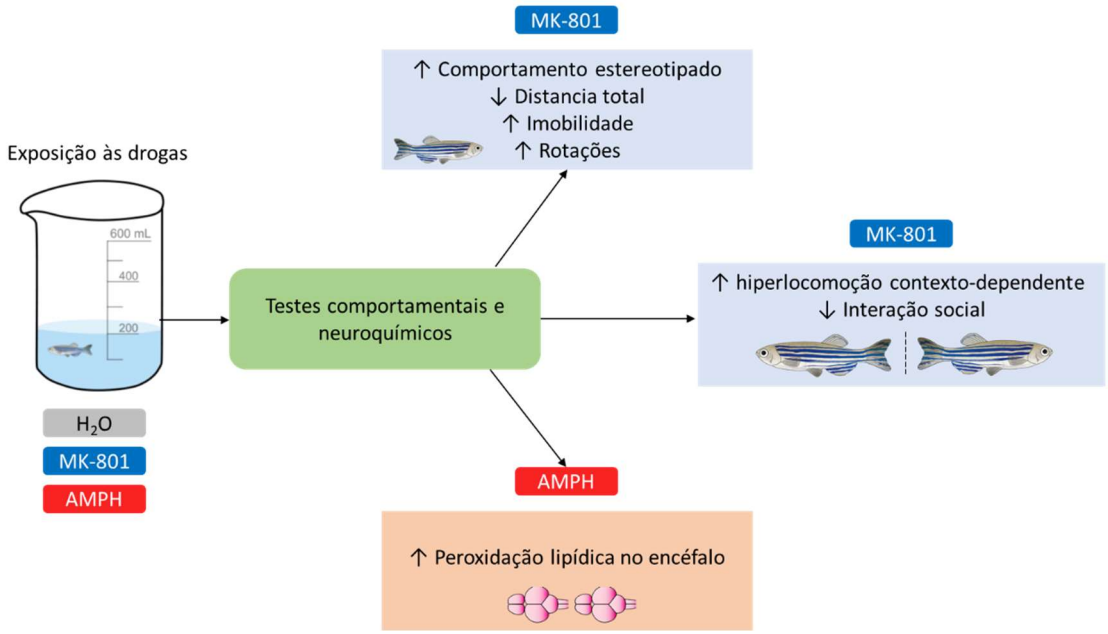

Figura 4. Esquema representando os principais resultados encontrados na avaliação dos efeitos de MK-801 e anfetamina em peixes-zebra adultos (Capítulo II). H_2O, água; MK-801, dizocilpina; AMPH, anfetamina.

5. CONCLUSÕES

Considerando os contínuos esforços científicos na modulação dos principais aspectos da esquizofrenia e desenvolvimento de novos tratamentos, avaliações comportamentais e neuroquímicas diretas relevantes para esta condição precisam ser investigadas. Nossos estudos corroboram com a hipótese inicial desse trabalho, ou seja, com a ideia de que endofenótipos de esquizofrenia podem ser modelados em peixes-zebra, um passo essencial para avançar no uso dessa espécie como organismo modelo para triagem de alto rendimento para o desenvolvimento de novos tratamentos. Embora mais estudos sejam necessários para confirmar nossos achados, concluímos que o MK-801, mais do que as outras drogas avaliadas nesse estudo, é uma ferramenta farmacológica útil na avaliação de endofenótipos comportamentais relevantes para a esquizofrenia em peixes-zebra.

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7. ANEXOS

Anexo 1. Carta de aprovação da Comissão de Ética no Uso de Animais (CEUA) da Universidade Federal do Rio Grande do Sul.

U F R G S
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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

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